Genetic and phenotypic characterization of small colony variant *Staphylococcus aureus* cystic fibrosis isolates

Mimi Rey Precit

A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2017

Reading Committee:
Lucas Hoffman, Chair
Caroline Harwood
Matthew Parsek

Program Authorized to Offer Degree:
Microbiology

University of Washington

Abstract

Genetic and phenotypic characterization of small colony variant *Staphylococcus aureus* cystic fibrosis isolates

Mimi Rey Precit

Chair of the Supervisory Committee:
Doctor Lucas Hoffman, MD,Phd
Adjunct Professor Microbiology
Associate Professor Pediatrics

Staphylococcus aureus small colony variants (SCVs) are recovered frequently from chronic, antibiotic refractory infections and are associated with worse disease outcomes and treatment failures. The defining SCV characteristic is slow growth *in vitro* resulting in small, atypical colony formation. SCVs arise following exposure to antimicrobials both *in vitro* and *in vivo,* however their unusual growth phenotype prevents the use of traditional laboratory diagnostics to guide treatment. In an effort to define SCV antibiotic resistance patterns, and provide empirical treatment options; we developed, optimized, and performed antibiotic susceptibility testing on a collection of clinical cystic fibrosis (CF) isolates. These findings are presented in Chapter 2. The SCV phenotype is caused by genetic changes in critical metabolic pathways that result in nutrient auxotrophies, most commonly for thymidine (THY), hemin, or menadione. THY–dependent SCVs have been associated with poor clinical outcomes in CF and we present evidence presented in Chapter 3 that the pro-inflammatory, small molecule cyclic-di-AMP may play a role in disease

progression. Additionally, although genetic mechanisms contributing to SCV formation have been reported, only a limited number of genes and pathways have been described. Furthermore, we and other groups have found that SCVs of the same auxotrophy type display morphological and colony size variability, suggesting additional mutations. Thus, we examined the genetic mechanisms behind SCV formation using whole-genome sequencing for a collection of clinical SCVs and normal colony (NC) *S. aureus* and have identified novel mutations presented in Chapter 4. Lastly, the traditional SCV designation, ~1/10 the size of NC *S. aureus*, is somewhat vague and a more precise definition would benefit both clinical as well as research laboratories working to categorize atypical, slow growing *S. aureus.* In an effort to ultimately provide a more rigorous, statistically driven definition of what constitutes an SCV, we examined the distribution of colony sizes that clinical *S. aureus* isolates display *in vitro* and performed preliminary statistical analyses, presented in Chapter 5. The full body of work presented here addresses important topics including antibiotic resistance, diagnostic development, pathogenesis, phenotypic, and genomic analyses of SCVs in an effort to further our understanding of this unusual variant of *S. aureus.*

“We sometimes underestimate the influence of little things”
- Charles W. Chesnutt

This work is dedicated to the little things that are all too often overlooked

Acknowledgements

I would like to extend my deepest appreciation and gratitude to everyone that contributed substantially to the work presented in this thesis as well as those who have supported me over the years.

First and foremost thank you to my brilliant, conscientious, and devoted advisor Dr. Lucas Hoffman, committee member and genius behind small colony variant susceptibility testing Dr. Jane Burns, committee members Dr. Matthew Parsek, Dr. Caroline Harwood, and Dr. Richard Darveau, the king of cyclic-di-AMP Dr. Joshua Woodward and fellow grad student Qing Tang, the master of the genome Dr. Stephen Salipante, and my dedicated mentor Dr. Daniel Wolter.

A special thank you to members of the Hoffman lab; to Dr. Christopher Pope for making me laugh everyday and reminding me that science is supposed to be fun, to fellow graduate student, now Dr., Catherine Armbruster for keeping me sane and for both blazing a trail and outfitting me with a beacon to follow in your footsteps.

Thank you to my husband Gavin for picking up all of the slack I unavoidably generated over the past 5+ years, my father for fostering tenacity, my mother for being consistently present for every panicked phone call and emotional collapse, and my animals for forcing me to go outside.

Lastly, I would like to thank the University of Washington Microbiology Department for presenting me with the opportunities to pursue my passions for microbiology and research.

Table of Contents

Chapter 1 Introduction...1

Chapter 2 An Optimized *in vitro* Antibiotic Susceptibility Testing Method for Small Colony Variant *Staphylococcus aureus*...19

Chapter 3 Thymidine-dependent *Staphylococcus aureus* Small Colony Variants Produce Excessive cyclic-di-AMP that Modulates STING-dependent Type I Interferon Production...76

Chapter 4 Preliminary Examination of Genetic Mechanisms Behind *S. aureus* Thymidine-Dependent and Hemin-Dependent Small-Colony Variant Formation in Cystic Fibrosis Clinical Isolates...110

Chapter 5 Preliminary Investigation of *in vitro* Colony Size Distribution of *Staphylococcus aureus* Cystic Fibrosis Isolates...131

Chapter 6 Discussions and Future Directions...157

Appendix A Genes with mutations present in *Staphylococcus aureus* thymidine-dependent small colony variants but absent in normal colony *S. aureus*...169

Appendix B Demographic data for patients represented in the *Staphylococcus aureus* colony size distribution study...216

List of Tables

Chapter 2

Table 1 Summary of media used in previous published studies of SCV AST....22

Table 2 (A) Complete list of strains/isolates used in this study....24

(B) List of isolates used for BD-MHA+Blood testing....24

Table 3 Complete list of antibiotics used in this study....29

Table 4 Comparison of disk-diffusion AST results of 14 selected normal colony (NC) clinical isolates....50

Table 5 Susceptibility profiles of NC and SCV clinical isolates determined by disk diffusion....52

Table 6 Atmospheric dependent susceptibility profiles of four isogenic THYD-SCVs....55

Table 7 Susceptibility profiles shown as percent susceptible of NC and SCV clinical isolates determined by etest....58

Table 8 Susceptibility profiles shown as percent susceptibilities of SCV and NC isolates....59

Table 9 Comparison of SCV and NC susceptibility profiles....61

Table 10 AST profiles among clonally-related SCVs for which those profiles differed....62

Table 11 Relationships between antibiotic exposure and *in vitro* susceptibilities....64

Chapter 3

Table 1 Primer sequences 5’-3’ for deletion constructs in *S. aureus*.........81

Chapter 4

Table 1 Defined mechanisms behind SCV formation in *S. aureus*..........112

Table 2 Primers 5’-3’ used for *thyA* and *hemA* deletion constructs.........118

Table 3 Genes with mutations in THYD-SCVs not present in NC isolates 120

Table 4 Mutations present in HEMD-SCVs but absent in isogenic, co-isolated NC isolates...122

Chapter 5

Table 1 Breakdown of auxotrophy types found in the isolate set...........137

Table 2 Normality Tests and generated values completed for each growth medium..139

Table 3 (A) Descriptive statistics of colony sizes on MHA (normally distributed)..143

(B) Descriptive statistics of colony sizes on media not modeled by a normally distributed..143

Table 4 Median and interquartile ranges for average colony sizes...........146

Table 5 Patient age corresponding to *S. aureus* cultures graphed in Figure 3 149

Table 6 (A) Descriptive statistics for Mueller-Hinton agar (MHA) to establish cut off values based on mean and standard deviation..150

(B) Colony size categorization cut offs based on distribution of colony sizes on MHA...150

Table 7 Isolate categorization based on colony size phenotype as determined by descriptive statistics for Mueller-Hinton agar.....152

List of Figures

Chapter 1

Figure 1 Distinguishing phenotypic features of SCVs..................................3

Figure 2 Summary schematic of work presented in this thesis....................13

Chapter 2

Figure 1 Distinguishing phenotypic features of SCVs.................................21

Figure 2 Interpretation of susceptibility to trimethoprim-sulfamethoxazole (SXT/TS) on media containing thymidine....................................31

Figure 3 PFGE dendrogram of all *S. aureus* clinical isolates used in this study..35

Figure 4 Growth of different SCV auxotrophic types on media containing various concentrations of supplements...37

Figure 5 Quality of SCV growth as determined by visual inspection of colony size on agar based media..39

Figure 6 Morphological variability indicative of reversion for SCVs grown on different media..42

Figure 7 Differentiation between SCVs and revertant NCs on LB agar......43

Figure 8 Percent reversion of SCVs on growth supportive media...............44

Figure 9 Gram's stain images of SCV cells with different auxotrophies after growth on indicated media..46

Figure 10 Average zone diameters for ATCC 25923 in air and CO_2.............48

Figure 11 Median MICs for ATCC 29213..56

Chapter 3

Figure 1 Auxotrophy test for thymidine and hemin dependence in the deletion strains..........82

Figure 2 Nucleotide sequences in thymidylate synthase (*thyA*) in thymidine dependent (THYD) isolates recovered from cystic fibrosis patients..........86

Figure 3 Δ*thyA* exhibited decreased survival ability in macrophages compared to *S. aureus* Newman and Δ*hemB* Newman..........90

Figure 4 Optimization of multiplicity of infection (MOI)..........91

Figure 5 Δ*thyA* induces more inflammation in macrophages compared to *S. aureus* Newman and Δ*hemB* Newman..........93

Figure 6 Δ*thyA* activates STING-dependent type I IFN response in macrophages..........94

Figure 7 Thymidine controls c-di-AMP levels in Δ*thyA*..........98

Figure 8 Δ*thyA* drives IFNβ production during lung infection..........101

Chapter 4

Figure 1 Demonstration of auxotrophy for thymidine and hemin in respective deletion strains..........123

Chapter 5

Figure 1 Frequency distribution of colony sizes of the isolate set on three growth media..........140

Figure 2 Scatter-dot plots showing spread of colony sizes..........145

Figure 3 Average colony size for three representative patients on three growth media..........148

Chapter 1:

Introduction

Small-colony variant bacteria

Small colony variants (SCVs) are a fastidious, slow-growing subpopulation of bacteria that have been described for a wide range of bacterial genera and species (1). SCV *Staphylococcus aureus Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Stenotrophomoas maltophila*, *Burkholderia cepacia* complex, *Vibrio cholerae, Neisseria gonorrhoeae, Escherichia coli, Salmonella enterica typhi*, *Brucella melitensis, Serratia marcescens, Shigella* species, and *Lactobacillus* species have been reported in the literature. Although SCVs have been observed across bacterial genera, SCV staphylococci are the most commonly studied.

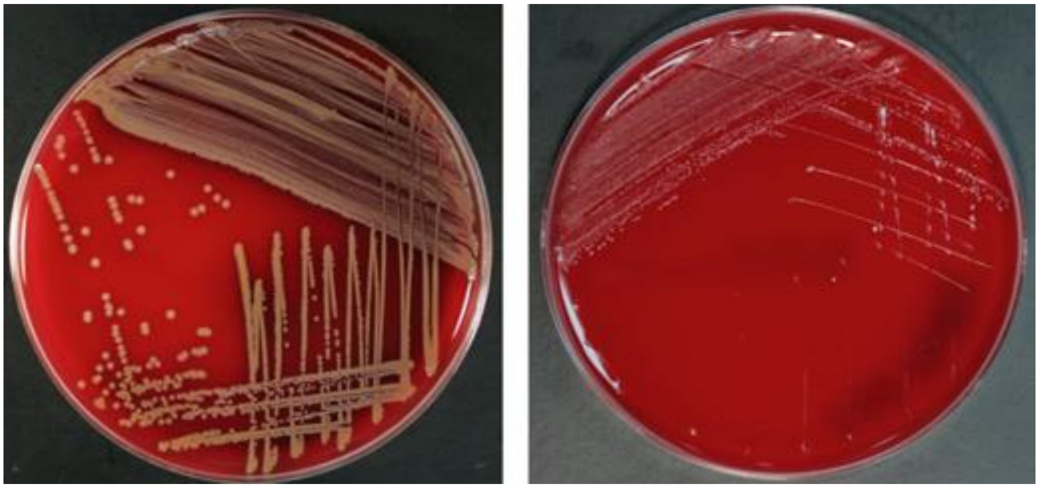
The first descriptive reports of SCV staphylococci, then termed "dwarf", "dissociate", or "G (gonidial)" colonies date back to the early 1900s (1-6). The first investigators studying this unusual morphotype observed that a combination of refrigeration and passage of *Staphylococcus aureus* or *S. epidermidis* in the laboratory could result in the formation of distinct colony variants (2, 6). They focused primarily on measuring growth rates and describing metabolic differences between various sized colonies within a clonal population (2, 3, 6). Reports commonly stated that small colonies had distinct morphologies such as atypical pigmentation on nutrient agar (4, 6) and significantly slower growth rates than larger colonies (2, 4) (Figure 1A). The propensity for small colonies to revert to a wild-type (WT), normal colony (NC) size was also often noted. Some groups expanded their experiments to survey growth compounds that enhanced *S. aureus* or *S. epidermidis* SCV growth, such as horse blood and ground animal tissues added to nutrient agar (3). It is now appreciated that many SCV staphylococci have metabolic defects that disrupt central metabolism and, as a result, typically become auxotrophic, requiring certain nutrients such as menadione, hemin, thiamine, unsaturated fatty acids,

A

Normal Colony (NC) / Wild Type

SCV–Small Colony Variant

B



Normal Colony / Wild Type

SCV - Small Colony Variant

Figure 1. Distinguishing phenotypic features of SCVs. (A) *In vitro* growth comparison of normal colony (NC) and a genetically related small colony variant (SCV) on nutrient agar showing distinct small colony size of an SCV. (B) *In vitro* comparison of NC and SCV on blood agar highlighting distinct SCV characteristics, including reduced hemolysis, small colony morphology, and decreased pigmentation.

thymidine, or CO_2 for growth (8). Supplementing SCV growth conditions with one or more of these compounds will complement growth to WT/NC levels.

Small-colony variant *S. aureus* associated infections

S. aureus is a versatile, opportunistic human pathogen that can cause disease in almost any tissue on the human body ranging from acute minor skin lesions to recurrent, antibiotic-refractory infections that can persist for years (7). Many reports in the last two decades have shown that chronic *S. aureus* infections of this type are often associated with the recovery of *S. aureus* SCVs (7, 19-21). Despite the observation that *S. aureus* SCVs are the most common SCV type associated with human and animal infections, interestingly, in 1910 the first reported SCV from a clinical sample was actually a *Salmonella typhi* variant recovered from primary blood cultures of a patient suffering from typhoid fever in 1910 (22). It wasn't until approximately 40 years later in the 1950's that the first clinical association between human infection and *S. aureus* SCVs was formally published when septic skin lesion samples from two distinct patients grew CO_2 dependent *S. aureus* SCVs (23).

Since their initial recovery from a human infection, *S. aureus* SCVs have been recovered from a wide variety of clinical specimens (8). However, the important association between *S. aureus* SCVs and chronic infections was not recognized until the 1990's. Studies carried out by Proctor *et. al* in 1994 and 1995 first described that SCVs were characteristically found in recurrent, relapsing *S. aureus* infections that had failed to respond to antibiotic treatment (24, 25). They reported on a range of clinical conditions including osteomyelitis, septic arthritis, bacteremia, and purulent sinusitis. Proctor and colleagues observed that SCVs recovered from these patients had electron-transport chain defects and were auxotrophic for critical electron-transport chain components such as hemin or menadione. Additionally, these investigators were the first to shed light on the concern that, due to abnormal growth characteristics of *S. aureus* SCVs (i.e., reduced colony size, reduced hemolysis on blood agar, and atypical pigmentation (Figure 1B), clinical microbiology laboratories were regularly misidentifying or not detecting potentially clinically important SCVs (7, 26). The concern of *S. aureus* SCVs being overlooked and/or undetected by many clinical microbiology laboratories still persists today (7, 27).

In the past 20 years, reports that underscore the importance of SCVs in infections that fail treatment have become common in the literature. It is now appreciated that *S. aureus* SCVs are frequently recovered from a variety of chronic infections such as osteomyelitis (28), device-related infections (19), soft-tissue infections (26), endocarditis (29), and most notoriously, respiratory tract infections in patients with the genetic disease cystic fibrosis (CF) (20, 30, 31).

Chronic infection with *S. aureus* is common among patients with CF

CF is a genetic disorder caused by a mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Normally, the CFTR protein regulates the flow of ions and water across epithelial cell membranes (32), but defects in this channel result in abnormal mucous secretions affecting multiple organ systems. CF is one of the most common hereditary diseases in the Caucasian population, affecting approximately 71,000 people worldwide (32). The hallmark clinical manifestation of CF is chronic pulmonary disease characterized by chronic airway infection. While the CF lung is susceptible to infection by an array of microorganisms, and lung infections are often polymicrobial, the bacterium detected earliest and most frequently is *S. aureus*

(CF patient registry 2015). The recovery of *S. aureus* and a specific *S. aureus* subpopulation, methicillin resistant *S. aureus* (MRSA), have been associated with poor clinical outcomes in CF patients (33, 34), and often the same clone of *S. aureus* can persist in the airways for years (32). Persistent infections of this type in the CF airway offers a unique niche where *S. aureus* faces numerous selective pressures such as neutrophil influx (35), hypoxia (36), competition with other bacteria (37), and antibiotics (38), which lead to various forms of bacterial adaptation, such as SCV emergence.

Chronic *S. aureus* infection in CF and the recovery of *S. aureus* SCVs

Antibiotic-refractory, chronic *S. aureus* infections in the CF lung are often associated with the recovery of *S. aureus* SCVs (1). The first study to report on *S. aureus* SCV prevalence in CF populations was published by a European group, Sparham *et al.*, in 1978. They found that ~50% of patients were culture positive for *S. aureus* SCVs (39). Subsequent work carried out by Gilligan *et. al.*, in 1987 and Kahl *et al.*, in 1998 reported SCV detection from CF secretions, specifically stressing the importance of using SCV specific detection methods (30, 40). Within the past decade, multiple groups in both Europe and the United States have shown that *S. aureus* SCVs are isolated from between 4-50% of CF patients, depending on the study population and isolation methods used (8, 26, 27, 41-43). Collectively, these studies reported that *S. aureus* SCVs recovered from the CF lung exhibit characteristic SCV features such as decreased pigmentation, and metabolic defects resulting in slow *in vitro* growth compared to WT or normal colony (NC) *S. aureus*. Additionally, the vast majority (80-95%) of the SCVs recovered from CF secretions were auxotrophic for thymidine (THY), a nucleoside with the DNA base thymine. THY-dependent (THYD) SCVs therefore require exogenous THY, either unphosphorylated or the monophosphate, for DNA synthesis to proceed. SCVs dependent on hemin (HEM), menadione (MEN), and/or CO_2 are also recovered from CF secretions but at lower frequencies (5-20%). HEM and MEN are defective for electron transport chain components critical for normal cellular respiration, while CO_2 is a non-specific stimulant for *S. aureus* growth. SCVs with undefined auxotrophies have also been described (1, 27, 41).

Antimicrobials can select for *S. aureus* SCVs both *in vitro* and *in vivo*

Given the relatively high frequency of detection and association with persistent lung infections, researchers and clinicians in the early 2000's began investigating how *S. aureus* SCVs arise in the CF lung as well as other chronic infections. Although it is likely that many selective pressures have not yet been described, it has been established that antibiotics commonly used to treat CF lung infections can select for *S. aureus* SCVs. Earlier reports focused on HEM-dependent (HEMD) and MEN-dependent (MEND) SCVs, which are more commonly isolated from bone and soft-tissue infections (7). A direct association between NC *S. aureus* exposure to either aminoglycosides, protein synthesis inhibitors commonly used to treat bacterial infections in CF, or toxic *Pseudomonas aeruginosa* exoproducts (known to frequently be present in CF secretions) and subsequent selection of HEMD or MEND SCVs *in vitro* has been established (12, 17). Further studies have also shown that aminoglycosides likely select HEMD or MEND SCVs *in vivo* since they are recovered from patients following treatment with the drug (7). Furthermore, these types of SCVs, both laboratory-derived and clinical isolates, show resistance to aminoglycosides compared to isogenic normal-colony strains *in vitro* (17). Aminoglycoside efficacy depends on an intact electron transport chain in *S. aureus,* and HEMD and MEND SCVs are defective in cellular respiration, preventing aminoglycoside uptake and therefore activity. Both MEND and HEMD-

SCVs have also been selected *in vitro* following exposure to *P. aeruginosa* exoproducts that suppress *S. aureus* respiration, including hydroxyquinoline n-oxide (HQNO) (12) and pyocyanin (44); these SCVs also show aminoglycoside resistance.

In contrast, selection of THY-dependent (THYD) SCVs, the primary type recovered from CF secretions, has been linked to a different antibiotic class: folate biosynthetic inhibitors such as trimethoprim-sulfamethoxazole (TMP-SMX). TMP-SMX interrupts two enzymatic steps in the metabolic pathway for folate, a cofactor essential for converting dUMP to dTMP, which is required for DNA synthesis. TMP-SMX, like the aminoglycosides, is often prescribed to treat CF lung infections, and THYD SCVs commonly arise following treatment with this drug (27, 40, 45). Additionally, THYD-SCVs can be selected *in vitro* following TMP-SMX exposure if external thymidine is available for uptake, which at least two separate studies have shown to be the case in CF sputum (46, 47). THYD-SCVs consequently show high levels of resistance to the TMP-SMX compared to isogenic normal colony *S. aureus* (48). Although similar phenotypes have been described for the different types of SCVs (i.e., slow growth, altered pigmentation, reduced expression of classic virulence factors, and reduced hemolysis), different genetic lesions in particular metabolic pathways are observed amongst HEMD, MEND, and THYD SCVs.

Known genetic mechanisms behind the SCV phenotype

Analyzing the causal mutations for small-colony formation in both clinical SCVs and those selected *in vitro* has proven challenging due to frequent phenotypic reversion *in vitro* of SCVs during analysis (7, 49). However, previous work using targeted gene sequencing and/or complementation experiments successfully identified the most common THYD-SCV causal mutations. In these previous studies, clinical THYD-SCVs were found to have mutations in the gene encoding thymidylate synthase (*thyA)*, a folate-dependent enzyme that catalyzes methylation of dUMP to dTMP (16, 45, 48). While these groups went on to show that a WT copy of *thyA* on a plasmid improved growth and susceptibilities to TMP-SMX (16, 45), neither group found that this complementation fully restored growth to WT levels, suggesting that additional genes are likely altered in clinical THYD-SCVs. By contrast, several groups have engineered strains with *thyA* gene deletions, showing that these strains display the THYD phenotype that can be complemented fully with an intact copy of the gene, unlike many clinical isolates (16, 48).

The genetic basis of SCV formation in clinical HEMD and MEND-SCVs has not been well studied, apparently due to phenotypic (and, presumably, genotypic) instability under the *in vitro* growth conditions (1). The few clinical isolates that have been characterized, and a handful selected *in vitro* by aminoglycoside exposure, were found to have mutations in a variety of MEN (50, 51) or HEM biosynthetic genes (17). In support of these mechanisms, Von Eiff *et al.* showed that a site-directed inactivating mutation in the HEM biosynthetic gene *hemB* conferred growth as an SCV *in vitro* (28). However, none of this work either analyzed HEMD or MEND isolates recovered from CF sputum or compared the characteristics of HEMD, MEND, and THYD-SCVs from CF patients.

***S. aureus* SCVs are associated with poor clinical outcomes in CF**

Later studies in the early/mid 2000's began to address whether *S. aureus* SCVs impacted clinical outcomes in CF patients; several European groups reported correlations between *S. aureus* SCV detection in respiratory specimens and reduced lung function, measured by forced expiratory volume (FEV_1), in CF patients (8, 52). However, the turning point however came in 2013 when Wolter and Emerson *et al.* at the University of Washington and Seattle Children's Hospital found that detection of *S. aureus* SCVs from children with CF was significantly and independently associated with poor clinical outcomes (27). The findings presented in that work provided a comprehensive statistical assessment employing multivariable regression analyses, demonstrating that controlling for many variables, such as age or the presence of other relevant CF pathogens like *Pseudomonas aeruginosa*, did not weaken the correlation between *S. aureus* SCV status and poor clinical outcome (27). These findings suggest that *S. aureus* SCVs are likely either an important CF pathogen or a marker for advanced lung disease.

Although SCV *S. aureus* may play a critical role in CF patient outcome and represent a form of antibiotic resistance to CF-relevant antimicrobials through mechanisms not found in co-isolated normal colony *S. aureus* (7), most clinical microbiology laboratories do not report on the presence of SCVs from clinical samples. Furthermore, because SCVs grow too slowly for standard *in vitro* antibiotic susceptibility testing, there has been very little available information until recently (53) (presented in Chapter 2), to help direct treatment of important SCV-related infections (27).

Expanding our understanding of key phenotypic and genetic features of *S. aureus* SCVs

The work presented in this thesis (Summarized in Figure 2) is focused on the phenotypic and genetic characterization of *S. aureus* SCV cystic fibrosis isolates, with the aim of addressing many of the knowledge gaps identified above. Starting in Chapter 2, I present the development and application of a novel antibiotic susceptibility testing method specifically designed and optimized for *S. aureus* SCVs; I also describe how this work is impacting and informing clinical practice.

Chapter 3 begins to address the difficult question of how *S. aureus* SCVs, specifically THYD-SCVs, contribute to poor clinical outcomes by examining the effects of a pro-inflammatory small molecule that is highly abundant in THYD-SCVs. In Chapter 4, I present preliminary whole-genome sequencing analysis of a collection of CF *S. aureus* isolates, with the aim of investigating the genetic changes responsible for THYD- and HEMD-SCV formation. Lastly, in Chapter 5, I present an exploratory examination of the distribution of colony sizes that clinical *S. aureus* CF isolates display *in vitro* in an effort to ultimately provide a more rigorous, statistically driven definition of what constitutes an SCV.

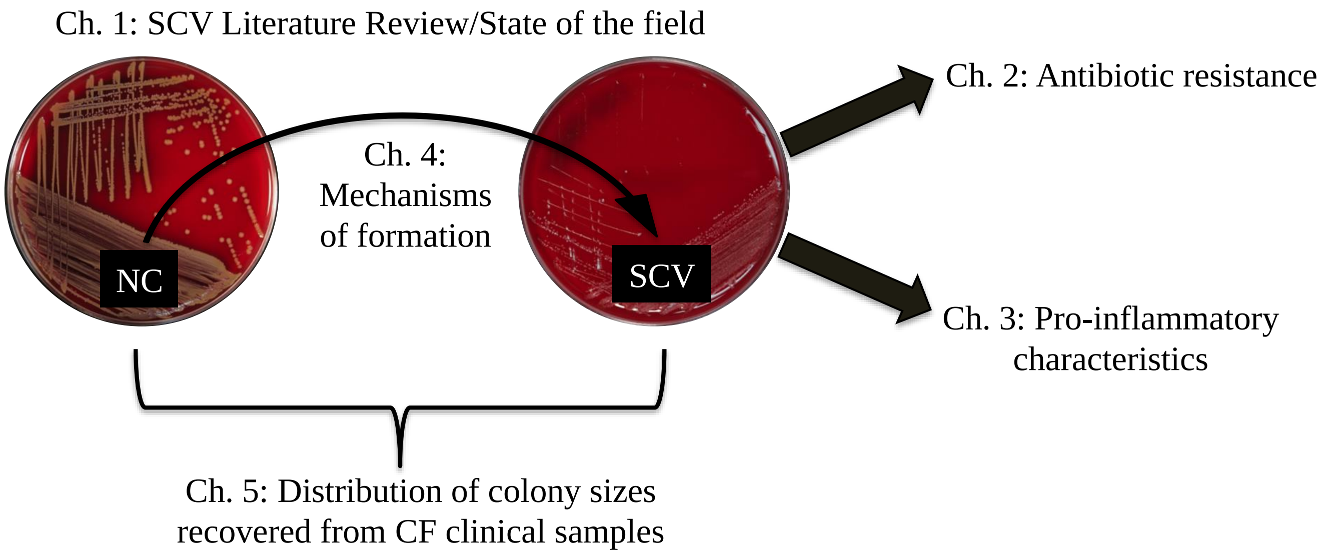


Figure 2. Summary schematic of work presented in this thesis. Chapter 1 focuses on presenting knowledge gaps in the small-colony variant (SCV) literature, highlighting both the history as well as the clinical relevance of *Staphylococcus aureus* SCVs. Chapter 2 encompasses defining SCV antibiotic susceptibility profiles using a novel method developed specifically for SCVs and the impact this work has had on how SCVs are currently treated by physicians. Chapter 3 focuses on the role the pro-inflammatory, small molecule cyclic-di-AMP may play in cystic fibrosis (CF) pathology. Chapters 4 and 5 focus on more preliminary analyses: Ch. 4 presents newly generated data on genetic mechanisms behind SCV formation and Ch. 5 explores the range of colony sizes clinical *S. aureus* isolates, including normal colony (NC), display *in vitro*.

References

1. **Kahl BC**, **Becker K**, **Löffler B**. 2016. Clinical Significance and Pathogenesis of Staphylococcal Small Colony Variants in Persistent Infections. Clin Microbiol Rev **29**:401–427.

2. **Hoffstadt RE**, **Youmans GP**. 1934. Dissociation of *Staphylococcus aureus*. J Bacteriol **27**:551–560.

3. **HALE JH**. 1947. Studies on Staphylococcus mutation; characteristics of the G (gonidial) variant and factors concerned in its production. Br J Exp Pathol **28**:202–210.

4. **Pinner M**, **Voldrich M**. 1932. Derivation of Staphylococcus albus, citreus and roseus from *Staphylococcus aureus*. Journal of Infectious Diseases.

5. **Swingle EL**. 1935. Studies on Small Colony Variants of *Staphylococcus aureus*. J Bacteriol **29**:467–489.

6. **Bigger JW**, **Boland CR**, **O'meara RAQ**. 1927. Variant colonies of *Staphylococcus aureus*. The Journal of Pathology **30**:261–269.

7. **Proctor RA**, **Kriegeskorte A**, **Kahl BC**, **Becker K**, **Löffler B**, **Peters G**. 2014. *Staphylococcus aureus* Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol **4**:99.

8. **Proctor RA**, **Eiff von C**, **Kahl BC**, **Becker K**, **McNamara P**, **Herrmann M**, **Peters G**. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol **4**:295–305.

9. **Gao W**, **Chua K**, **Davies JK**, **Newton HJ**, **Seemann T**, **Harrison PF**, **Holmes NE**, **Rhee H-W**, **Hong J-I**, **Hartland EL**, **Stinear TP**, **Howden BP**. 2010. Two novel point mutations in clinical *Staphylococcus aureus* reduce linezolid susceptibility and switch on the stringent response to promote persistent infection. PLoS Pathog **6**:e1000944.

10. **Onyango LA**, **Dunstan RH**, **Gottfries J**, **Eiff von C**, **Roberts TK**. 2012. Effect of Low Temperature on Growth and Ultra-Structure of Staphylococcus spp. PLoS ONE **7**:e29031.

11. **Karatzas KAG**, **Zervos A**, **Tassou CC**, **Mallidis CG**, **Humphrey TJ**. 2007. Piezotolerant small-colony variants with increased thermotolerance, antibiotic susceptibility, and low invasiveness in a clonal *Staphylococcus aureus* population. Appl Environ Microbiol **73**:1873–1881.

12. **Hoffman LR**, **Déziel E**, **D'Argenio DA**, **Lépine F**, **Emerson J**, **McNamara S**, **Gibson RL**, **Ramsey BW**, **Miller SI**. 2006. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA **103**:19890–19895.

13. **Vesga O**, **Groeschel MC**, **Otten MF**. 1996. *Staphylococcus aureus* small colony variants are induced by the endothelial cell intracellular milieu. Journal of Infectious ….

14. **Tuchscherr L**, **Medina E**, **Hussain M**, **Völker W**, **Heitmann V**, **Niemann S**, **Holzinger D**, **Roth J**, **Proctor RA**, **Becker K**, **Peters G**, **Löffler B**. 2011. *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med **3**:129–141.

15. **Bayston R**, **Ashraf W**, **Smith T**. 2007. Triclosan resistance in methicillin-resistant *Staphylococcus aureus* expressed as small colony variants: a novel mode of evasion of susceptibility to antiseptics. Journal of antimicrobial ….

16. **Chatterjee I**, **Kriegeskorte A**, **Fischer A**, **Deiwick S**, **Theimann N**, **Proctor RA**, **Peters G**, **Herrmann M**, **Kahl BC**. 2008. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. J Bacteriol **190**:834–842.

17. **Schaaff F**, **Bierbaum G**, **Baumert N**, **Bartmann P**, **Sahl H-G**. 2003. Mutations are involved in emergence of aminoglycoside-induced small colony variants of *Staphylococcus aureus*. Int J Med Microbiol **293**:427–435.

18. **Annear DI**, **Grubb WB**. 1973. Stimulation of variants of *Staphylococcus aureus* by penicillin. Lancet **1**:1189–1190.

19. **Mirani ZA**, **Aziz M**, **Khan SI**. 2014. Small colony variants have a major role in stability and persistence of *Staphylococcus aureus* biofilms. J Antibiot.

20. **Sadowska B**, **Bonar A**, **Eiff von C**, **Proctor RA**, **Chmiela M**, **Rudnicka W**, **Róźalska B**. 2002. Characteristics of *Staphylococcus aureus*, isolated from airways of cystic fibrosis patients, and their small colony variants. FEMS Immunol Med Microbiol **32**:191–197.

21. **Abele-Horn M**, **Schupfner B**, **Emmerling P**, **Waldner H**, **Göring H**. 2000. Persistent wound infection after herniotomy associated with small-colony variants of *Staphylococcus aureus*. Infection **28**:53–54.

22. **Jacobsen KA**. 1910. Mitteilungen über einen variablen Typhusstamm (Bacterium typhi mutabile), sowie über eine eigentümliche hemmende Wirkung des gewöhnlichen agar, …. Zentralbl. Bakteriol.[Orig. A].

23. **SHERRIS JC**. 1952. Two small colony variants of Staph. aureus isolated in pure culture from closed infected lesions and their carbon dioxide requirements. J Clin Pathol **5**:354–355.

24. **Proctor RA**, **Balwit JM**, **Vesga O**. 1994. Variant subpopulations of *Staphylococcus aureus* as cause of persistent and recurrent infections. Infect Agents Dis **3**:302–312.

25. **Proctor RA**, **van Langevelde P**, **Kristjansson M**, **Maslow JN**, **Arbeit RD**. 1995. Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. Clin Infect Dis **20**:95–102.

26. **Garcia LG**, **Lemaire S**, **Kahl BC**, **Becker K**, **Proctor RA**, **Denis O**, **Tulkens PM**, **Van**

Bambeke F. 2013. Antibiotic activity against small-colony variants of *Staphylococcus aureus*: review of in vitro, animal and clinical data. J Antimicrob Chemother **68**:1455–1464.

27. **Wolter DJ**, **Emerson JC**, **McNamara S**, **Buccat AM**, **Qin X**, **Cochrane E**, **Houston LS**, **Rogers GB**, **Marsh P**, **Prehar K**, **Pope CE**, **Blackledge M**, **Déziel E**, **Bruce KD**, **Ramsey BW**, **Gibson RL**, **Burns JL**, **Hoffman LR**. 2013. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis **57**:384–391.

28. **Eiff von C**, **Bettin D**, **Proctor RA**, **Rolauffs B**, **Lindner N**, **Winkelmann W**, **Peters G**. 1997. Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. Clin Infect Dis **25**:1250–1251.

29. **Baddour LM**, **Christensen GD**. 1987. Prosthetic valve endocarditis due to small-colony staphylococcal variants. Rev Infect Dis **9**:1168–1174.

30. **Kahl B**, **Herrmann M**, **Everding AS**, **Koch HG**, **Becker K**, **Harms E**, **Proctor RA**, **Peters G**. 1998. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. J Infect Dis **177**:1023–1029.

31. **Goerke C**, **Wolz C**. 2010. Adaptation of *Staphylococcus aureus* to the cystic fibrosis lung. Int J Med Microbiol **300**:520–525.

32. **Goss CH**, **Muhlebach MS**. 2011. Review: *Staphylococcus aureus* and MRSA in cystic fibrosis. J Cyst Fibros **10**:298–306.

33. **Pillarisetti N**, **Williamson E**, **Linnane B**, **Skoric B**, **Robertson CF**, **Robinson P**, **Massie J**, **Hall GL**, **Sly P**, **Stick S**, **Ranganathan S**, **AREST CF OBOTARESTFCF**. 2012. Infection, Inflammation, and Lung Function Decline in Infants with Cystic Fibrosis. American Journal of Respiratory and Critical Care Medicine **184**:75–81.

34. **Sawicki GS**, **Rasouliyan L**, **Ren CL**. 2012. The Impact of MRSA on Lung Function in Patients with Cystic Fibrosis. American Journal of Respiratory and Critical Care Medicine **179**:734–735.

35. **Cohen TS**, **Prince A**. 2012. Cystic fibrosis: a mucosal immunodeficiency syndrome. Nature Medicine **18**:509–519.

36. **Worlitzsch D**, **Tarran R**, **Ulrich M**, **Schwab U**, **Cekici A**. 2002. Reduced oxygen concentrations in airway mucus contribute to the early and late pathogenesis of Pseudomonas aeruginosa cystic fibrosis airway infection. J Clin Invest.

37. **Hogardt M**, **Heesemann J**. 2013. Microevolution of. Pseudomonas aeruginosa.

38. **Prunier A-L**, **Malbruny B**, **Laurans M**, **Brouard J**, **Duhamel J-F**, **Leclercq R**. 2003. High Rate of Macrolide Resistance in *Staphylococcus aureus* Strains from Patients with Cystic Fibrosis Reveals High Proportions of Hypermutable Strains. J Infect Dis **187**:1709–

1716.

39. **Sparham PD**, **Lobban DI**, **Speller DC**. 1978. Isolation of *Staphylococcus aureus* from sputum in cystic fibrosis. J Clin Pathol **31**:913–918.

40. **Gilligan PH**, **Gage PA**, **Welch DF**, **Muszynski MJ**, **Wait KR**. 1987. Prevalence of thymidine-dependent *Staphylococcus aureus* in patients with cystic fibrosis. J Clin Microbiol **25**:1258–1261.

41. **Yagci S**, **Hascelik G**, **Dogru D**, **Ozcelik U**, **Sener B**. 2013. Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. Clin Microbiol Infect **19**:77–84.

42. **Besier S**, **Smaczny C**, **Mallinckrodt von C**, **Krahl A**, **Ackermann H**, **Brade V**, **Wichelhaus TA**. 2007. Prevalence and clinical significance of *Staphylococcus aureus* small-colony variants in cystic fibrosis lung disease. J Clin Microbiol **45**:168–172.

43. **Vergison A**, **Denis O**, **Deplano A**. 2007. National survey of molecular epidemiology of *Staphylococcus aureus* colonization in Belgian cystic fibrosis patients. Journal of ….

44. **Biswas L**, **Biswas R**, **Schlag M**, **Bertram R**, **Götz F**. 2009. Small-colony variant selection as a survival strategy for *Staphylococcus aureus* in the presence of Pseudomonas aeruginosa. Appl Environ Microbiol **75**:6910–6912.

45. **Besier S**, **Ludwig A**, **Ohlsen K**, **Brade V**, **Wichelhaus TA**. 2007. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. Int J Med Microbiol **297**:217–225.

46. **Besier S**, **Zander J**, **Siegel E**, **Saum SH**, **Hunfeld K-P**, **Ehrhart A**, **Brade V**, **Wichelhaus TA**. 2008. Thymidine-dependent *Staphylococcus aureus* small-colony variants: human pathogens that are relevant not only in cases of cystic fibrosis lung disease. J Clin Microbiol **46**:3829–3832.

47. **Zander J**, **Besier S**, **Faetke S**, **Saum SH**, **Müller V**, **Wichelhaus TA**. 2010. Antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2'-deoxyuridine and rifampicin against *Staphylococcus aureus*. Int J Antimicrob Agents **36**:562–565.

48. **Kriegeskorte A**, **Block D**, **Drescher M**, **Windmüller N**, **Mellmann A**, **Baum C**, **Neumann C**, **Lorè NI**, **Bragonzi A**, **Liebau E**, **Hertel P**, **Seggewiss J**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2014. Inactivation of thyA in *Staphylococcus aureus* attenuates virulence and has a strong impact on metabolism and virulence gene expression. MBio **5**:e01447–14.

49. **Kahl BC**. 2014. Small colony variants (SCVs) of *Staphylococcus aureus*--a bacterial survival strategy. Infect Genet Evol **21**:515–522.

50. **Dean MA**, **Olsen RJ**, **Long SW**, **Rosato AE**, **Musser JM**. 2014. Identification of point mutations in clinical *Staphylococcus aureus* strains that produce small-colony variants

auxotrophic for menadione. Infect Immun **82**:1600–1605.

51. **Lannergård J**, **Eiff von C**, **Sander G**, **Cordes T**, **Seggewiss J**, **Peters G**, **Proctor RA**, **Becker K**, **Hughes D**. 2008. Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother **52**:4017–4022.

52. **Stone A**, **Saiman L**. 2007. Update on the epidemiology and management of *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus*, in patients with cystic fibrosis. Curr Opin Pulm Med **13**:515–521.

53. **Precit MR**, **Wolter DJ**, **Griffith A**, **Emerson J**, **Burns JL**, **Hoffman LR**. 2016. Optimized In Vitro Antibiotic Susceptibility Testing Method for Small-Colony Variant *Staphylococcus aureus*. Antimicrob Agents Chemother **60**:1725–1735.

Chapter 2:

An Optimized *in vitro* Antibiotic Susceptibility Testing Method for Small Colony Variant *Staphylococcus aureus*

The majority of the work presented in this chapter is published as:

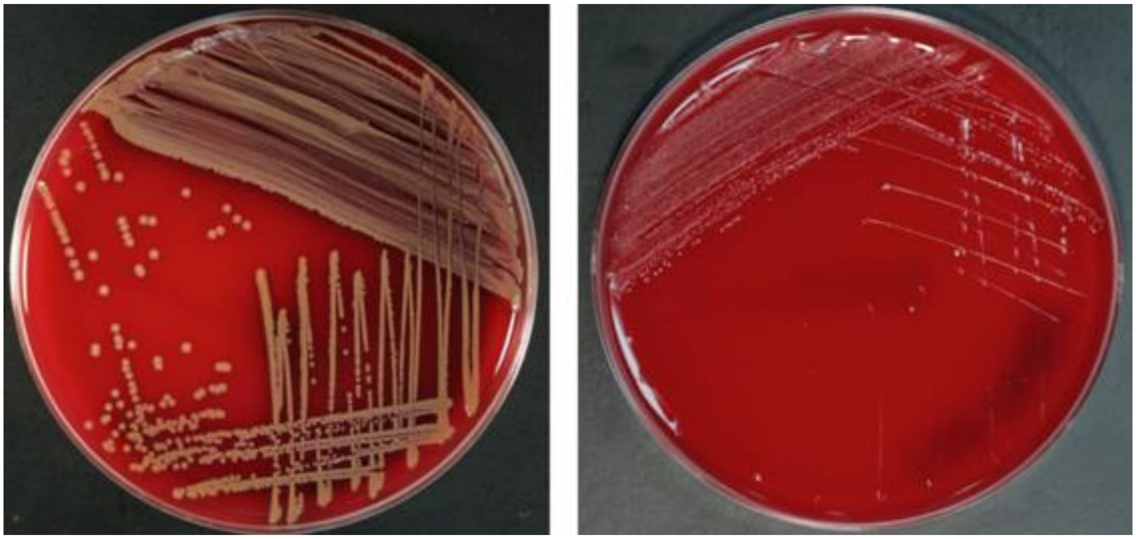
Precit, M., Wolter, D., Griffith, A., Emerson, J., Burns, J., Hoffman, L. An optimized *in vitro* antibiotic susceptibility testing method for *Staphylococcus aureus* small colony variants. *Antimicrob Agents Chemother.* 2016 Jan 4;60(3):1725-35. doi: 10.1128/AAC.02330-15.PMID: 26729501

Introduction

Staphylococcus aureus small-colony variant (SCV) infections are particularly prevalent in the lungs of people with cystic fibrosis (CF) often following prolonged antibiotic treatment (1-3). In children with CF, SCV respiratory infection is independently associated with worse lung function and faster lung function decline (3).

SCVs are typically identified by their distinctive phenotypic traits when grown *in vitro* on most agar-based media (Figure 1). Despite sharing similar phenotypic appearances on agar surfaces, SCVs can carry mutational defects in different metabolic pathways, most commonly electron transport or DNA biosynthesis, which result in auxotrophy for specific nutrients such as menadione, hemin, or thymidine (4).

Because of impaired *in vitro* growth and non-classical phenotypic characteristics, SCVs are difficult to detect using conventional clinical microbiological approaches, and there are no approved methods for SCV antimicrobial susceptibility testing (AST), hindering the selection of treatments for infected patients. AST was initially developed for the analysis of rapidly growing, aerobic bacteria within 24 hours using a standard medium (Mueller-Hinton); SCV growth under these conditions is generally insufficient to provide meaningful results. As such, a variety of different methods using diverse media (e.g. Mueller-Hinton supplemented with blood, brain heart infusion, Columbia blood agar) and incubation times (48-72 hr) have been utilized for AST of SCVs (1, 5-14) (Table 1). These conditions were often chosen empirically because they supported the growth of the specific SCVs being characterized.



Normal Colony SCV - Small Colony Variant

Figure 1. Distinguishing phenotypic features of SCVs. *In vitro* growth comparison of NC (left) and a genetically related SCV (right) on blood agar showing distinct SCV characteristics, including reduced hemolysis, small colony morphology, and decreased pigmentation.

Table 1. Summary of media used in previous published studies of SCV AST

SCV Auxotrophy	AST Method	Media	Reference
7 menadione-dependent SCVs	Microbroth dilution Etest Agar dilution	MH[b] Base medium not specified but supplemented with 1.5ug/mL menadione	(5-7, 13,14)
5 hemin-dependent SCVs	Microbroth dilution Disk diffusion	BHI MH with longer incubation	(1,8,13,14)
23 thymidine-dependent SCVs	Microbroth dilution Etest Agar dilution	BHI MH supplemented with sheep blood MH supplemented with 10ug/mL thymidine	(1,5,9-11)
Unspecified auxotrophies[a]	Disk Diffusion Microbroth dilution Agar dilution Etest	MH MH supplemented with sheep blood MH supplemented with thiamine and menadione	(1, 7, 8, 12)

[a] SCVs with unspecified auxotrophies were isolated from clinical samples recovered from CF, osteomyelitis, sepsis, and soft-tissue infections

[b] Mueller-Hinton (MH), Brain Heart Infusion (BHI)

To further complicate SCV testing, SCVs frequently revert during *in vitro* growth to a NC phenotype, potentially altering susceptibilities and limiting interpretation. The primary goal of this study was to identify optimal *in vitro* conditions that would adequately support growth, maintain genotypic stability (i.e., minimize reversion to NC), and allow us to define the *in vitro* susceptibilities of a diverse collection of *S. aureus* SCV clinical isolates. Using susceptibility results from this method, we also sought to determine whether *in vitro* susceptibilities of CF SCVs could be reliably predicted from 1: their auxotrophy type and methicillin resistance (MRSA) status, 2: the susceptibilities of their clonally-related NC isolates from the same patients, or 3: the source patient's antibiotic treatment history, as a complementary or alternative method to SCV AST. Our results indicate that this AST method, if validated in a clinical study, could be useful in directing antibiotic treatment for infections with SCV *S. aureus.*

Materials and Methods

Strains used in this study. The *S. aureus* isolates for this study comprised 85 SCVs and, for each SCV lineage defined by pulsed-field gel electrophoresis (PFGE methodology described below), at least 1 preceding or co-isolated NC isolate (n=27) (Table 2A). All clinical isolates were obtained from a single-center study of children (n=23) with CF (3). Since a menadione-dependent clinical SCV was not available, an *in vitro* selected menadione-dependent SCV from parental strain *S. aureus* Newman (15) was used. *S. aureus* ATCC 25923 and ATCC 29213 were used as controls in AST, and USA300 MRSA strain JE2 (16) served as a positive control in *mecA* PCR and for β-lactam resistance in AST.

Table 2A. Complete list of strains/isolates used in this study

Number of isolates or Strain Designation	Phenotype/auxotrophy	Source	Reference
79 (31 MRSA & 48 MSSA)[a]	SCV/THYD[b]	CF Respiratory Secretions	(3)
5 (3 MRSA & 2 MSSA)	SCV/HEMD	CF Respiratory Secretions	(3)
27 (9 MRSA & 19 MSSA)	NC/None	CF Respiratory Secretions	(3)
1 (MSSA)	SCV/Undefined	CF Respiratory Secretions	(3)
1 (MSSA)	SCV/MEND	*In vitro* selection	(15)
S. aureus Newman	Parent strain for MEND SCV/None	-	(15)
S. aureus JE2 – MRSA	MRSA Control Strain/None	Lab strain, NARSA[c]	(16)
S. aureus ATCC 25923	NC Control Strain/None	University of Washington Clinical Lab	-
S. aureus ATCC 29213	NC Control Strain/None	Seattle Children's Clinical Lab	-

Table 2B. List of isolates used for BD-MHA+Blood testing

Number of Isolates or Strain Designation	Phenotype/auxotrophy	Source	Reference
2 (1 MRSA & 1 MSSA)[a]	SCV/ THYD	CF Respiratory Secretions	(3)
3 (2 MRSA & 1 MSSA)	SCV/ HEMD	CF Respiratory Secretions	(3)
1 (MSSA)	SCV/ MEND	*In vitro* selection	(15)
4 (2 MRSA & 2 MSSA)	None/isogenic-paired NC isolates/None	CF Secretions	(3)
Newman	Parent for MEND-SCV / None	Lab strain	(15)
USA 300 JE2-MRSA	MRSA Control Strain/None	Lab strain, NARSA[c]	(16)
ATCC 25923	NC Control Strain/None	University of Washington Clinical Lab	-
ATCC 29213	NC Control Strain/None	Seattle Children's Clinical Lab	-

[a] MRSA refers to Methicillin Resistant *S. aureus* and MSSA refers to Methicillin Susceptible

[b] THYD (thymidine-dependent), HEMD (hemin-dependent), MEND (menadione-depdenedent)

[c] Network on Antimicrobial Resistance in *Staphylococcus aureus*

Media used in this study. Various growth media were used in this study where indicated, including: Luria-Bertani (LB, Becton Dickinson [BD], Sparks, MD), trypticase soy agar (TSA, BD), trypticase soy agar with 5% sheep blood (BAP, Remel, Lenexa, KS), mannitol salt agar (BD), Mueller-Hinton agar (MHA, Oxoid, Basingstoke, Hampshire, UK and Teknova), brain heart infusion (BHI, BD), and Chocolate agar (Remel). Commercially available BHI agar was also obtained from Hardy Diagnostics (Hardy-BHI, Santa Maria, CA) and Teknova (Teknova-BHI, Hollister, CA). A synthetic CF sputum media, formulated to mimic the nutritional composition of CF sputum, was prepared as described (17), with the addition of 0.1 mg/mL herring sperm DNA (Sigma) (SCFMD).

Additionally, in later experiments we assessed MHA with 5% sheep blood (MHA+Blood) from three manufactures Remel, BD, and Teknova per request of the CLSI, atypical *S. aureus* group, as a candidate media for SCV AST.

Media Supplementation with hemin, menadione, and thymidine. The final concentrations of thymidine, hemin, and menadione for media supplementation were chosen empirically by evaluating the growth of each SCV type (i.e. thymidine-, hemin-, and menadione -dependent SCVs) on MHA with increasing concentrations of supplements ranging from 0ug/mL to 10ug/mL. The SCVs were cultured directly onto the agar media from -80°C freezer stocks (TSA with 15% glycerol). Supplemented media consisted of the lowest concentration of each supplement that met two criteria: 1) significantly improved the *in vitro* growth of SCVs auxotrophic for that supplement, and 2) didn’t inhibit growth of NC *S. aureus* and SCVs of other auxotrophic types.

To test whether results of a commercially prepared sup-MHA would be similar to that medium prepared in our laboratory, we custom-ordered sup-MHA from Teknova; this medium is referred to as THM-MHA (Thymidine-Hemin-Menadione Mueller-Hinton Agar), to distinguish it from our in-house sup-MHA.

Evaluation of SCV growth. Growth of a subset of SCVs (n=10, including 1 menadione-, 2 hemin-, and 7 thymidine-dependent SCVs) and paired, isogenic NCs (n=10) was assessed on 11 agar media: LB, TSA, BAP, BHI, mannitol, MHA, SCFMD, sup-TSA, sup-BHI, sup-MHA, and sup-SCFMD. SCVs were cultured directly from frozen stocks (TSA with 15% glycerol) onto 11 different media: Plates were incubated for 24h at 35°C in both aerobic and microaerobic (5% CO_2) conditions. SCVs with colonies similar in size and appearance to NCs (≥1mm in diameter), when grown on the same media, indicated adequate growth (large colonies), while SCV colonies that were ≤1 mm indicated poor growth (small colonies).

At a later date, the same above methodology above was used to evaluate MHA+Blood from three different manufacturers, BD, Remel, and Teknova, however only 5 SCVs were scored for growth. Furthermore, an additional parameter was added to evaluate colony size, SCVs with colonies similar in size and appearance to NCs (≥1mm in diameter), when grown on the same media, indicated adequate growth (large colonies), SCV colonies that were ≤1 mm indicated poor growth (small colonies), and SCV colonies that were ≤0.5 mm were also observed (pin-point colonies).

Evaluation of SCV phenotypic stability. SCV stability was evaluated on SCV growth-supportive media in two ways: 1) assigning a media-stability score based on visual inspection of colony morphology variation on each medium, and 2) measuring frequency of reversion of SCVs to stable NC growth. Specifically, SCVs were plated from frozen stocks onto media that adequately supported SCV growth (sup-MHA, BHI, sup-BHI, TSA, sup-TSA, and THM-MHA) incubated at 35˚C for 24h, and colonies were inspected for consistency. Stability scores reflected number of colony types observed on each medium: a score of 1 indicated a single colony type, 2 indicated 2 colony types, etc. Media with one colony type (sup-MHA, THM-MHA, BD-BHI, sup-BD-BHI) for each SCV were chosen for subsequent experiments. The frequency of reversion was determined by sub-culturing 150 randomly chosen colonies ≥1mm from the test media onto LB agar, which identifies SCVs incubating at 35°C for 24h and measuring colony size. Frequency of reversion was calculated by dividing the number of colonies that were ≥1mm in diameter on LB agar by the total colony number.

At a later date, the same above methodology was used to evaluate reversion frequencies of a subet of 5 SCVs on MHA+Blood that received a stability score of 1, therefore supporting SCV growth (BD-MHA+Blood). This was completed alongside sup-MHA and MHA, however only 50 randomly chosen colonies were selected and assessed using LB agar as a discriminatory reference medium.

Antibiotic susceptibility testing methodology. SCVs, paired isogenic NC isolates, and ATCC control strains were sub-cultured onto chocolate agar plates from frozen stocks and grown aerobically overnight at 35°C. Colonies from individual isolates were suspended in 0.85% saline to an optical density equivalent to 0.5 McFarland (~1x10^8 cfu/mL). Lawn cultures were prepared from the inoculated saline, and antibiotic disks or Etests were dispensed onto the agar surfaces of either sup-MHA, BD-BHI, THM-MHA, or MHA (for NC and control strains). Plates were incubated aerobically or microaerobically (with 5% CO_2) at 35°C for 18-24 hr. All antibiotic disks were obtained from BD except fusidic acid, which was prepared with powdered antibiotic from Sigma Chemical Co. (St. Louis, MO) and filter disks from BD (10 µg disk content). All Etests were obtained from Biomerieux, Durham, NC.

Disk diffusion was conducted on all SCVs using 24 antibiotics (Table 3) according to CLSI guidelines (18) except that BHI and sup-MHA were substituted for MHA. *S. aureus* ATCC 25923 and NC isolates were also tested on MHA, BHI, and sup-MHA to compare the effects of different media and supplementation on zone diameters. For a subset of isolates (26 SCVs, 13 corresponding NCs, and ATCC 25923), disk diffusion was also performed under microaerobic conditions (5% CO_2). AST results were interpreted according to CLSI guidelines (18) for all antibiotics except fusidic acid, for which EUCAST guidelines were used (19).

Minimum inhibitory concentrations (MICs) were determined with Etest for 13 antibiotics (Table 3) using a subset of the isolates (28 SCVs and 24 respective NCs) and ATCC 29213 on THM-MHA (Teknova). In parallel, MICs were determined for ATCC 29213 on standard MHA from Teknova to compare the effects of supplementation on susceptibility results.

Table 3. Complete list of antibiotics used in this study

Antibiotic	Class	Abbreviation	Disk Diffusion	Etest	MHA+ Blood[a]

Cefoxition	β-lactam	FOX	✓	-	✓
Oxacillin	β-lactam	OX	✓	-	-
Cephalothin	β-lactam	CE	-	✓	-
Piperacillin-Tazobactam	β-lactam	TZP	✓	✓	-
Amoxicillin-Clavulanate	β-lactam	AMC	✓	✓	-
Cefazolin	β-lactam	CZ	✓	-	-
Imipenem	β-lactam	IP	-	✓	-
Meropenem	β-lactam	MEM	✓	-	-
Ticarcillin-Clavulanate	β-lactam	TIM	✓	-	-
Levofloxacin	Fluoroquinolone	LVX	✓	✓	✓
Moxifloxacin	Fluoroquinolone	MXF	✓	-	-
Tobramycin	Aminoglycoside	NN	✓	✓	✓
Amikacin	Aminoglycoside	AN	✓	-	-
Trimethoprim/ Sulfamethoxazole	Antifolate	SXT	✓	✓	✓
Minocycline	Tetracycline	MI	✓	✓	-
Chloramphenicol	Amphenicol	C	✓	✓	-
Azithromycin	Macrolide	AZM	✓	-	-
Clindamycin	Lincosamide	CC	✓	✓	✓
Quinupristin/ Dalfopristin	Streptogramin	SYN	✓	✓	-
Telithromycin	Ketolides	TEL	✓	-	-
Linezolid	Oxazolidinone	LZD	✓	✓	-
Rifampin	Ansamycin	RA	✓	-	-
Tigecycline	Glycylcycline	TGC	✓	-	-
Fosfomycin	Phosphonic	FOS	✓	-	-
Vancomycin	Glycopeptide	VA	✓	✓	✓ (Etest)
Fusidic Acid	Fusidane	FA	✓	-	-

[a] MHA+Blood was only tested at a later date for a small subset of antibiotics from select classes for evaluation as per request of the CLSI

At a later date, susceptibility testing using the above methodologies designed to assess BD-MHA+Blood was carried out. However only a limited number isolates were tested (Table 2B), using a limited number of antibiotics (Table 3) by both disk diffusion and Etest methods.

Due to the presence of antagonists (e.g. thymidine) in AST media, the Clinical Laboratory Standards Institute (CLSI) recommends that slight growth (≤ 20% of lawn growth) around a trimethoprim-sulfamethoxazole disk or strip, if present, should be ignored, and the zone diameter or growth up to the disk/strip should be measured at the margin of heaviest growth (18). We always observed slight growth of susceptible organisms around trimethoprim-sulfamethoxazole disks/strips (Figure 2) on sup-MHA and BD-BHI, the latter of which presumably contains thymidine, and so we applied the ≤ 20% guideline for both media. Isolates that were resistant to trimethoprim-sulfamethoxazole grew uniformly up to the disk/strip (Figure 2).

Validation of MHA manufactured by Teknova. In order to validate the MHA manufactured by Teknova, an additional control using disk diffusion was completed for a subset of 5 clinical SCVs (2 THYD-SCVs, 2 HEMD-SCVs, and 1 MEND-SCV) and corresponding isogenic NC isolates on Teknova-MHA (used only for NCs), THM-MHA, sup-MHA, and BD-BHI. Isolates exhibited identical susceptibilities on all of these media.

MRSA detection in clinical *S. aureus* isolates. MRSA were identified using the PBP2’ latex agglutination kit (Oxoid), with confirmation by *mecA* gene detection using PCR as described (20, 21).

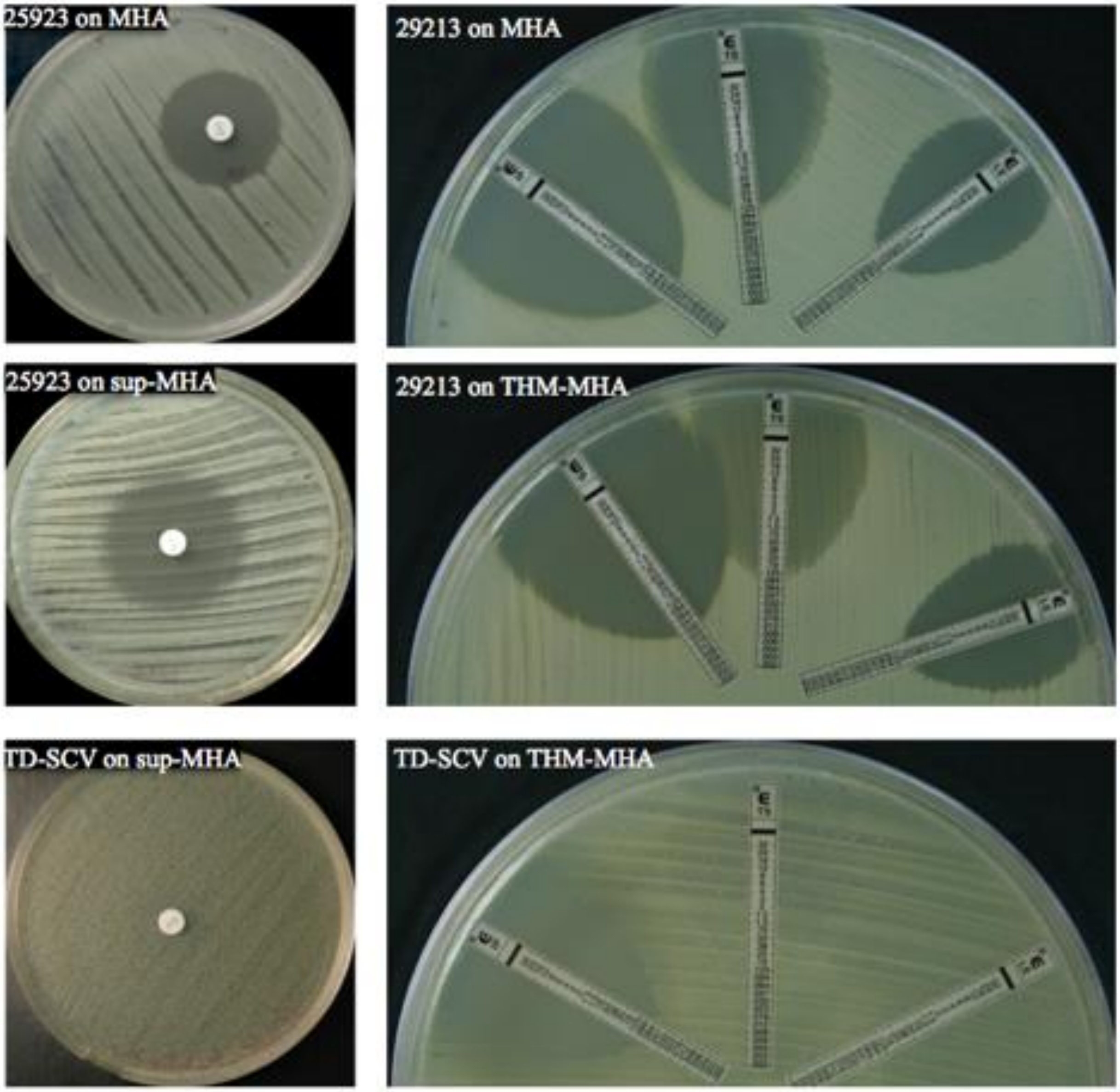

25923 on MHA
29213 on MHA
25923 on sup-MHA
29213 on THM-MHA
TD-SCV on sup-MHA
TD-SCV on THM-MHA

Figure 2. Interpretation of susceptibility to trimethoprim-sulfamethoxazole (SXT/TS) on media containing thymidine. ATCC 25923 and ATCC 29213 were grown on MHA (top row) and sup-MHA/THM-MHA (middle row) for AST. Control isolates displayed a clear zone of inhibition around the SXT disk and TS Etest strip in the absence of thymidine but showed slight growth (≤20%) when media contained thymidine. The slight growth was ignored for interpretation of susceptibilities as recommended by the CLSI. A thymidine-dependent SCV showed uniform growth up to the disk/strip on sup-MHA and THM-MHA (bottom row), and was considered resistant.

Determining genetic relatedness using pulsed-field gel electrophoresis (PFGE). Genetic relatedness of the clinical isolates was determined by PFGE according to the Centers for Disease Control and Prevention PulseNet protocol (22). Briefly, chromosomal DNA was digested with 50 U of *Sma*I (Roche) at room temperature for 3 hr, and DNA restriction fragments were separated in a 1% SeaKem Gold agarose gel using a CHEF DR-III (Bio-Rad, Hercules, California). DNA restriction patterns were analyzed using BioNumerics software (v. 6.5; Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram was constructed by the UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering method and Dice similarity coefficients with an optimization setting of 1.0% and position tolerance of 1.25%.

Correlation of susceptibilities with antibiotic exposure. Antibiotic exposure was defined as present if the end date for treatment with that antibiotic provided in study logs maintained by study subjects occurred within 90 days prior to the culture date. Antibiotic end dates were missing for 15 of 407 antibiotic courses identified; in those cases, end dates were assigned a value equal to (start date +28 days) for inhaled tobramycin, (start date + 180 days) for oral azithromycin taken on a continuing basis, or (start date + 14 days) for other antibiotics. Logistic regression models accounted for multiple isolates per subject and used robust variance estimates. The odds ratio with 95% confidence interval was calculated as the measure of association between the predictor (antibiotic exposure within 90 days prior to culture date) and the outcome (isolate not susceptible to the antibiotic tested). Models could not be fit when the antibiotic exposure perfectly predicted isolate status as susceptible or not susceptible (these observations were dropped from the model leaving no variability in the predictor variable). This analysis excluded 3 replicate SCVs from the same culture as another SCV and 11 isolates for which antibiotic treatment data were incomplete. Thus, the total data set consisted of 71 isolates from 21 subjects (analysis dataset).

Results

Genetic relatedness of clinical isolates determined by PFGE. Clinical CF respiratory isolates belonged to 15 distinct PFGE groups and included both MSSA (n=71) and MRSA (n=43) (Figure 3). MSSA isolates belonged to 9 different PFGE groups, and MRSA isolates comprised 6 distinct PFGE groups with just over half (n=25) belonging to the USA100 classification scheme (Figure 3B). A single NC-MRSA and SCV-MRSA isogenic pair was found to have the USA700 pulsotype (Figure 3B). All SCVs except a single hemin-dependent SCV isolate 116-15, were co-cultured with a genetically related normal colony isolate with an identical PFGE banding pattern.

MHA supplementation with hemin, menadione, and thymidine. Representative SCVs, belonging to each auxotrophy type and a laboratory strain ATCC 25923 were evaluated for growth on MHA with various concentrations of thymidine, hemin, menadione (Figure 4). ATCC 25923 grew well on all media except MHA supplemented with 5ug/mL and hemin and menadione, this was expected since these compounds can be toxic to bacteria at these concentrations (4). Collectively, media supplemented with 5-10 ug/mL thymidine and 1ug/mL each of hemin and menadione best supported SCV growth while not preventing growth of the control strain (Figure 4). The final concentrations chosen were based upon previous studies showing that 5µg/mL thymidine would approximate the sum of the concentrations of thymidine and its biologically-active metabolite, dTMP, observed in CF sputum (23, 24), while those for hemin and menadione were chosen empirically based on *in vitro* performance. Therefore, our

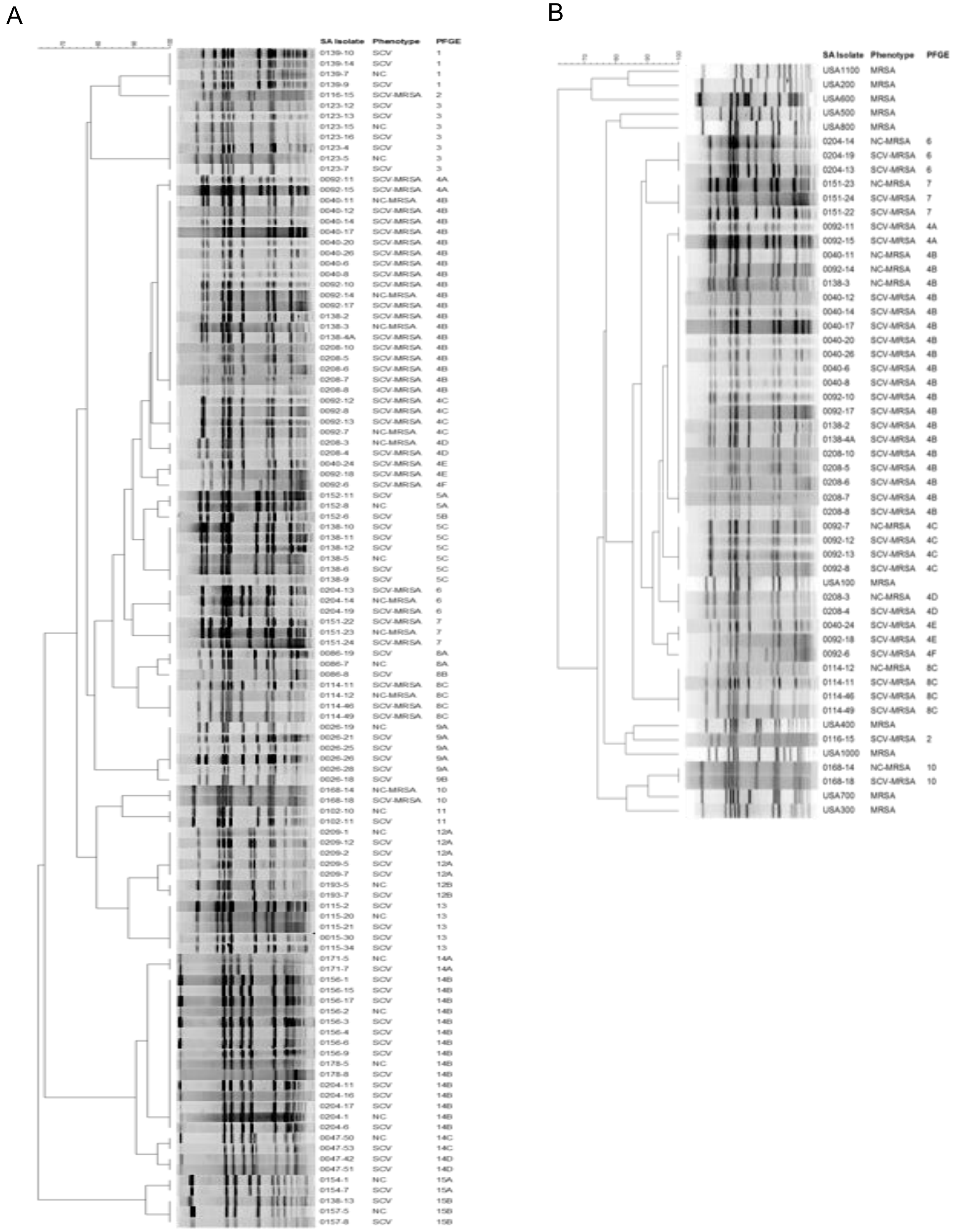

A
SA Isolate Phenotype PFGE
0139-10 SCV 1
0139-14 SCV 1
0139-7 NC 1
0139-9 SCV 1
0116-15 SCV-MRSA 2
0123-12 SCV 3
0123-13 SCV 3
0123-15 NC 3
0123-16 SCV 3
0123-4 SCV 3
0123-5 NC 3
0123-7 SCV 3
0092-11 SCV-MRSA 4A
0092-15 SCV-MRSA 4A
0040-11 NC-MRSA 4B
0040-12 SCV-MRSA 4B
0040-14 SCV-MRSA 4B
0040-17 SCV-MRSA 4B
0040-20 SCV-MRSA 4B
0040-26 SCV-MRSA 4B
0040-6 SCV-MRSA 4B
0040-8 SCV-MRSA 4B
0092-10 SCV-MRSA 4B
0092-14 NC-MRSA 4B
0092-17 SCV-MRSA 4B
0138-2 SCV-MRSA 4B
0138-3 NC-MRSA 4B
0138-4A SCV-MRSA 4B
0208-10 SCV-MRSA 4B
0208-5 SCV-MRSA 4B
0208-6 SCV-MRSA 4B
0208-7 SCV-MRSA 4B
0208-8 SCV-MRSA 4B
0092-12 SCV-MRSA 4C
0092-8 SCV-MRSA 4C
0092-13 SCV-MRSA 4C
0092-7 NC-MRSA 4C
0208-3 NC-MRSA 4D
0208-4 SCV-MRSA 4D
0040-24 SCV-MRSA 4E
0092-18 SCV-MRSA 4E
0092-6 SCV-MRSA 4F
0152-11 SCV 5A
0152-8 NC 5A
0152-6 SCV 5B
0138-10 SCV 5C
0138-11 SCV 5C
0138-12 SCV 5C
0138-5 NC 5C
0138-6 SCV 5C
0138-9 SCV 5C
0204-13 SCV-MRSA 6
0204-14 NC-MRSA 6
0204-19 SCV-MRSA 6
0151-22 SCV-MRSA 7
0151-23 NC-MRSA 7
0151-24 SCV-MRSA 7
0086-19 SCV 8A
0086-7 NC 8A
0086-8 SCV 8B
0114-11 SCV-MRSA 8C
0114-12 NC-MRSA 8C
0114-46 SCV-MRSA 8C
0114-49 SCV-MRSA 8C
0026-19 NC 9A
0026-21 SCV 9A
0026-25 SCV 9A
0026-26 SCV 9A
0026-28 SCV 9A
0026-18 SCV 9B
0168-14 NC-MRSA 10
0168-18 SCV-MRSA 10
0102-10 NC 11
0102-11 SCV 11
0209-1 NC 12A
0209-12 SCV 12A
0209-2 SCV 12A
0209-5 SCV 12A
0209-7 SCV 12A
0193-5 NC 12B
0193-7 SCV 12B
0115-2 SCV 13
0115-20 NC 13
0115-21 SCV 13
0015-30 SCV 13
0115-34 SCV 13
0171-5 NC 14A
0171-7 SCV 14A
0156-1 SCV 14B
0156-15 SCV 14B
0156-17 SCV 14B
0156-2 NC 14B
0156-3 SCV 14B
0156-4 SCV 14B
0156-6 SCV 14B
0156-9 SCV 14B
0178-5 NC 14B
0178-8 SCV 14B
0204-11 SCV 14B
0204-16 SCV 14B
0204-17 SCV 14B
0204-1 NC 14B
0204-6 SCV 14B
0047-50 NC 14C
0047-53 SCV 14C
0047-42 SCV 14D
0047-51 SCV 14D
0154-1 NC 15A
0154-7 SCV 15A
0138-13 SCV 15B
0157-5 NC 15B
0157-8 SCV 15B
B
SA Isolate Phenotype PFGE
USA1100 MRSA
USA200 MRSA
USA600 MRSA
USA500 MRSA
USA800 MRSA
0204-14 NC-MRSA 6
0204-19 SCV-MRSA 6
0204-13 SCV-MRSA 6
0151-23 NC-MRSA 7
0151-24 SCV-MRSA 7
0151-22 SCV-MRSA 7
0092-11 SCV-MRSA 4A
0092-15 SCV-MRSA 4A
0040-11 NC-MRSA 4B
0092-14 NC-MRSA 4B
0138-3 NC-MRSA 4B
0040-12 SCV-MRSA 4B
0040-14 SCV-MRSA 4B
0040-17 SCV-MRSA 4B
0040-20 SCV-MRSA 4B
0040-26 SCV-MRSA 4B
0040-6 SCV-MRSA 4B
0040-8 SCV-MRSA 4B
0092-10 SCV-MRSA 4B
0092-17 SCV-MRSA 4B
0138-2 SCV-MRSA 4B
0138-4A SCV-MRSA 4B
0208-10 SCV-MRSA 4B
0208-5 SCV-MRSA 4B
0208-6 SCV-MRSA 4B
0208-7 SCV-MRSA 4B
0208-8 SCV-MRSA 4B
0092-7 NC-MRSA 4C
0092-12 SCV-MRSA 4C
0092-13 SCV-MRSA 4C
0092-8 SCV-MRSA 4C
USA100 MRSA
0208-3 NC-MRSA 4D
0208-4 SCV-MRSA 4D
0040-24 SCV-MRSA 4E
0092-18 SCV-MRSA 4E
0092-6 SCV-MRSA 4F
0114-12 NC-MRSA 8C
0114-11 SCV-MRSA 8C
0114-46 SCV-MRSA 8C
0114-49 SCV-MRSA 8C
USA400 MRSA
0116-15 SCV-MRSA 2
USA1000 MRSA
0168-14 NC-MRSA 10
0168-18 SCV-MRSA 10
USA700 MRSA
USA300 MRSA

Figure 3. PFGE dendrogram of all *S. aureus* clinical isolates used in this study. (A) Dendogram showing genetic relatedness of all NC and SCV clinical isolates. (B) Dendrogram of the MRSA (NC-MRSA and SCV-MRSA) clinical isolates compared to USA type MRSA. Each dendrogram, indicating genetic relatedness of isolates, is shown on the left portion of each figure and was generated with Bionumerics software. The scale for the dendrograms represents the percent genetic relatedness. PFGE groups were defined by isolates that were at least 90% genetically similar. *Salmonella* serotype *Braenderup* H9812 was used for gel-to-gel normalization. USA type MRSA patterns were kindly provided by Linda McDougal.

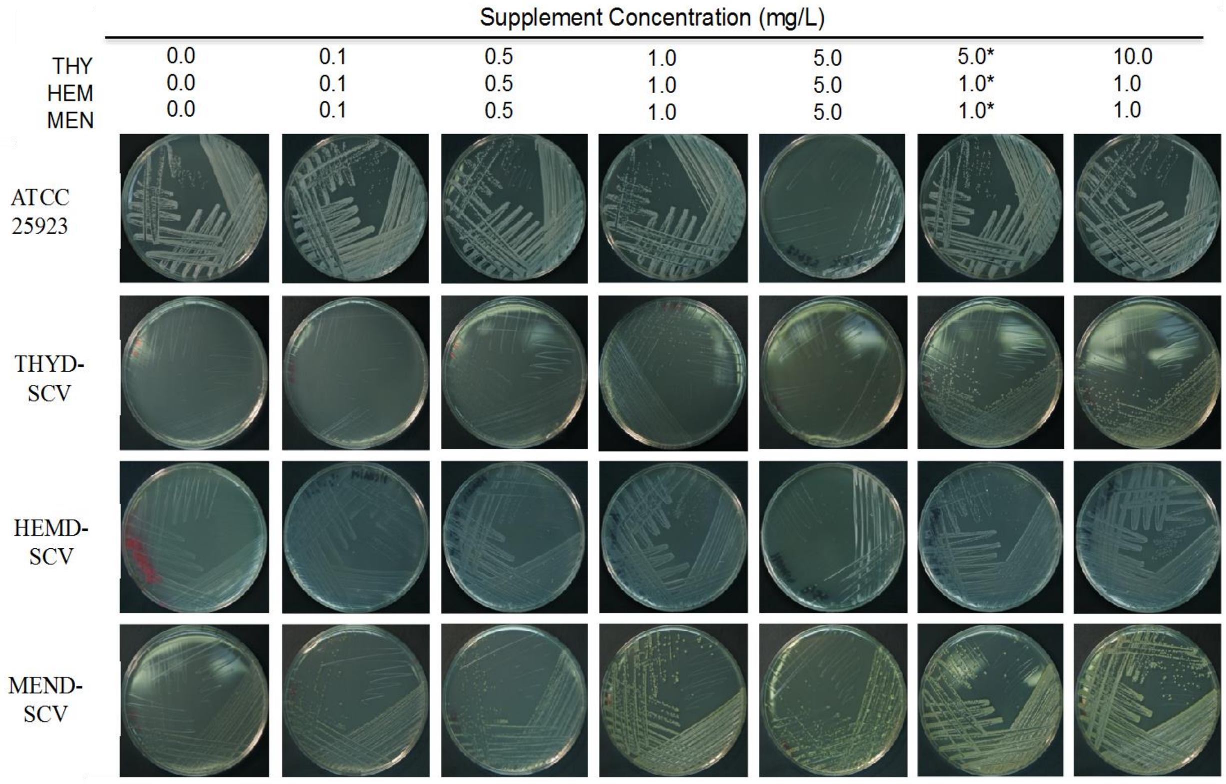


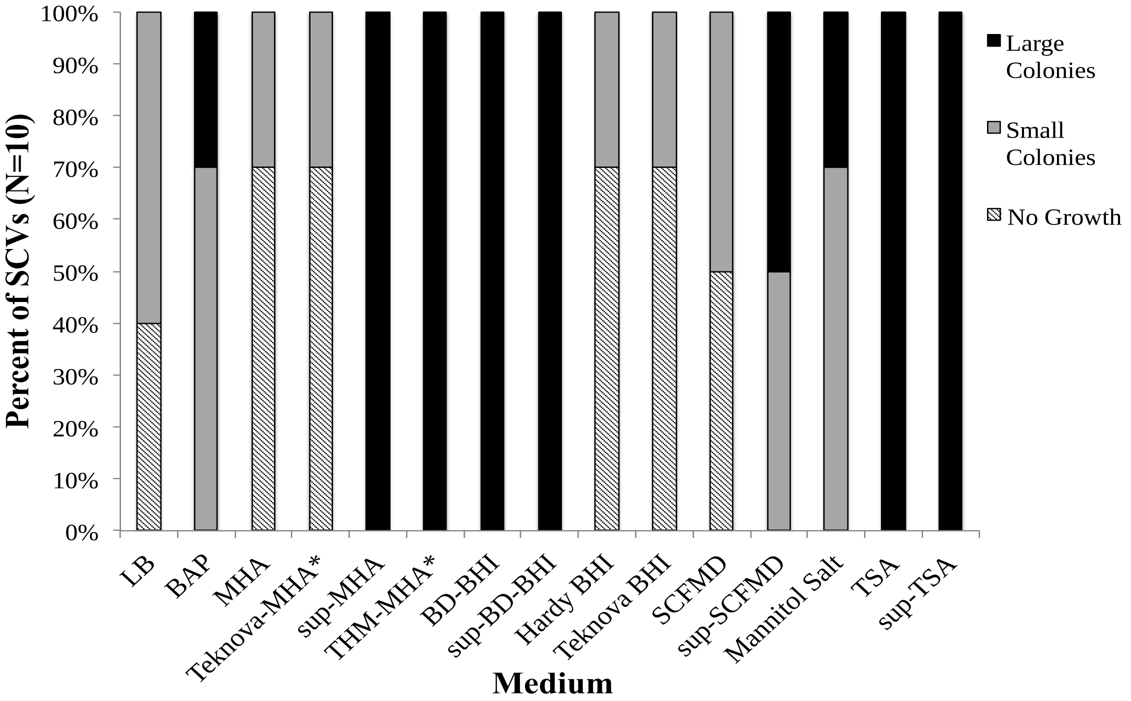
Figure 4. Growth of different SCV auxotrophic types on media containing various concentrations of supplements. Thymidine (THY), hemin (HEM), and menadione (MEN) were added into MHA at the concentrations indicated above each column. Each row represents a separate *S. aureus* isolate or strain, including each of the three most common SCV types: THYD (Thymidine-dependent), HEMD (Hemin-dependent), MEND (Menadione-dependent). ATCC 25923 served a representative NC control strain for growth comparison. *Column 6 depicts the final concentrations that were chosen for supplementation.

final supplement concentrations that were chosen were 5ug/mL thymidine, 1ug/mL hemin, and 1ug/mL menadione.

Evaluation of SCV Growth and phenotypic stability on various media. Eleven different agar media were screened for their ability to support adequate growth and minimize phenotypic reversion with a subset of SCVs, chosen to represent the three primary auxotrophic types. All SCVs grew very poorly (menadione-dependent and hemin-dependent SCVs) or were unable to grow (thymidine-dependent SCVs) on MHA, the CLSI recommended AST medium (Figure 5A). By comparison, all SCVs grew similar in size to isogenic NC isolates on 5 media: supplemented and unsupplemented TSA and BD-BHI agars, and sup-MHA (Figure 5A). Surprisingly, thymidine-dependent SCVs were unable to grow on prepared BHI agar from two other companies (Hardy-BHI and Teknova-BHI), indicating that BHI differs between manufacturers. The synthetic medium formulated to mimic CF sputum, SCFMD, did not sufficiently support growth of all SCVs, even when supplemented (Figure 5A). Growth of the SCVs on the tested media was comparable under aerobic and microaerobic atmospheric growth conditions.

Later experiments testing MHA+blood from different manufacturers using a small subset of SCVs (n=5) compared to isogenic NC isolates (NC), to represent the 3 auxotrophy types, showed that MHA+blood did not adequately complement the growth of SCVs back to NC levels as does sup-MHA/THM-MHA and BD-BHI (Figure 5B). However MHA+blood manufactured by BD performed better compared to MHA+blood from other manufacturers. Therefore BD-MHA+blood was chosen for subsequent experiments.

A


Percent of SCVs (N=10)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
LB
BAP
MHA
Teknova-MHA*
sup-MHA
THM-MHA*
BD-BHI
sup-BD-BHI
Hardy BHI
Teknova BHI
SCFMD
sup-SCFMD
Mannitol Salt
TSA
sup-TSA
Medium
Large Colonies
Small Colonies
No Growth

B

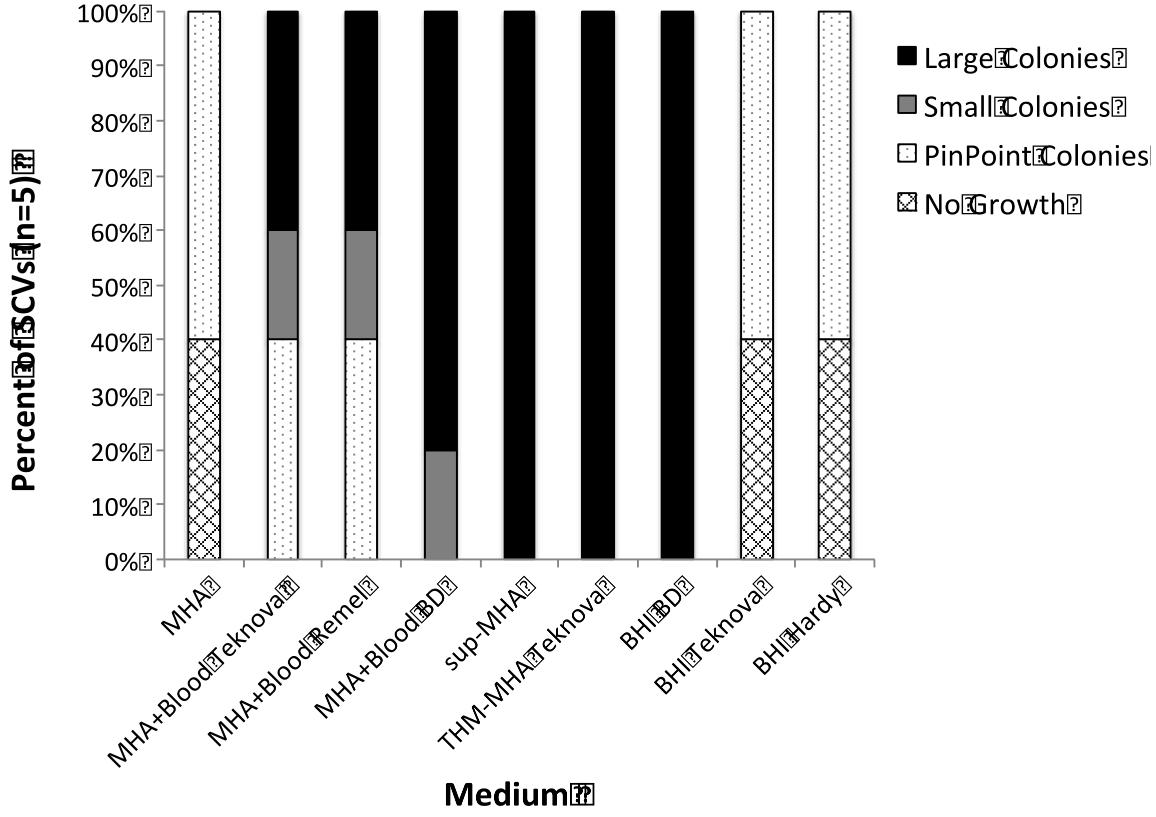

Percent of SCVs (n=5)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
MHA
MHA+Blood Teknova
MHA+Blood Remel
MHA+Blood BD
sup-MHA
THM-MHA Teknova
BHI BD
BHI Teknova
BHI Hardy
Medium
Large Colonies
Small Colonies
PinPoint Colonies
No Growth

Figure 5. Quality of SCV growth as determined by visual inspection of colony size on agar based media. (A) 10 SCVs were characterized by colony size as an indicator of adequate growth on various media. *SCV and NC colony growth was tested at a later date on THM-MHA (Thymidine, Hemin, Menadione-MHA, which is chemically identical to sup-MHA, but manufactured by Teknova) and Teknova manufactured unsupplemented MHA (Teknova-MHA) to confirm that isolates grew comparably on commercially available and in-house prepared sup- and unsupplemented MHA. (B) 5 SCVs were characterized by colony size as an indicator of adequate growth on various media. Large colonies were ≥1 mm in diameter, small colonies were <1 mm in diameter, and pinpoint colonies were ≤0.5 mm in diameter. All tests were done in both aerobic and CO_2 conditions, results were the same under both atmospheric conditions.

Abbreviations: Luria-Bertani (LB), trypticase soy agar + 5% sheep blood (BAP), Muellar-Hinton Agar (MHA), trypticase soy agar (TSA), brain heart infusion (BHI), synthetic CF sputum media supplemented herring sperm DNA (SCFMD). Media designated with “sup-” indicates base media (MHA, TSA, BHI, SCFMD) supplemented with SCV growth supportive nutrients thymidine, hemin, and menadione. MHA+blood is supplemented with 5% sheep blood by the manufacturer.

Note: Identical findings were observed in microaerobic 5% CO_2 incubation conditions therefore only aerobic data is shown.

Improved growth of SCVs on the above media may either be due to the presence of growth-supportive nutrients or to genetic reversion. In the first case, the *in vitro* susceptibilities of SCVs would be measured under chemically complementary conditions. In the latter case, the results would reflect the susceptibilities of *in vitro*-selected revertants, rather than SCVs. SCVs grew with substantial morphological variability (i.e., received a stability score greater than 1) on some media (e.g., TSA and sup-TSA), indicative of genetic instability and reversion, while other media supported uniform growth (e.g., sup-MHA) (Figure 6). To determine whether uniform colony sizes indicated SCV stability, we compared reversion frequencies for each SCV on the media that supported uniform growth and on blood agar (BAP), a medium previously used in SCV AST (1, 23). For each SCV on each medium, 150 randomly selected colonies were sub-cultured onto LB agar, on which SCVs grow small or not at all; large colonies represented revertants (Figure 7). Reversion frequencies were lowest on sup-MHA, followed by BD-BHI and sup-BD-BHI agars, under both aerobic and microaerobic conditions (Figure 8A). BAP yielded the highest rates of reversion under both atmospheric conditions and, on this medium, thymidine-dependent SCVs, in particular, exhibited a distinctive, anucleate cellular morphology upon Gram's staining (25), indicating poor growth (Figure 9). Sup-MHA and BD-BHI were therefore chosen for AST as they best supported growth and maintained phenotypic stability of SCVs. While SCVs grow with colony sizes approaching those of NC isolates on these media, these isolates retain SCV phenotypes upon sub-culturing to LB, and for simplicity we will refer to these mutants as SCVs even when cultivated on growth-complementing media.

Later experiments assessing stability of 5 SCVs on BD-MHA+blood showed that reversion rates were higher on this BD-MHA+blood compared to sup-MHA in both aerobic and microaerobic

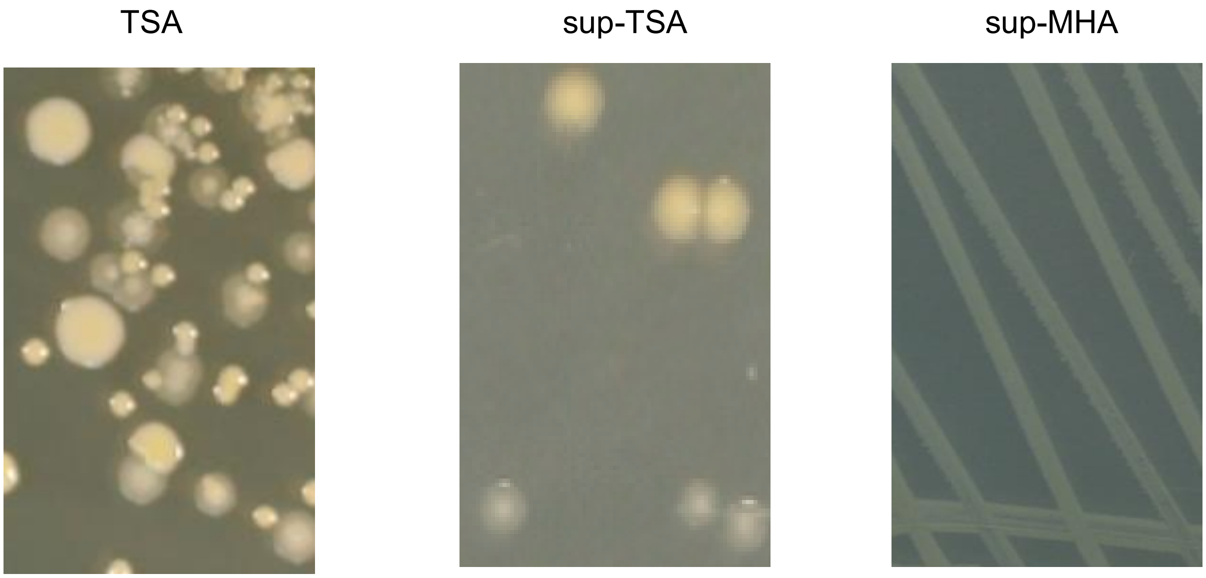


Figure 6. Morphological variability indicative of reversion for SCVs grown on different media. A representative thymidine-dependent SCV grown on trypticase soy-agar (TSA) (left), sup-TSA (middle), and sup-MHA (right) exhibited variable colony morphology on TSA and sup-TSA, indicative of reversion on those two media but not on sup-MHA. SCVs showed comparable, uniform growth on sup-MHA, sup-BHI, and BHI (only sup-MHA shown).

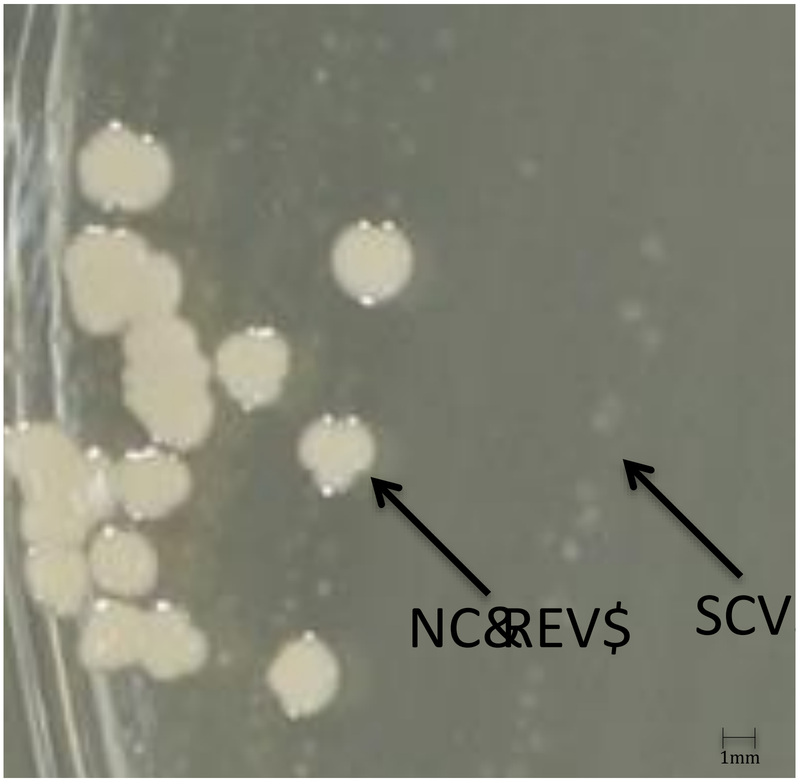


Figure 7. Differentiation between SCVs and revertant NCs on LB agar. A representative hemin-dependent SCV (labeled SCV on the figure) displaying reversion to NC growth (NC-REV) is shown on LB agar. On this medium, SCVs either do not grow at all (in the case of thymidine-dependent SCVs) or grow poorly (in the case of hemin- and menadione-dependent SCVs) compared to NC.

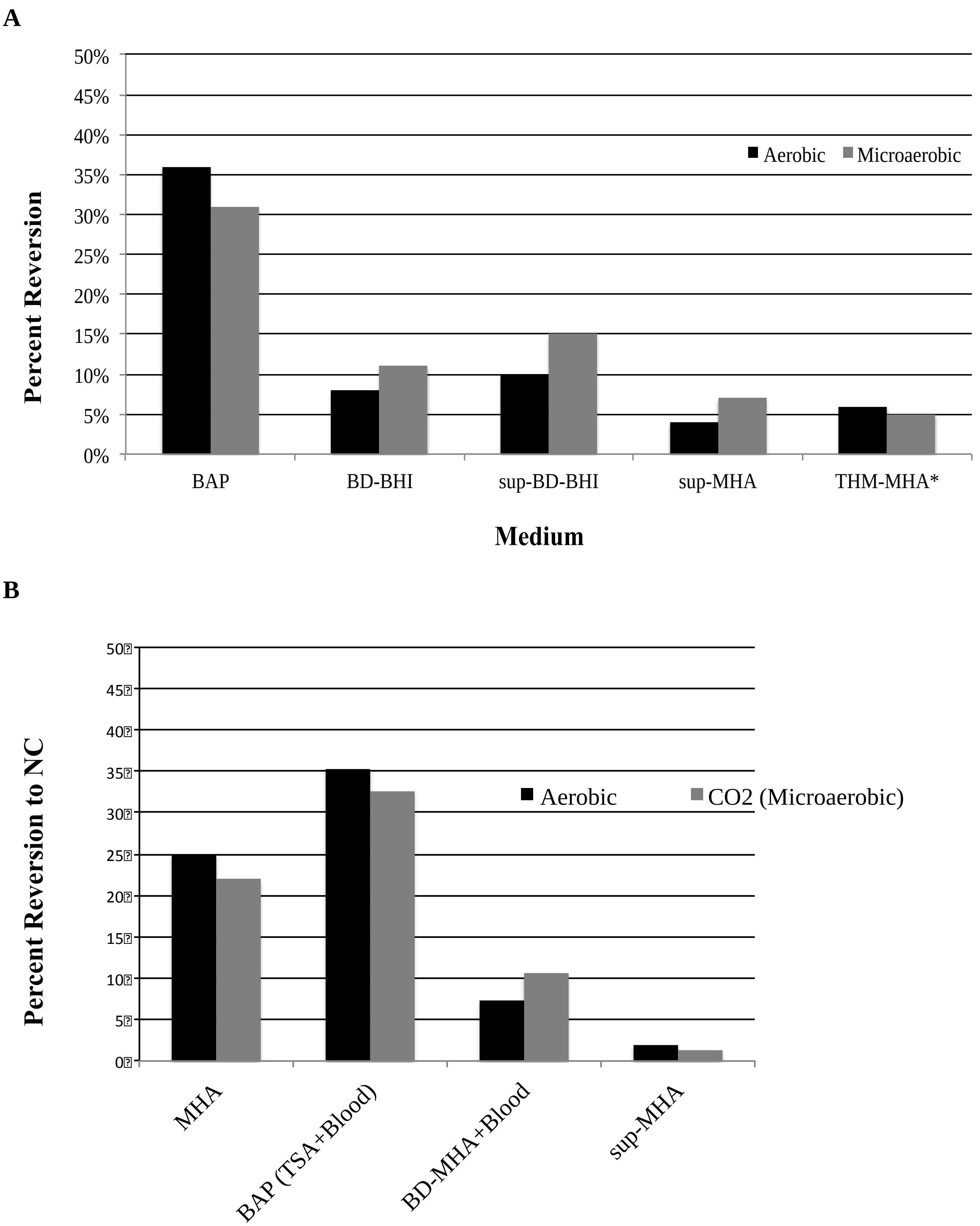

A
Percent Reversion
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Aerobic
Microaerobic
BAP
BD-BHI
sup-BD-BHI
sup-MHA
THM-MHA*
Medium
B
Percent Reversion to NC
50
45
40
35
30
25
20
15
10
5
0
Aerobic
CO2 (Microaerobic)
MHA
BAP (TSA+Blood)
BD-MHA+Blood
sup-MHA
Medium

Figure 8. Percent reversion of SCVs on growth supportive media. (A)SCV isolates were grown on media on the x-axis overnight after which colonies selected at random were sub-cultured onto LB (discriminatory medium) to distinguish between reversion to NC growth and complementation by medium chemical content. Percent reversion is shown on the y-axis. *Commercially prepared sup-MHA (THM-MHA) was validated at a later date. (B) After SCVs were grown on the media designated on the x-axis overnight, colonies were selected at random and sub-cultured to LB to distinguish between reversion to NC growth and complementation by medium chemical content.

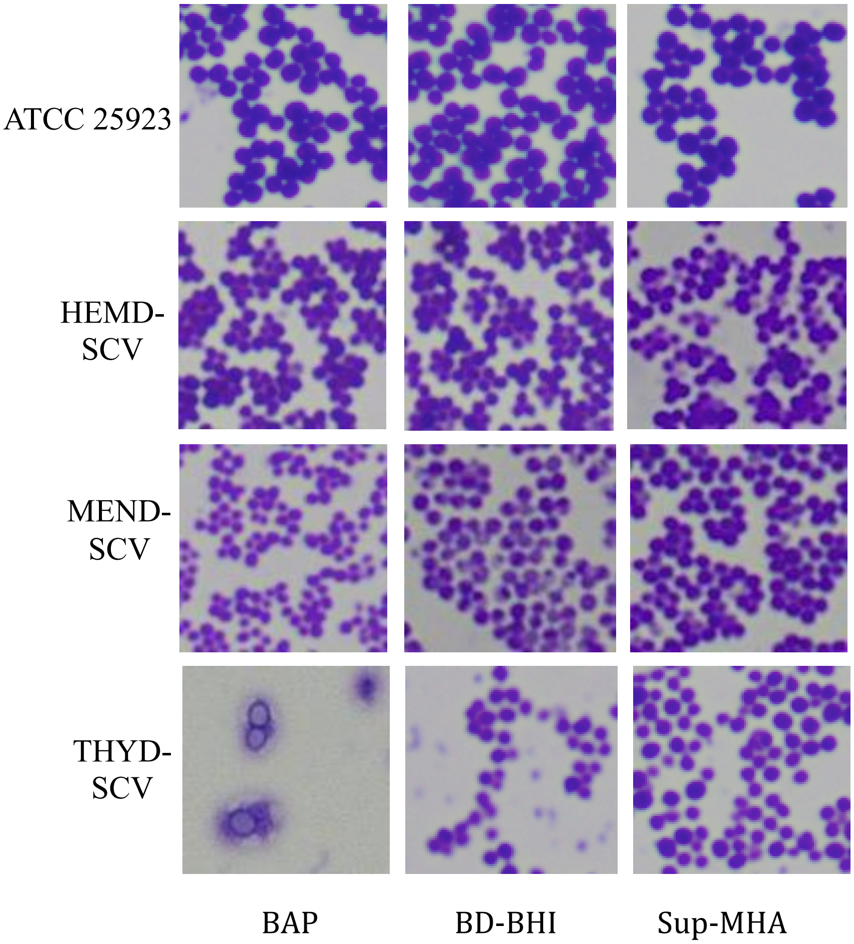


Figure 9. Gram's stain images of SCV cells with different auxotrophies after growth on indicated media. Cells of representative hemin-dependent (HEMD) and menadione-dependent (MEND) SCVs looked similar to those of Normal Colony (ATCC 25923) after growth on BAP, BD-BHI, and sup-MHA, indicative of growth complementation. However, thymidine-dependent (THYD) SCVs displayed abnormal cellular morphology on BAP, consistent with their slow growth and abnormal colony morphology on that medium.

conditions (Figure 8B). However, reversion frequencies were considerably lower on BD-MHA+blood compared to BAP as well as MHA and AST for a small subset of isolates with a handful of antibiotics was completed on BD-MHA+blood.

Antibiotic susceptibility testing under aerobic conditions by disk diffusion

To determine how closely antibiotic disk diffusion zone diameters on our test media would overlap with the acceptable range limits defined by the CLSI, we tested the susceptibility of control strain ATCC 25923 to 24 antibiotics on MHA, sup-MHA, and BD-BHI. The average zone diameters and standard deviations for ATCC 25923 fell within the CLSI acceptable range limits for each antibiotic on all media with a few exceptions, all involving BD-BHI (Figure 10A). Susceptibilities were also tested for a subset of 14 NC clinical isolates (8 MSSA and 6 MRSA) on MHA (CLSI recommended media), sup-MHA, and BD-BHI. Susceptibilities to the various antibiotics were identical on all media (Table 4). Therefore, supplementation of MHA with thymidine, hemin, and menadione did not significantly alter the antibiotic susceptibilities for the control isolates, suggesting that this media can be used for AST of all *S. aureus* isolates. Although results with the control strain fell outside acceptable limits for several antibiotics on BD-BHI, susceptibility testing was conducted with BD-BHI and sup-MHA for the complete set of isolates.

AST was performed on 112 clinical isolates (SCVs and their isogenic NCs), a menadione-dependent SCV selected *in vitro* from *S. aureus* strain Newman, and *S. aureus* Newman. All isolates grew on both BD-BHI and sup-MHA, and zone diameters were measurable after 18-24h incubation. No discrepancies in AST results were observed between BD-BHI and sup-MHA for

A

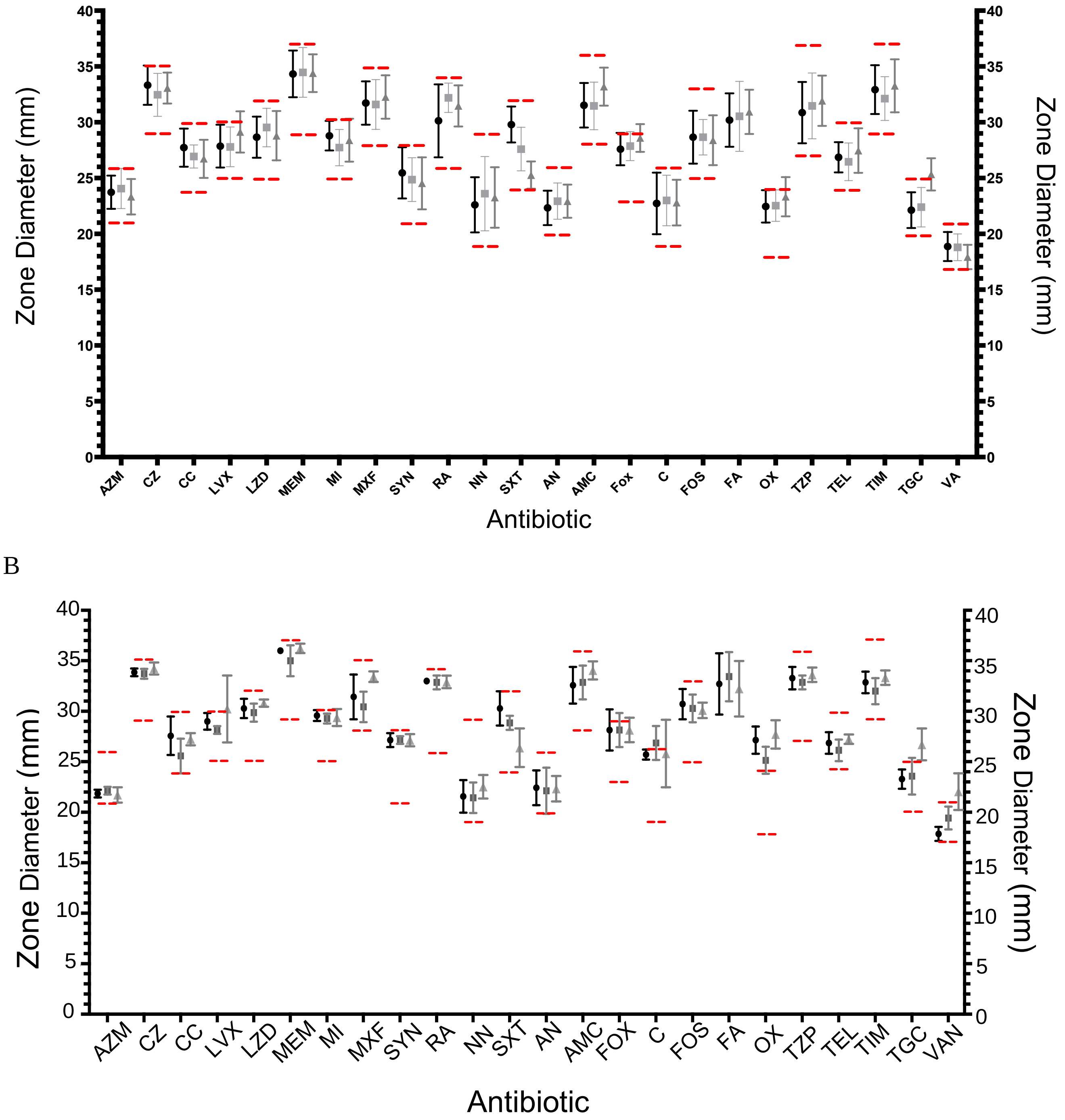

Zone Diameter (mm)
40
35
30
25
20
15
10
5
0
AZM
CZ
CC
LVX
LZD
MEM
MI
MXF
SYN
RA
NN
SXT
AN
AMC
Fox
C
FOS
FA
OX
TZP
TEL
TIM
TGC
VA
Antibiotic
B
Zone Diameter (mm)
AZM
CZ
CC
LVX
LZD
MEM
MI
MXF
SYN
RA
NN
SXT
AN
AMC
FOX
C
FOS
FA
OX
TZP
TEL
TIM
TGC
VAN
Antibiotic

Figure 10. Average zone diameters for ATCC 25923 in air and CO_2. (A) The average zone size for the control strain ATCC 25923 fell within CLSI accepted ranges (horizontal red lines) for MHA (black circles) and sup-MHA (light grey squares) for all antibiotics tested, and for the majority of antibiotics tested on BD-BHI (dark grey triangles). Error bars depict the standard deviation for 15 replicates. The average zone diameter for tigecycline (TGC) and the standard deviations for levofloxacin (LVX), cefoxitin (FOX), and oxacillin (OX) fell outside of the CLSI-defined acceptable range-limits on BHI. Fusidic acid (FA) does not have CLSI-defined range-limits; therefore, no red bars are shown. Although a range limit for ATCC 25923 has not been reported for fusidic acid, average zone diameters and standard deviations for this antibiotic were comparable on all three media and well above the EUCAST breakpoint (≥ 24 mm). (B) The average zone size for the control strain ATCC 25923 fell within CLSI-defined accepted ranges (horizontal red lines) for MHA (black circles) and sup-MHA (dark grey squares) for the majority of antibiotics tested and, to a lesser extent, BD-BHI (dark grey triangles). Specific discrepancies are noted in the text. Fusidic acid does not have CLSI-defined range-limits, and therefore, no red bars are shown. Error bars depict the standard deviation for 5 replicates.

X-axis, Antibiotics: azithromycin (AZM), cefazolin (CZ), clindamycin (CC), levofloxacin (LVX), linezolid (LZD), meropenem (MEM), minocycline (MI), moxifloxacin (MXF), quinupristin-dalfopristin (SYN), rifampin (RA), tobramycin (NN), trimethoprim/sulfamethoxazole (SXT), amikacin (AN), amoxicillin-clavulanate (AMC), cefoxitin (FOX), chloramphenicol (C), fosfomycin (FOS), fusidic acid (FA), oxacillin (OX), piperacillin-tazobactam (TZP), telithromycin (TEL), ticarcillin-clavulanate (TIM), vancomycin (VA).

Table 4. Comparison of disk-diffusion AST results of 14 selected NC clinical isolates using BHI, sup-MHA, and the CLSI-recommended medium, MHA.

	% Susceptible (Disk Test)					
	NC-MSSA (n=8)			NC-MRSA (n=6)		
Antibiotic	**BHI**	**sup-MHA**	**CLSI-MHA**	**BHI**	**sup-MHA**	**CLSI-MHA**
FOX[a]	100.0	100.0	100.0	0.0	0.0	0.0
OX[a]	100.0	100.0	100.0	66.7	66.7	66.7
TZP	100.0	100.0	100.0	ND	ND	ND
AMC	100.0	100.0	100.0	ND	ND	ND
CZ	100.0	100.0	100.0	ND	ND	ND
MEM	100.0	100.0	100.0	ND	ND	ND
TIM	100.0	100.0	100.0	ND	ND	ND
LVX	87.5	87.5	87.5	0.0	0.0	0.0
MXF	87.5	87.5	87.5	0.0	0.0	0.0
NN	87.5	87.5	87.5	0.0	0.0	0.0
AN	100.0	100.0	100.0	16.7	16.7	16.7
SXT	100.0	100.0	100.0	100.0	100.0	100.0
MI	100.0	100.0	100.0	100.0	100.0	100.0
C	100.0	100.0	100.0	100.0	100.0	100.0
AZM	62.5	62.5	62.5	0.0	0.0	0.0
CC	100.0	100.0	100.0	66.7	66.7	66.7
SYN	100.0	100.0	100.0	100.0	100.0	100.0
TEL	100.0	100.0	100.0	66.7	66.7	66.7
LZD	100.0	100.0	100.0	100.0	100.0	100.0
RA	87.5	87.5	87.5	33.3	33.3	33.3
TGC	100.0	100.0	100.0	100.0	100.0	100.0
FA	100.0	100.0	100.0	100.0	100.0	100.0

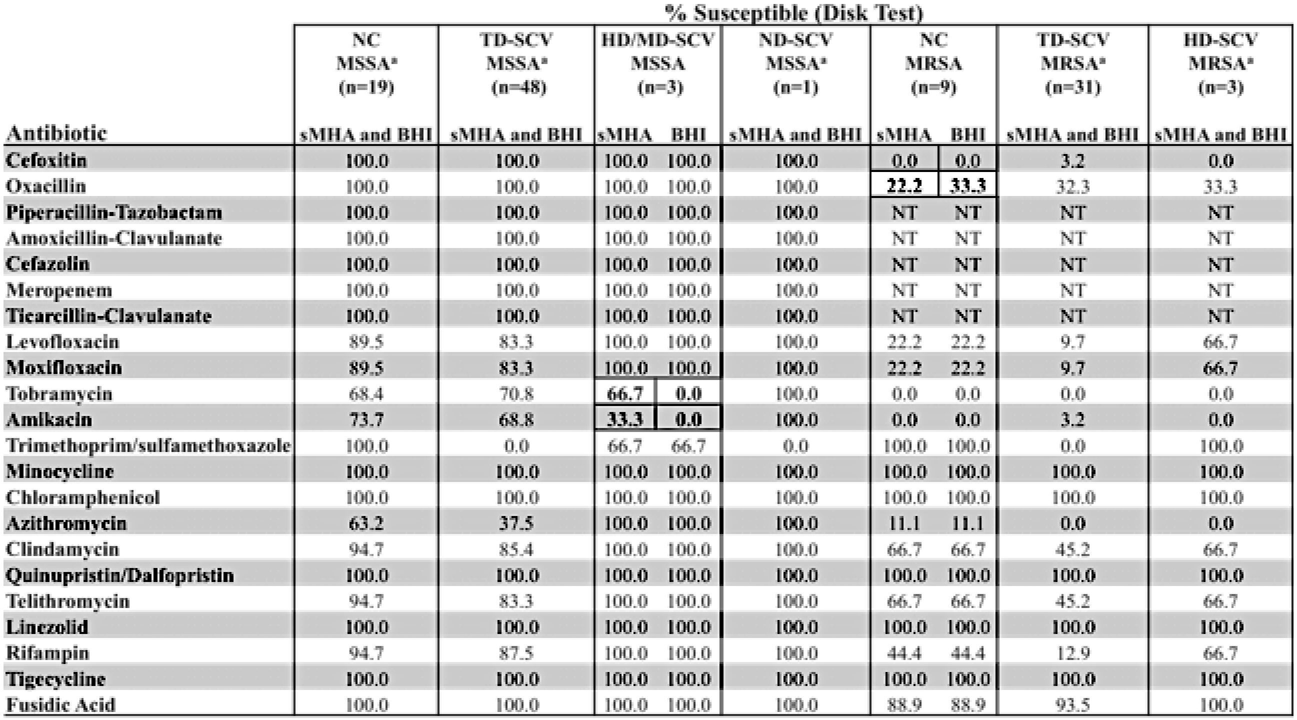
[a] Only FOX (cefoxitin) and OX (oxacillin) were tested against NC-MRSA isolates for this control, other β-lactams were Not Done (ND)

the thymidine-dependent SCVs (MSSA and MRSA). All thymidine-dependent SCVs were resistant to trimethoprim-sulfamethoxazole, while all NC isolates were susceptible (Table 5). Collectively, thymidine-dependent SCVs tended to be non-susceptible to azithromycin. A small percentage (~12-30%) of thymidine-dependent SCV-MSSA were also non-susceptible to the fluoroquinolones, aminoglycosides, clindamycin, telithromycin, and rifampin (Table 5). Fewer thymidine-dependent SCV-MRSA were susceptible to these antibiotics than were thymidine-dependent SCV-MSSA (Table 5).

In contrast with thymidine-dependent SCVs, hemin- and menadione-dependent SCV-MSSA exhibited different susceptibilities on BD-BHI and sup-MHA. While these SCVs were generally susceptible to most antibiotics, they were all non-susceptible to the aminoglycosides tobramycin and amikacin on BD-BHI (Table 5). However, these SCVs exhibited slightly larger zone diameters to these drugs (by 2-8 mm) on sup-MHA that, in some cases, changed the interpretation to susceptible. The hemin-dependent SCV-MRSA were uniformly non-susceptible to both aminoglycosides (Table 5). A single SCV with an undetermined auxotrophy was resistant only to trimethoprim-sulfamethoxazole (Table 5).

MRSA control strain JE2 and all *S. aureus* isolates were screened for methicillin resistance using oxacillin and cefoxitin disks. JE2 was resistant to both oxacillin and cefoxitin, with zone diameters comparable (± 1 mm) between MHA, BD-BHI, and sup-MHA. Cefoxitin resistance was detected for all NC *mecA*-positive isolates on both media but, interestingly, resistance to oxacillin was only observed for 67% of these isolates on BD-BHI, and 77% on sup-MHA. Similarly, of the 31 *mecA*-positive thymidine-dependent SCVs, 97% were resistant to cefoxitin

Table 5. Susceptibility profiles of NC and SCV clinical isolates determined by disk diffusion.



	% Susceptible (Disk Test)								
	NC MSSA[a] (n=19)	TD-SCV MSSA[a] (n=48)	HD/MD-SCV MSSA (n=3)		ND-SCV MSSA[a] (n=1)	NC MRSA (n=9)		TD-SCV MRSA[a] (n=31)	HD-SCV MRSA[a] (n=3)
Antibiotic	sMHA and BHI	sMHA and BHI	sMHA	BHI	sMHA and BHI	sMHA	BHI	sMHA and BHI	sMHA and BHI
Cefoxitin	100.0	100.0	100.0	100.0	100.0	0.0	0.0	3.2	0.0
Oxacillin	100.0	100.0	100.0	100.0	100.0	22.2	33.3	32.3	33.3
Piperacillin-Tazobactam	100.0	100.0	100.0	100.0	100.0	NT	NT	NT	NT
Amoxicillin-Clavulanate	100.0	100.0	100.0	100.0	100.0	NT	NT	NT	NT
Cefazolin	100.0	100.0	100.0	100.0	100.0	NT	NT	NT	NT
Meropenem	100.0	100.0	100.0	100.0	100.0	NT	NT	NT	NT
Ticarcillin-Clavulanate	100.0	100.0	100.0	100.0	100.0	NT	NT	NT	NT
Levofloxacin	89.5	83.3	100.0	100.0	100.0	22.2	22.2	9.7	66.7
Moxifloxacin	89.5	83.3	100.0	100.0	100.0	22.2	22.2	9.7	66.7
Tobramycin	68.4	70.8	66.7	0.0	100.0	0.0	0.0	0.0	0.0
Amikacin	73.7	68.8	33.3	0.0	100.0	0.0	0.0	3.2	0.0
Trimethoprim/sulfamethoxazole	100.0	0.0	66.7	66.7	0.0	100.0	100.0	0.0	100.0
Minocycline	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Chloramphenicol	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Azithromycin	63.2	37.5	100.0	100.0	100.0	11.1	11.1	0.0	0.0
Clindamycin	94.7	85.4	100.0	100.0	100.0	66.7	66.7	45.2	66.7
Quinupristin/Dalfopristin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Telithromycin	94.7	83.3	100.0	100.0	100.0	66.7	66.7	45.2	66.7
Linezolid	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Rifampin	94.7	87.5	100.0	100.0	100.0	44.4	44.4	12.9	66.7
Tigecycline	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fusidic Acid	100.0	100.0	100.0	100.0	100.0	88.9	88.9	93.5	100.0

[a] These isolates displayed identical percent susceptibilities on both sup-MHA (specified in table as sMHA) and BHI; therefore percent susceptibilities are shown as a single column representing both media.

Note. Discrepancies between media for the same group of isolates is noted by bold boxes and discussed in the text.

ND-SCV refers to an SCV with a Non-Defined auxotrophy.

NT in the rows corresponding to the β-lactams indicates that these drugs were Not Tested for the corresponding MRSA isolates.

and 67.7% were resistant to oxacillin on both media. A single *mecA*-positive, thymidine-dependent SCV was susceptible to both cefoxitin and oxacillin on BD-BHI and sup-MHA, as well as on BAP. All hemin-dependent SCV-MRSAs were cefoxitin-resistant on both media, and 2/3 of these isolates were oxacillin-resistant (Table 5). In contrast, all of the MSSA-SCVs were susceptible to each of the β-lactams tested. Therefore, MRSA status correlated well with cefoxitin resistance for both NC and SCV isolates, with a single exception.

All isolates, regardless of phenotype (i.e. NC/SCV, MSSA/MRSA), were susceptible to minocycline, chloramphenicol, quinupristin/dalfopristin, linezolid, and tigecycline (Table 5). Almost all *S. aureus* isolates, except 3 MRSA isolates (1 NC and 2 SCVs), were susceptible to fusidic acid. While MRSA status correlated well with β-lactam resistance and auxotrophy for thymidine correlated well with trimethoprim-sulfamethoxazole resistance, susceptibility to many other clinically-useful classes (including aminoglycosides, fluoroquinolones, macrolides, clindamycin, and rifampin) could not be reliably predicted from those two characteristics.

Antibiotic susceptibility testing under microaerobic conditions by disk diffusion. The CF lung is known to include areas with low oxygen (26). Since SCVs frequently exhibit metabolic characteristics different from their NC counterparts (27) that may be impacted by atmospheric conditions, we compared AST results when performed under microaerobic versus aerobic conditions. ATCC 25923 gave similar results under both conditions, with standard deviations for levofloxacin, cefoxitin, and tigecycline falling outside of the CLSI acceptable range on BD-BHI (Figure 10B). A few antibiotics yielded microaerobic, but not aerobic, zone sizes out of CLSI range limits on both MHA and sup-MHA, including cefoxitin, chloramphenicol, oxacillin, and tigecycline (Figure 10B).

Microaerobic susceptibilities for the vast majority of clinical isolates (subset of 26 SCVs and 13 NC isogenic pairs) were identical to those from aerobic conditions with one exception. Four clonally-related, thymidine-dependent, *mecA*-positive isolates were non-susceptible to cefoxitin and oxacillin under aerobic conditions, but were susceptible to both of these antibiotics under microaerobic conditions (Table 6).

Antibiotic susceptibility testing by Etest. Etest was performed using a commercially-prepared supplemented MHA (THM-MHA) to obtain MICs. As a first quality-control step, we compared the susceptibilities of *S. aureus* ATCC 29213 to specific antibiotics tested on THM-MHA and MHA from the same company (Teknova-MHA). MICs fell within the CLSI acceptable range limits for all drugs tested (Figure 11) with similar results between THM-MHA and Teknova-MHA. Thus, supplementation of the CLSI-recommended media did not influence the susceptibilities of the *S. aureus* control strain.

We performed Etest on a subset of the isolates (24 NC and 26 SCVs) that had been analyzed by disk diffusion. All isolates grew on THM-MHA, and MICs were measurable after 24 hr incubation. No discrepancies were observed between antibiograms determined by disk diffusion and Etest. Cefazolin and meropenem Etest strips are not available, therefore cephalothin and imipenem were used as surrogate markers for susceptibility, respectively. Additionally, because there are no established breakpoints for vancomycin by disk diffusion, susceptibility to this drug

Table 6. Atmospheric dependent susceptibility profiles of four isogenic THYD-SCVs.

	Isolate Number	0208-05	0208-05	0208-05	0208-05	0208-07	0208-07	0208-07	0208-07	0208-08	0208-08	0208-08	0208-08	0208-10	0208-10	0208-10	0208-10
	Phenotype	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA	SCV-MRSA
	Auxotrophy	THYD	THYD	THYD	THYD	THYD	THYD	THYD	THYD	Thy	Thy	Thy	Thy	Thy	Thy	Thy	Thy
	PFGE Group	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B	4B
	Media	BHI	BHI	sup-MHA	sup-MHA	BHI	BHI	sup-MHA	sup-MHA	BHI	BHI	sup-MHA	sup-MHA	BHI	BHI	sup-MHA	sup-MHA
	Atmosphere	Air	5 % CO_2	Air	5 % CO_2	Air	5 % CO_2	Air	5 % CO_2	Air	5 % CO_2	Air	5 % CO_2	Air	5 % CO_2	Air	5 % CO_2
Zone size (mm) for antibiotics tested	FOX	6	32	6	33	6	33	6	36	6	32	6	34	6	29	6	30
	LVX	10	10	10	10	9	7	8	6	10	12	13	11	14	12	13	12
	MXF	22	21	22	22	20	20	19	18	22	22	23	21	23	22	23	22
	NN	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	AN	14	12	15	12	12	12	16	13	15	16	12	14	15	10	10	15
	SXT	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	MI	39	38	42	38	38	39	34	42	40	38	36	39	39	41	37	42
	C	28	24	28	29	26	27	25	27	26	26	28	23	26	26	24	28
	AZM	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	CC	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	SYN	24	25	28	25	25	27	23	29	33	32	34	34	32	34	32	34
	TEL	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	LZD	29	29	32	32	25	25	29	30	38	36	41	38	38	37	39	37
	RA	9	10	10	9	10	12	10	11	17	17	19	19	19	19	19	17
	TGC	36	34	33	34	32	33	31	31	34	32	30	32	33	34	34	31
	FOS	29	33	34	39	31	30	33	38	28	30	30	28	28	35	29	33
	VA	25	23	27	26	24	24	26	26	23	23	25	22	23	26	24	25
	FA	34	36	36	38	34	33	36	38	36	36	34	33	36	38	36	37

Shaded areas indicate where isolates had conflicting cefoxitin (FOX) susceptibilities in aerobic (air) verses 5% CO_2 atmospheric conditions. Susceptibility profiles were the same for all other antibiotics.

Antibiotic abbreviations; azithromycin (AZM), cefazolin (CZ), clindamycin (CC), levofloxacin (LVX), linezolid (LZD), meropenem (MEM), minocycline (MI), moxifloxacin (MXF), quinupristin-dalfopristin (SYN), rifampin (RA), tobramycin (NN), trimethoprim/sulfamethoxazole (SXT), amikacin (AN), amoxicillin-clavulanate (AMC), cefoxitin (FOX), chloramphenicol (C), fosfomycin (FOS), fusidic acid (FA), oxacillin (OX), piperacillin-tazobactam (TZP), telithromycin (TEL), ticarcillin-clavulanate (TIM), vancomycin (VA).

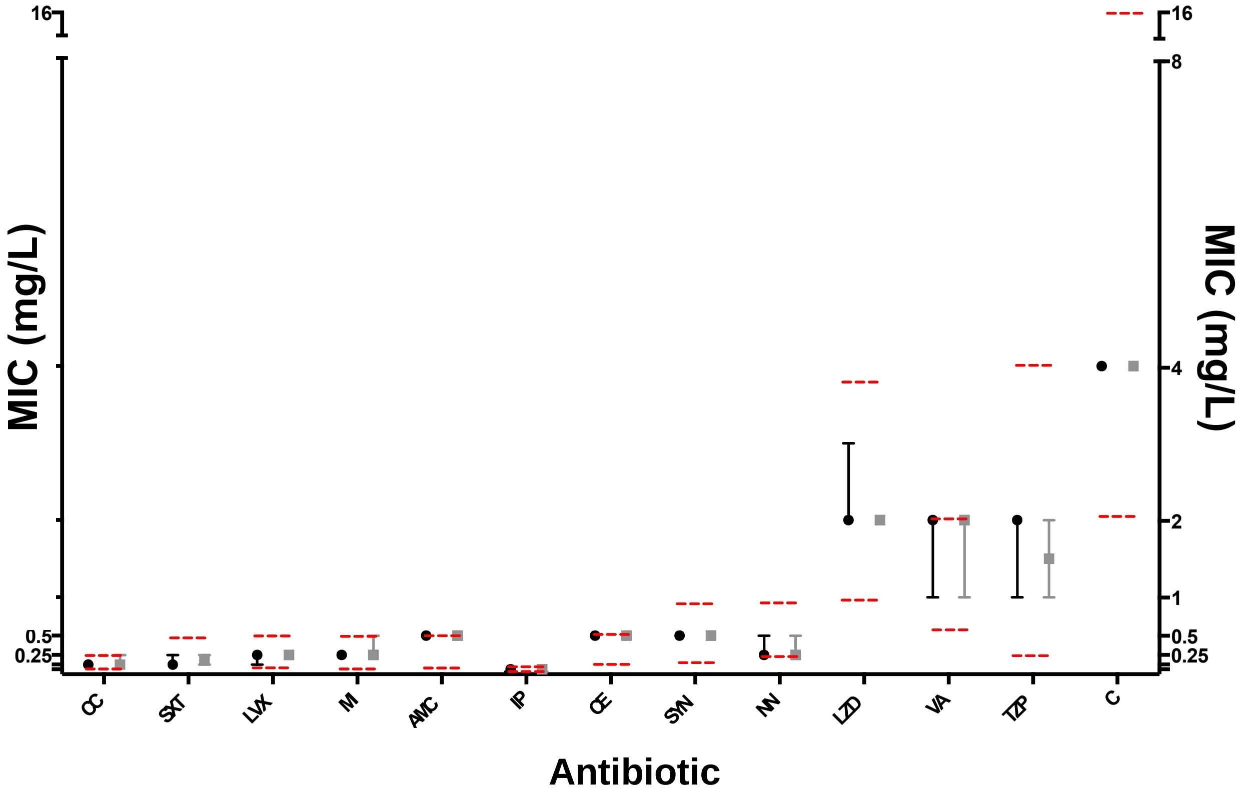


Figure 11. Median MICs for ATCC 29213. The median MICs as determined by Etest for control strain ATCC 29213 fell within CLSI acceptable ranges (horizontal red lines) for MHA (black circles) and sup-MHA (grey squares) for all the antibiotics tested. Errors bars depict the lower and upper range-limits detected for 4 replicates.

X-axis Antibiotics: clindamycin (CC), trimethoprim/sulfamethoxazole (SXT), levofloxacin (LVX), minocycline (MI), amoxicillin-clavulanate (AMC), imipenem (IP), cephalothin (CE), quinupristin-dalfopristin (SYN), tobramycin (NN), linezolid (LZD), vancomycin (VA), piperacillin-tazobactam (TZP), chloramphenicol (C).

was determined only by Etest; all isolates were susceptible to vancomycin (Table 7). As with disk diffusion, all isolates were susceptible to chloramphenicol, linezolid, minocycline, and quinupristin-dalfopristin regardless of phenotype (Table 7).

Additional antibiotic susceptibility testing to evaluate BD-MHA+blood agar media. After the above experiments were performed and published, a group led by the Clinical Laboratory Standards Institute (CLSI) asked us to compare the AST performance for SCVs of THM-MHA with another medium they were exploring, Mueller-Hinton agar supplemented with whole blood (BD-MHA+blood). We performed comparative AST on these media by disk diffusion for a small subset of antibiotics (Table 3) and Etest (vancomycin only), using a subset of 11 clinical isolates representing the three auxotrophy types along with isogenic NC paired isolates on BD-MHA+blood in both aerobic and microaerobic conditions. While all isolates grew on BD-MHA+blood media after 24 hours, the MEND-SCV isolates tested failed to grow to NC levels on this medium. Additionally, there were discrepancies in susceptibilities for the hemin and menadione-dependent MSSA SCV isolates in both air and CO_2 conditions (Table 8). Both hemin and menadione-dependent MSSA SCVs were susceptible to trimethoprim/sulfamethoxazole using BD-MHA+blood but the hemin-dependent SCV tested on sup-MHA was resistant to trimethoprim/sulfamethoxazole. (Table 8).

Oddly, both the hemin and menadione dependent MSSA SCV isolates were susceptible to tobramycin only on BD-MHA+blood under aerobic conditions, but they were non-susceptible under microaerobic conditions on this medium. These isolates were non-susceptible to tobramycin on sup-MHA under both aerobic and microaerobic conditions. This observation

Table 7. Susceptibility profiles shown as percent susceptible of NC and SCV clinical isolates determined by Etest

	% Susceptible (Etest)						
Antibiotic	**NC MSSA (n=17)**	**TD-SCV MSSA (n=15)**	**HD-SCV MSSA (n=2)**	**MD-SCV MSSA (n=1)**	**NC MRSA (n=7)**	**TD-SCV MRSA (n=6)**	**HD-SCV MRSA (n=2)**
Clindamycin	94.1	80.0	100.0	100.0	57.1	33.3	50.0
Trimethoprim/ Sulfamethoxazole	100.0	0.0	50.0	100.0	100.0	0.0	100.0
Levofloxacin	88.2	80.0	100.0	100.0	14.3	0.0	50.0
Chloramphenicol	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Linezolid	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Vancomycin	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Minocycline	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Quinupristin/ Dalfopristin	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Cephalothin	100.0	100.0	100.0	100.0	NT	NT	NT
Amoxicillin/ Calvulanate	100.0	100.0	100.0	100.0	NT	NT	NT
Imipenem	100.0	100.0	100.0	100.0	NT	NT	NT
Piperacillin Tazobactam	100.0	100.0	100.0	100.0	NT	NT	NT
Tobramycin	64.7	73.3	50.0	100.0	0.0	0.0	0.0

Note. NT in the rows corresponding to the β-lactams indicates that these drugs were Not Tested for the corresponding MRSA isolates. All testing was completed on THM-MHA manufactured by Teknova.

Table 8. Susceptibility profiles shown as percent susceptibilities of SCV and NC isolates

	NC-MSSA n=3				TD-MSSA n=1				HD/MD-MSSA n=2				NC-MRSA n=2				TD-MRSA n=1				HD-MRSA n=2			
	MHAB	MHAB	sMHA	sMHA	MHAB	MHAB	sMHA	sMHA	MHAB	MHAB	sMHA	sMHA	MHAB	MHAB	sMHA	sMHA	MHAB	MHAB	sMHA	sMHA	MHAB	MHAB	sMHA	sMH
Cefoxitin	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0	0	0	0	0	0	0	0	0
Trimethoprim/ Sulfamethoxazole	100	100	100	100	0	0	0	0	**100**	**100**	**50**	**50**	100	100	100	100	0	0	0	0	100	100	100	100
Tobramycin	100	100	100	100	100	100	100	100	**100**	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
Clindamycin	100	100	100	100	100	100	100	100	100	100	100	100	50	50	50	50	100	100	100	100	100	100	100	100
Levofloxicin	100	100	100	100	100	100	100	100	100	100	100	100	50	50	50	50	0	0	0	0	100	100	100	100
Vancomycin (Etest)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

All antibiotics besides vancomycin were tested using the disk diffusion method. Vancomycin was tested using Etest strips.

White columns correspond to percent susceptibilities from aerobic conditions and grey columns correspond to percent susceptibilities from microaerobic (5% CO_2) conditions

Discrepancies (only seen for trimethoprim/sulfamethoxazole and tobramycin) are bolded.

Abbreviations: MHAB (BD-MHA+blood), sMHA (in house prepared Sup-MHA), NC (Normal Colony), TD (Thymidine-Dependent), HD (Hemin-dependent), MD (Menadione-dependent). MSSA (methicillin susceptible *Staphylococcus aureus*), MRSA (methicillin resistant *Staphylococcus aureus*).

Not Shown: Zone diameters for ATCC 25923 fell within acceptable CLSI ranges on all media in all incubation conditions, 2 replicates. Vancomycin minimum inhibitory concentration for ATCC 29213 fell within CLSI breakpoints on all media in all in all incubation conditions, 2 replicates. The MRSA control strain JE2 showed resistance to cefoxitin on all media in all conditions tested.

could perhaps be due to the presence of different concentrations of hemin and menadione between BD-MHA+blood plates. Cefoxitin disk testing predicted MRSA status (confirmed by *mecA* PCR) for all isolates tested on both BD-MHA+blood and sup-MHA (Table 8). As expected, all thymidine-dependent SCVs were resistant to trimethoprim/sulfamethoxazole on both BD-MHA+blood and sup-MHA. These isolates have mutations, confirmed by sequencing, in thymidylate synthase (*thyA*) that are predicted to be inactivating; previous work by our lab as well as other groups have shown that inactivation of *thyA* confers resistance to trimethoprim/sulfamethoxazole, as we observed on both BD-MHA+blood and sup-MHA.

NC Susceptibilities as a predictor of SCV susceptibilities. We evaluated whether *in vitro* susceptibilities of SCVs could be predicted based on the antibiograms of isogenic NC *S. aureus* isolated before or concurrently with SCVs from the same patients. In addition to expected differences in trimethoprim-sulfamethoxazole and aminoglycoside susceptibility, some SCVs differed in their susceptibilities to other drugs compared to their genetically-related NC counterparts (Table 9), and the susceptibilities of genetically-related SCVs from the same patient occasionally differed from each other (Table 10). Therefore, the susceptibilities of SCVs cannot consistently be predicted by isogenic NC isolates from the same patients (or even from other SCVs), indicating that *in vitro* susceptibilities for an SCV can only be reliably defined by testing that isolate.

Previous antibiotic exposure as a predictor of SCV susceptibilities. To determine whether a recent exposure history to a specific antibiotic reliably predicts resistance to that antibiotic (or others in its class) for SCVs, we analyzed the relationship between SCV *in vitro* susceptibilities

Table 9. Comparison of SCV and NC susceptibility profiles.

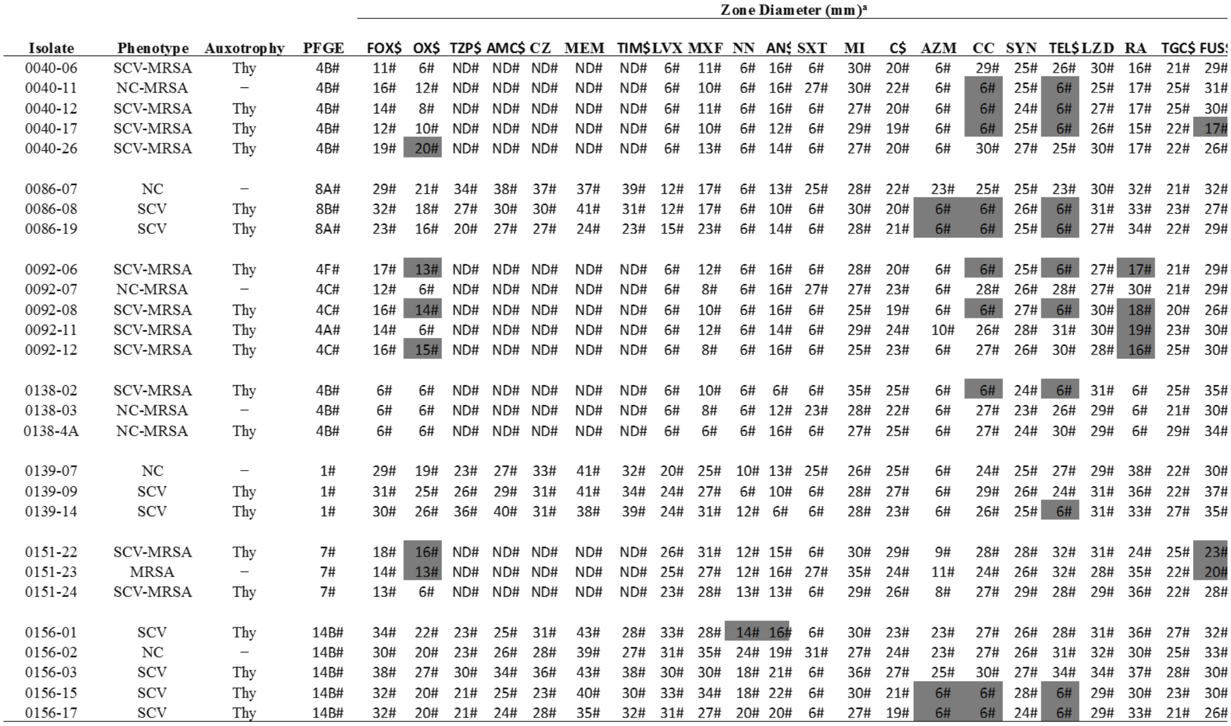
	Percent of SCVs with same susceptibility profile as paired NC isolate			
Antibiotic	**TD-MSSA[a] (n=48)**	**TD-MRSA[a] (n=31)**	**HD/MD-MSSA (n=3)**	**HD-MRSA (n=2)**
Cefoxitin	100	97	100	100
Levofloxacin	100	97	100	100
Moxifloxacin	100	97	100	100
Tobramycin[b]	96	100	100 (sup-MHA) 33 (BHI)	100
Amikacin[b]	90	97	0 (sup-MHA) 33 (BHI)	100
Trimethoprim-Sulfamethoxazole	0	0	67	100
Minocycline	100	100	100	100
Chloramphenicol	100	100	100	100
Azithromycin	88	100	100	50
Clindamycin	92	84	100	100
Quinupristin/Dalfopristin	100	100	100	100
Telithromycin	89	84	100	100
Linezolid	100	100	100	100
Rifampin	100	90	100	100
Tigecycline	100	100	100	100
Fusidic Acid	100	96	100	100

[a] The isolate collection was primarily composed of thymidine-dependent (TD) SCVs. Hemin- and menadione-dependent (HD/MD) SCVs made up the minority of clinical isolates.

[b] Exposure to these drugs has been linked to the emergence of SCVs (NN and AN with HD/MD-SCVs and SXT with TD-SCVs), and therefore, discrepancies between NC/SCV susceptibilities are expected

Note. Bolded cells highlight any value(s) less than 100% similarity between SCV and NC susceptibilities to an antibiotic.

Table 10. AST profiles among clonally-related SCVs for which those profiles differed



				Zone Diameter (mm)[a]																					
Isolate	**Phenotype**	**Auxotrophy**	**PFGE**	**FOX$**	**OX$**	**TZP$**	**AMC$**	**CZ**	**MEM**	**TIM$**	**LVX**	**MXF**	**NN**	**AN$**	**SXT**	**MI**	**C$**	**AZM**	**CC**	**SYN**	**TEL$**	**LZD**	**RA**	**TGC$**	**FUS**
0040-06	SCV-MRSA	Thy	4B#	11#	6#	ND#	ND#	ND#	ND#	ND#	6#	11#	6#	16#	6#	30#	20#	6#	29#	25#	26#	30#	16#	21#	29#
0040-11	NC-MRSA	–	4B#	16#	12#	ND#	ND#	ND#	ND#	ND#	6#	10#	6#	16#	27#	30#	22#	6#	6#	25#	6#	25#	17#	25#	31#
0040-12	SCV-MRSA	Thy	4B#	14#	8#	ND#	ND#	ND#	ND#	ND#	6#	11#	6#	16#	6#	27#	20#	6#	6#	24#	6#	27#	17#	25#	30#
0040-17	SCV-MRSA	Thy	4B#	12#	10#	ND#	ND#	ND#	ND#	ND#	6#	10#	6#	12#	6#	29#	19#	6#	6#	25#	6#	26#	15#	22#	17#
0040-26	SCV-MRSA	Thy	4B#	19#	20#	ND#	ND#	ND#	ND#	ND#	6#	13#	6#	14#	6#	27#	20#	6#	30#	27#	25#	30#	17#	22#	26#
0086-07	NC	–	8A#	29#	21#	34#	38#	37#	37#	39#	12#	17#	6#	13#	25#	28#	22#	23#	25#	25#	23#	30#	32#	21#	32#
0086-08	SCV	Thy	8B#	32#	18#	27#	30#	30#	41#	31#	12#	17#	6#	10#	6#	30#	20#	6#	6#	26#	6#	31#	33#	23#	27#
0086-19	SCV	Thy	8A#	23#	16#	20#	27#	27#	24#	23#	15#	23#	6#	14#	6#	28#	21#	6#	6#	25#	6#	27#	34#	22#	29#
0092-06	SCV-MRSA	Thy	4F#	17#	13#	ND#	ND#	ND#	ND#	ND#	6#	12#	6#	16#	6#	28#	20#	6#	6#	25#	6#	27#	17#	21#	29#
0092-07	NC-MRSA	–	4C#	12#	6#	ND#	ND#	ND#	ND#	ND#	6#	8#	6#	16#	27#	27#	23#	6#	28#	26#	28#	27#	30#	21#	29#
0092-08	SCV-MRSA	Thy	4C#	16#	14#	ND#	ND#	ND#	ND#	ND#	6#	10#	6#	16#	6#	25#	19#	6#	6#	27#	6#	30#	18#	20#	26#
0092-11	SCV-MRSA	Thy	4A#	14#	6#	ND#	ND#	ND#	ND#	ND#	6#	12#	6#	14#	6#	29#	24#	10#	26#	28#	31#	30#	19#	23#	30#
0092-12	SCV-MRSA	Thy	4C#	16#	15#	ND#	ND#	ND#	ND#	ND#	6#	8#	6#	16#	6#	25#	23#	6#	27#	26#	30#	28#	16#	25#	30#
0138-02	SCV-MRSA	Thy	4B#	6#	6#	ND#	ND#	ND#	ND#	ND#	6#	10#	6#	6#	6#	35#	25#	6#	6#	24#	6#	31#	6#	25#	35#
0138-03	NC-MRSA	–	4B#	6#	6#	ND#	ND#	ND#	ND#	ND#	6#	8#	6#	12#	23#	28#	22#	6#	27#	23#	26#	29#	6#	21#	30#
0138-4A	NC-MRSA	Thy	4B#	6#	6#	ND#	ND#	ND#	ND#	ND#	6#	6#	6#	16#	6#	27#	25#	6#	27#	24#	30#	29#	6#	29#	34#
0139-07	NC	–	1#	29#	19#	23#	27#	33#	41#	32#	20#	25#	10#	13#	25#	26#	25#	6#	24#	25#	27#	29#	38#	22#	30#
0139-09	SCV	Thy	1#	31#	25#	26#	29#	31#	41#	34#	24#	27#	6#	10#	6#	28#	27#	6#	29#	26#	24#	31#	36#	22#	37#
0139-14	SCV	Thy	1#	30#	26#	36#	40#	31#	38#	39#	24#	31#	12#	6#	6#	28#	23#	6#	26#	25#	6#	31#	33#	27#	35#
0151-22	SCV-MRSA	Thy	7#	18#	16#	ND#	ND#	ND#	ND#	ND#	26#	31#	12#	15#	6#	30#	29#	9#	28#	28#	32#	31#	24#	25#	23#
0151-23	MRSA	–	7#	14#	13#	ND#	ND#	ND#	ND#	ND#	25#	27#	12#	16#	27#	35#	24#	11#	24#	26#	32#	28#	35#	22#	20#
0151-24	SCV-MRSA	Thy	7#	13#	6#	ND#	ND#	ND#	ND#	ND#	23#	28#	13#	13#	6#	29#	26#	8#	27#	29#	28#	29#	36#	22#	28#
0156-01	SCV	Thy	14B#	34#	22#	23#	25#	31#	43#	28#	33#	28#	14#	16#	6#	30#	23#	23#	27#	26#	28#	31#	36#	27#	32#
0156-02	NC	–	14B#	30#	20#	23#	26#	28#	39#	27#	31#	35#	24#	19#	31#	27#	24#	23#	27#	26#	31#	32#	30#	25#	33#
0156-03	SCV	Thy	14B#	38#	27#	30#	34#	36#	43#	38#	30#	30#	18#	21#	6#	36#	27#	25#	30#	27#	34#	34#	37#	28#	30#
0156-15	SCV	Thy	14B#	32#	20#	21#	25#	23#	40#	30#	33#	34#	18#	22#	6#	30#	21#	6#	6#	28#	6#	29#	30#	23#	30#
0156-17	SCV	Thy	14B#	32#	20#	21#	24#	28#	35#	32#	31#	27#	20#	20#	6#	27#	19#	6#	6#	24#	6#	29#	33#	21#	26#

[a] Zone sizes were comparable on sup-MHA and BD-BHI. Values measured from sup-MHA only are shown.

Note: Shading highlights discrepancies in disk diameters that altered the susceptibility reading (based on CLSI breakpoints) of genetically-related strains.

on sup-MHA and antibiotic treatment data from the parent study. The analysis showed that SCVs were frequently, but not always, non-susceptible to antibiotics to which the source patient was recently exposed. As shown in Table 11, exposure to tobramycin and trimethoprim-sulfamethoxazole were each associated with significantly higher odds that subsequent SCVs would be non-susceptible to those antibiotics on logistic regression (OR (95% CI) 12.4 (2.3, 68.3) and 2.2 (0.3, 17.6), respectively). However, the correlation was not 100% for either antibiotic. While treatment with other antibiotics was relatively less frequent prior to SCV isolation, those isolated after exposure to azithromycin, clindamycin, and rifampin were invariably resistant to those drugs; by contrast, SCVs isolated after fluoroquinolone treatment were uniformly susceptible to levofloxacin. There were no other antibiotic exposures identified in SCV-positive subjects. Therefore, history of exposure to all antibiotics but fluoroquinolones may provide a clinically meaningful rationale for avoiding those drugs when treating SCV-positive patients, assuming *in vitro* susceptibilities are accurate predictors of clinical response. Nevertheless, these results indicate that treatment history, as for other potential predictors, has limited utility in predicting *in vitro* susceptibility results.

Discussion

This work shows that SCVs have distinct antibiotic resistance profiles, at least *in vitro*. Additionally, it’s appreciated that SCVs have been selected *in vitro* following antibiotic exposure, and antibiotic treatment has been associated with the subsequent detection of SCVs (4, 28). Therefore, antibiotic selection plays a key role in SCV emergence and subsequent SCV antibiotic susceptibilities. While AST is the standard technique for identifying appropriate

Table 11: Relationships between antibiotic exposure and *in vitro* susceptibilities

All antibiotics to which subjects culture-positive for SCVs during the study reported exposure within 90 days prior to cultures are included.

Antibiotic tested	Susceptibility findings for 71 isolates in the analysis dataset	Antibiotic exposure within 90 days prior to culture date [a]	Logistic model results [b]
Amikacin	29 susceptible 42 not susceptible	Amikacin – No instances of exposure	Not applicable
		Tobramycin – 27 isolates with prior exposure; 25 isolates not susceptible	OR=19.9 (95% CI 3.5, 111.9; p=0.001)
Azithromycin	13 susceptible 58 not susceptible	Clarithromycin – No instances of exposure	Not applicable
		Azithromycin – 8 isolates (from 5 subjects) with prior exposure; all 8 isolates not susceptible	Unable to fit
		Azithromycin or Clindamycin – 11 isolates (from 6 subjects) with prior exposure; all 11 isolates not susceptible	Unable to fit
Clindamycin	49 susceptible 22 not susceptible	Clindamycin – 3 isolates (from 1 subject) with prior exposure; all 3 isolates not susceptible	Unable to fit
		Azithromycin or Clindamycin – 11 isolates with prior exposure; 7 isolates were not susceptible	OR=5.2 (95% CI 0.8, 34.0; p=0.08)
Levofloxacin	38 susceptible 33 not susceptible	Quinolones (ciprofloxacin, levofloxacin, or moxifloxacin)– 8 isolates with prior exposure; 7 isolates susceptible	OR=0.14 (95% CI 0.02, 1.1; p=0.06)
Rifampin	41 susceptible 30 not susceptible	Rifampin – 12 isolates (from 4 subjects) with prior exposure; all 12 isolates not susceptible	Unable to fit
Tobramycin	28 susceptible 43 not susceptible	Tobramycin – 27 isolates with prior exposure; 25 isolates not susceptible	OR=18.1 (95% CI 3.2, 100.9; p=0.001)
Trimethoprim/ Sulfamethoxazole	4 susceptible 67 not susceptible	Trimethoprim/Sulfamethoxazole (SXT) – 50 isolates with prior exposure; 48 isolates not susceptible	OR=2.5 (95% CI 0.3, 20.6; p=0.39)

[a] Previous antibiotic exposure was determined by evaluating antibiotic start and end dates in relation to the culture date. Antibiotic exposure was defined as present if the antibiotic was used within the 90 days prior to the culture date. [b] Logistic regression models accounted for multiple isolates per subject and used robust variance estimates. Logistic models were unable to be fit when presence of prior antibiotic exposure perfectly predicted isolate susceptibility category.

therapeutic options for infections, the growth characteristics of SCVs (29) render them incompatible with recommended AST methods. Several studies have reported susceptibilities for a limited number of SCVs using a variety of different *in vitro* methods (Table 1), but as shown in this study, different media (including the same medium from different manufacturers) can vary substantially in their abilities to support SCV growth and minimize genotypic reversion. Here, we show that two media, BD-BHI and sup-MHA (commercially, THM-MHA), allowed for reproducible AST determination within 24h while minimizing reversion. Additionally our results indicate that BD-MHA+blood may also be useful in performing AST on SCVs, but more rigorous testing is warranted. In either case, the resulting *in vitro* susceptibilities could not be reliably predicted from either clinical or biochemical characteristics, or from the susceptibilities of genetically-related NC isolates from the same patient. BD-MHA+blood yielded reproducible AST results within 24 hours for some of the antibiotics tested, and we also observed low rates of reversion to NC growth on this medium.

As expected, all thymidine-dependent SCVs were resistant to trimethoprim-sulfamethoxazole on all media tested, and hemin- and menadione-dependent SCVs were non-susceptible to the aminoglycosides (at least on BD-BHI and BD-MHA+blood under microaerobic conditions). Similarly, MRSA isolates were generally resistant to □□lactams. Therefore, our results indicate that some specific elements of AST may be reliably predicted from either SCV auxotrophy type or MRSA status. However, discrepancies existed even for these simple comparisons (discussed in detail below). The SCV antibiograms were often similar to those of their genetically-related NC counterparts, but not always (Table 9), indicating that NC isolates, when available, may be used as a rough guide for SCV treatment at best. Our results also indicate that SCVs from the same patients can exhibit diverse susceptibilities (Table 10). Similar diversity within CF patients has been identified for *P. aeruginosa* (30, 31).

While azithromycin resistance was common among all SCVs, independent of auxotrophy type, prior work found no association between long-term azithromycin usage and SCV detection (32), indicating SCVs were not likely selected by azithromycin. Rather, these results probably reflect the common use of macrolides in the CF population (33). Similarly, SCV-MRSA were more often multi-drug resistant than were SCV-MSSA, likely reflecting higher intensities of antibiotic exposure among CF patients with MRSA (34, 35). Many of these SCV-MRSA were related to the USA100 PFGE type, a group previously regarded as causing health-care associated infections and exhibiting multi-drug resistance (36).

The identification of MRSA is an important function of the hospital clinical laboratory, as these isolates are difficult to treat, have infection control implications, and may be associated with worse patient outcomes (35). Broth microdilution using oxacillin or disk diffusion with cefoxitin are recommended assays for the primary detection of MRSA. The Pbp2a latex agglutination test and *mecA* PCR, which we used as primary tests, generally serve as confirmatory tests when susceptibility testing is possible. However, Kipp *et al.* showed that Pbp2a agglutination assays can be problematic when insufficient numbers of cells are used, as is often the case for SCVs grown *in vitro* (37). Disk diffusion on BD-BHA and sup-BHI, both of which supported SCV growth, correctly identified 33/34 thymidine- and hemin-dependent SCV-MRSA using cefoxitin disks. Additional false negatives were encountered when oxacillin disks were used for screening. A single thymidine-dependent SCV-MRSA isolate was susceptible to both cefoxitin and oxacillin on

BD-BHI and sup-MHA, yet remained susceptible to both drugs when tested on a third medium, BAP. *mecA*-positive, oxacillin-susceptible MRSA has previously been reported and shown to be uncommon (38), which may explain this isolate. During the preparation of this manuscript, another study by Horiochu *et al.* reported the detection of thymidine-dependent SCV-MRSA through supplementation of the susceptibility testing or agar screening media with 10 µg/mL of thymidine (39). Our study supports this approach (albeit at a lower thymidine concentration of 5 µg/mL) and extends it to other auxotrophic types through the addition of menadione and hemin. Additionally, disk diffusion for a very small subset of isolates carried out on BD-MHA+Blood correctly identified 3/3 thymidine and hemin dependent SCV-MRSA confirmed by *mecA* PCR. Work published by Miller *et. al.* following publication of our work showed that MHA+blood agar was able to correctly identify 27/27 SCV-MRSA isolates using cefoxitin disk testing (40); however, no other antibiotics were used in this work. Our results further show that AST with supplemented media is reproducible and useful beyond simply detecting methicillin resistance.

While SCVs generally grew well on BD-BHI and sup-MHA with minimal reversion rates, there were noted limitations for AST with each medium. The control strain, ATCC 29523, did not always exhibit susceptibilities within the CLSI acceptable range limits for certain antibiotics, such as tigecycline, and this strain was often more susceptible to specific drugs on BD-BHI than on sup-MHA. Thymidine-dependent SCVs grew on BHI agar from one manufacturer but not from two others, suggesting formula variations between manufacturers that limit its utility. Similar results was observed for MHA+blood agar, which varied among the products of 3 separate manufacturers in their capacities to support SCV growth. Therefore, MHA+blood, like BHI, is limited due to manufacturer variability. Neither MHA+blood nor BHI are prepared to the same rigorous specifications as MHA, and lot-to-lot variations may exist. The media developed in this study (sup-MHA and THM-MHA) used the CLSI-recommended base medium, MHA, supplemented with compounds required by SCVs for optimal growth. Concentrations were chosen based on the best available knowledge about their abundance in CF sputum as well as their empirically-determined abilities to support CF *S. aureus* SCV growth and phenotypic stability. This medium eliminated the issue of manufacturer-to-manufacturer differences in formulation. Most importantly, supplementation of MHA did not impact the susceptibilities of ATCC 29523 (disk diffusion) and ATCC 29213 (Etest), as zone diameters and MICs fell within the CLSI acceptable range limits and were comparable with values from unsupplemented MHA. Furthermore, AST results were similar with MHA and sup-MHA for the subset of NC isolates tested.

Interpreting trimethoprim-sulfamethoxazole susceptibilities under these conditions merits special comment. Zone diameters and MICs for this drug combination always required the CLSI-recommended guideline of 80% growth inhibition for interpretation. The slight growth (≤ 20%) around trimethoprim-sulfamethoxazole in these thymidine-containing media by many isolates may be attributed to uptake of thymidine (perhaps by the pyrimidine transporter NupC (41)), which would circumvent the inhibition of tetrahydrofolic acid production by this drug combination. SCVs that were resistant to trimethoprim-sulfamethoxazole grew uniformly up to the disk or Etest strip.

The only susceptibility differences exhibited between BD-BHI and sup-MHA were for the aminoglycosides among hemin- and menadione-dependent SCVs, both of which have defects in electron transport and, consequently, in aminoglycoside uptake (27). Therefore, the observed differences in aminoglycoside susceptibilities on these two media likely reflect differences in concentrations of hemin and menadione. In contrast with thymidine, the concentrations of neither hemin nor the natural menadione analog (menaquinone) have been defined for CF sputum. These two compounds may be sufficiently abundant in CF airways to support the growth of the respective SCVs. This possibility could explain the observation that thymidine-dependent SCVs are more prevalent than hemin- and menadione-dependent SCVs in CF populations among whom antibiotics known to select for the latter two types are used more often than is trimethoprim-sulfamethoxazole, which selects for thymidine-deficient SCVs (1, 38, 42).

The only susceptibility differences observed on BD-MHA+blood compared to in-house prepared sup-MHA under both aerobic and microaerobic conditions were observed for hemin– and menadione-dependent SCVs. These isolates were resistant to tobramycin under microaerobic conditions but susceptible under aerobic conditions on BD-MHA+blood. Additionally, a hemin dependent SCV was susceptible to trimethoprim/sulfamethoxazole on BD-MHA+blood yet resistant on sup-MHA. We have performed whole genome sequencing on this hemin-dependent isolate and identified a mutation predicted to be inactivating in the gene that encodes dihydropteroate synthetase (the enzymatic drug target for sulfamethoxazole). Although this prediction needs to be confirmed using more rigorous testing, it is likely this mutation confers resistance to trimpethoprim/sulfamethoxazole (as determined by sequence and modeling) and, therefore, susceptibility to this drug identified on BD-MHA+blood may have been false.

One could argue that stimulating the growth of SCVs towards an NC phenotype through media supplementation masks their true susceptibilities. However, SCVs reach sputum densities in CF children similar to those of NC *S. aureus* (3), and thymidine concentrations have been detected in CF sputum at similar levels to those in sup-MHA (23, 43); as above, less is known about the *in* *vivo* availability of heme and menaquinone. Thus, the actual *in vivo* growth characteristics and antibiotic susceptibilities of SCVs are not known, but they could be reflected by media supplementation.

While the AST method developed for this study was applied to CF clinical respiratory SCVs, this methodology could be used to assess *in vitro* susceptibilities of SCVs from a variety of *S. aureus* infections. While the AST method described here may or may not reflect *in vivo* antibiotic response of SCVs, our application of this method to a large collection of clinical SCV isolates identified 100% susceptibility to a number of antibiotics (specifically, linezolid, tigecycline, chloramphenicol, minocycline, quinupristin/dalfopristin, and vancomycin) that may therefore prove to be ideal empirical choices for treating SCV infections in the absence of AST. In fact, following publication, this work has informed clinical practice at Seattle Children's Hospital (SCH) CF treatment center. New guidelines for antibiotic treatment of *S. aureus* SCVs in CF patients were developed in September 2016 in effort to standardize the approach to antibiotic management of CF patients culture positive for SCV *S. aureus*. These guidelines recommend that antibiotics for which all isolates were susceptible be used as first line treatment agents for the management of SCV CF respiratory infections. Lastly, we suggest that antibiotic treatment of *S. aureus* SCV infection using the results (for testing empirical choices) and methods (for testing AST-guided choices) described here should form the basis of prospective clinical trials, preferably measuring both clinical and microbiological outcomes.

References

1. **Kahl B**, **Herrmann M**, **Everding AS**, **Koch HG**, **Becker K**, **Harms E**, **Proctor RA**, **Peters G**. 1998. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. J Infect Dis **177**:1023–1029.

2. **Proctor RA**, **van Langevelde P**, **Kristjansson M**, **Maslow JN**, **Arbeit RD**. 1995. Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. Clin Infect Dis **20**:95–102.

3. **Wolter DJ**, **Emerson JC**, **McNamara S**, **Buccat AM**, **Qin X**, **Cochrane E**, **Houston LS**,

Rogers GB, **Marsh P**, **Prehar K**, **Pope CE**, **Blackledge M**, **Déziel E**, **Bruce KD**, **Ramsey BW**, **Gibson RL**, **Burns JL**, **Hoffman LR**. 2013. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis **57**:384–391.

4. **Proctor RA**, **Eiff von C**, **Kahl BC**, **Becker K**, **McNamara P**, **Herrmann M**, **Peters G**. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol **4**:295–305.

5. **Lannergård J**, **Eiff von C**, **Sander G**, **Cordes T**, **Seggewiss J**, **Peters G**, **Proctor RA**, **Becker K**, **Hughes D**. 2008. Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother **52**:4017–4022.

6. **Eiff von C**, **Heilmann C**, **Proctor RA**, **Woltz C**, **Peters G**, **Götz F**. 1997. A site-directed *Staphylococcus aureus* hemB mutant is a small-colony variant which persists intracellularly. J Bacteriol **179**:4706–4712.

7. **Acar JF**, **Goldstein FW**, **Lagrange P**. 1978. Human infections caused by thiamine- or menadione-requiring *Staphylococcus aureus*. J Clin Microbiol **8**:142–147.

8. **Miller MH**, **Wexler MA**, **Steigbigel NH**. 1978. Single and combination antibiotic therapy of *Staphylococcus aureus* experimental endocarditis: emergence of gentamicin-resistant mutants. Antimicrob Agents Chemother **14**:336–343.

9. **Yagci S**, **Hascelik G**, **Dogru D**, **Ozcelik U**, **Sener B**. 2013. Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. Clin Microbiol Infect **19**:77–84.

10. **Sadowska B**, **Bonar A**, **Eiff von C**, **Proctor RA**, **Chmiela M**, **Rudnicka W**, **Różalska B**. 2002. Characteristics of *Staphylococcus aureus*, isolated from airways of cystic fibrosis patients, and their small colony variants. FEMS Immunol Med Microbiol **32**:191–197.

11. **Horiuchi K**, **Matsumoto T**, **Ota Y**, **Kasuga E**, **Negishi T**, **Yaguchi T**, **Sugano M**, **Honda T**. 2015. Addition of thymidine to culture media for accurate examination of thymidine-dependent small-colony variants of methicillin-resistant *Staphylococcus aureus*: A pilot study. J Microbiol Methods **110**:40–44.

12. **Gao W**, **Chua K**, **Davies JK**, **Newton HJ**, **Seemann T**, **Harrison PF**, **Holmes NE**, **Rhee H-W**, **Hong J-I**, **Hartland EL**, **Stinear TP**, **Howden BP**. 2010. Two novel point mutations in clinical Staphylococcus aureus reduce linezolid susceptibility and switch on the stringent response to promote persistent infection. PLoS Pathog **6**:e1000944.

13. **Singh R**, **Ray P**, **Das A**, **Sharma M**. 2010. Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic *Staphylococcus aureus* small-colony variant. J Med Microbiol **59**:521–527.

14. **Chuard C**, **Vaudaux PE**, **Proctor RA**, **Lew DP**. 1997. Decreased susceptibility to

antibiotic killing of a stable small colony variant of *Staphylococcus aureus* in fluid phase and on fibronectin-coated surfaces. J Antimicrob Chemother **39**:603–608.

15. **Hoffman LR**, **Déziel E**, **D'Argenio DA**, **Lépine F**, **Emerson J**, **McNamara S**, **Gibson RL**, **Ramsey BW**, **Miller SI**. 2006. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of Pseudomonas aeruginosa. Proc Natl Acad Sci USA **103**:19890–19895.

16. **Diep BA**, **Gill SR**, **Chang RF**, **Phan TH**, **Chen JH**, **Davidson MG**, **Lin F**, **Lin J**, **Carleton HA**, **Mongodin EF**, **Sensabaugh GF**, **Perdreau-Remington F**. 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet **367**:731–739.

17. **Palmer KL**, **Aye LM**, **Whiteley M**. 2007. Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum. J Bacteriol **189**:8079–8087.

18. **Clinical and Laboratory Standards Institute**. 2013. Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement;[... provides updated tables for... M02-A11, M07-A9, ….

19. **Matuschek E**, **Brown DFJ**, **Kahlmeter G**. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect **20**:O255–66.

20. **Mantion B**, **Cavalié L**, **Prère M-F**. 2015. Evaluation of an immunochromatographic assay for detection of PBP2a on non-*Staphylococcus aureus* clinical isolates. J Microbiol Methods **112**:46–48.

21. **Adaleti R**, **Nakipoglu Y**, **Karahan ZC**, **Tasdemir C**, **Kaya F**. 2008. Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant *Staphylococcus aureus*. J Infect Dev Ctries **2**:46–50.

22. **McDougal LK**, **Steward CD**, **Killgore GE**, **Chaitram JM**, **McAllister SK**, **Tenover FC**. 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol **41**:5113–5120.

23. **Besier S**, **Ludwig A**, **Ohlsen K**, **Brade V**, **Wichelhaus TA**. 2007. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. Int J Med Microbiol **297**:217–225.

24. **Zander J**, **Besier S**, **Saum SH**, **Dehghani F**, **Loitsch S**, **Brade V**, **Wichelhaus TA**. 2008. Influence of dTMP on the phenotypic appearance and intracellular persistence of *Staphylococcus aureus*. Infect Immun **76**:1333–1339.

25. **Osawa K**, **Kataoka N**, **Maruo T**. 2011. [Comparison of the quick Gram stain method to the B&M modified and favor methods]. Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi **22**:23–25.

26. **Worlitzsch D**, **Tarran R**, **Ulrich M**, **Schwab U**, **Cekici A**. 2002. Reduced oxygen concentrations in airway mucus contribute to the early and late pathogenesis of Pseudomonas aeruginosa cystic fibrosis airway infection. J Clin Invest.

27. **Proctor RA**, **Kriegeskorte A**, **Kahl BC**, **Becker K**, **Löffler B**, **Peters G**. 2014. *Staphylococcus aureus* Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol **4**:99.

28. **Kahl BC**. 2014. Small colony variants (SCVs) of *Staphylococcus aureus*--a bacterial survival strategy. Infect Genet Evol **21**:515–522.

29. **Tuchscherr L**, **Medina E**, **Hussain M**, **Völker W**, **Heitmann V**, **Niemann S**, **Holzinger D**, **Roth J**, **Proctor RA**, **Becker K**, **Peters G**, **Löffler B**. 2011. *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med **3**:129–141.

30. **Foweraker JE**, **Laughton CR**, **Brown DFJ**, **Bilton D**. 2005. Phenotypic variability of Pseudomonas aeruginosa in sputa from patients with acute infective exacerbation of cystic fibrosis and its impact on the validity of antimicrobial susceptibility testing. J Antimicrob Chemother **55**:921–927.

31. **Mayer-Hamblett N**, **Rosenfeld M**, **Gibson RL**, **Ramsey BW**, **Kulasekara HD**, **Retsch-Bogart GZ**, **Morgan W**, **Wolter DJ**, **Pope CE**, **Houston LS**, **Kulasekara BR**, **Khan U**, **Burns JL**, **Miller SI**, **Hoffman LR**. 2014. Pseudomonas aeruginosa In Vitro Phenotypes Distinguish Cystic Fibrosis Infection Stages and Outcomes. American Journal of Respiratory and Critical Care Medicine 140617081504001.

32. **Green N**, **Burns JL**, **Mayer-Hamblett N**, **Kloster M**, **Lands LC**, **Anstead M**, **Ratjen F**, **Saiman L**. 2011. Lack of association of small-colony-variant *Staphylococcus aureus* strains with long-term use of azithromycin in patients with cystic fibrosis. J Clin Microbiol **49**:2772–2773.

33. **Prunier A-L**, **Malbruny B**, **Laurans M**, **Brouard J**, **Duhamel J-F**, **Leclercq R**. 2003. High Rate of Macrolide Resistance in *Staphylococcus aureus* Strains from Patients with Cystic Fibrosis Reveals High Proportions of Hypermutable Strains. J Infect Dis **187**:1709–1716.

34. **Ren CL**, **Morgan WJ**, **Konstan MW**, **Schechter MS**, **Wagener JS**, **Fisher KA**, **Regelmann WE**. 2007. Presence of methicillin resistant *Staphylococcus aureus* in respiratory cultures from cystic fibrosis patients is associated with lower lung function. Pediatric Pulmonology **42**:513–518.

35. **Sawicki GS**, **Rasouliyan L**, **Ren CL**. 2012. The Impact of MRSA on Lung Function in Patients with Cystic Fibrosis. American Journal of Respiratory and Critical Care Medicine **179**:734–735.

36. **Roberts JC**. 2013. Community-associated methicillin-resistant *staphylococcus aureus* epidemic clone USA100; more than a nosocomial pathogen. Springerplus **2**:133.

37. **Kipp F**, **Becker K**, **Peters G**, **Eiff von C**. 2004. Evaluation of different methods to detect methicillin resistance in small-colony variants of *Staphylococcus aureus*. J Clin Microbiol **42**:1277–1279.

38. **Kumar VA**, **Steffy K**, **Chatterjee M**, **Sugumar M**, **Dinesh KR**, **Manoharan A**, **Karim S**, **Biswas R**. 2013. Detection of oxacillin-susceptible mecA-positive *Staphylococcus aureus* isolates by use of chromogenic medium MRSA ID. J Clin Microbiol **51**:318–319.

39. **Horiuchi K**, **Matsumoto T**, **Ota Y**, **Kasuga E**, **Negishi T**, **Yaguchi T**, **Sugano M**, **Honda T**. 2015. Addition of thymidine to culture media for accurate examination of thymidine-dependent small-colony variants of methicillin-resistant *Staphylococcus aureus*: A pilot study. J Microbiol Methods **110**:40–44.

40. **Miller SA**, **Karichu J**, **Kohner P**, **Cole N**, **Hindler JA**, **Patel R**, **Richter S**, **Humphries RM**. 2017. Multicenter Evaluation of a Modified Cefoxitin Disk Diffusion Method and PBP2a Testing To Predict mecA-Mediated Oxacillin Resistance in Atypical *Staphylococcus aureus*. J Clin Microbiol **55**:485–494.

41. **Kriegeskorte A**, **Block D**, **Drescher M**, **Windmüller N**, **Mellmann A**, **Baum C**, **Neumann C**, **Lorè NI**, **Bragonzi A**, **Liebau E**, **Hertel P**, **Seggewiss J**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2014. Inactivation of thyA in *Staphylococcus aureus* attenuates virulence and has a strong impact on metabolism and virulence gene expression. MBio **5**:e01447–14.

42. **Kahl BC**, **Belling G**, **Reichelt R**, **Herrmann M**, **Proctor RA**, **Peters G**. 2003. Thymidine-dependent small-colony variants of *Staphylococcus aureus* exhibit gross morphological and ultrastructural changes consistent with impaired cell separation. J Clin Microbiol **41**:410–413.

43. **Zander J**, **Besier S**, **Faetke S**, **Saum SH**, **Müller V**, **Wichelhaus TA**. 2010. Antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2'-deoxyuridine and rifampicin against *Staphylococcus aureus*. Int J Antimicrob Agents **36**:562–565.

Chapter 3:

Thymidine-dependent *Staphylococcus aureus* Small Colony Variants Produce Excessive cyclic-di-AMP that Modulates STING-dependent Type I Interferon Production

Note: The work in this chapter has been performed by Qing Tang, Mimi Precit, Fariha Ahmed-Qadri, Adelle McFarland, Lucas Hoffman, and Joshua Woodward and is being prepared as a manuscript for publication.

Introduction

Thymidine-dependent SCVs (THYD-SCVs) are the most common SCV type isolated from CF lung secretions and their recovery has been independently associated with more severe respiratory outcomes (1).

Thymidine dependence in SCVs has been attributed to inactivating mutations in the thymidylate synthase-encoding gene *thyA*, leading to a lack of endogenous dTMP (thymidylate) production and subsequent dependence on exogenous thymidine sources (2, 3). Thymidylate production requires folate, and THYD-SCVs are highly resistant to folate pathway inhibitors such as trimethoprim-sulfamethoxazole (4, 5) (TMP-SMX). Accordingly, previous work has shown that THYD-SCVs with inactivating mutations in *thyA* emerge following TMP-SMX exposure both *in vitro* and *in vivo* (6). SCV infections often persist for years, and treatment of chronic *S. aureus* infections of the CF lung presents a serious challenge for patients and clinicians. Despite their clinical and therapeutic implications, little is known either about why THYD-SCVs emerge so frequently, or why they are associated with worse outcomes.

Bacteria, especially bacterial pathogens, depend on signal molecules to modulate their metabolism pathways in response to changing environments. Cyclic-di-AMP (c-di-AMP) is a recently discovered bacterial secondary messenger involved in the regulation of bacterial physiological processes and the interaction between pathogen and host (7). Broadly among bacteria, c-di-AMP is required for cell wall homeostasis, fatty acid metabolism, antibiotic and osmotic stress susceptibility, virulence, as well as the establishment of infection (8-11). Specifically relating to *S. aureus*, c-di-AMP is central to bacterial growth, cell size, osmolyte import, envelope stress, antibiotic resistance and innate immune recognition (12-15). Therefore, c-di-AMP is known to regulate many of the features associated with *S. aureus* SCVs, including bacterial metabolism, antibiotic susceptibility, and host response.

C-di-AMP from *Listeria monocytogenes, Chlamydia trachomatis, Mycobacterium tuberculosis, Group B Streptococcus* and *S. aureus* can be directly detected by the host innate immune system though receptor STING (stimulator of interferon genes) (12, 16-19). STING is localized in the endoplasmic reticulum and can bind cyclic dinucleotides to induce the production of Type I interferons and inflammatory cytokines. Canonical cyclic dinucleotides, including c-di-AMP, c-di-GMP and 3'3'-cGAMP, directly bind and activate STING to induce transcription of Type I interferons (20). Cytosolic DNA derived either from host or pathogens can be directly sensed by cGAS, which synthesizes the non-canonical second messenger 2'3'-cGAMP and subsequently binds to STING to initiate downstream signaling (20). C-di-AMP can activate interferon production in immune cells including dendritic cells, monocytes, macrophages and granulocytes in mice (21). Moreover, c-di-AMP can be directly sensed by the majority of human STING alleles (22), supporting that c-di-AMP is a broadly conserved molecule detected by the immune system.

In our study, we show that THYD-SCVs elicit elevated inflammation during macrophage infection, and that both clinical and laboratory-derived THYD-SCVs produce higher levels of c-di-AMP than do wild-type (WT) *S. aureus* cells, in turn promoting inflammation during murine lung infection through the STING signaling pathway. These results indicate an important role for c-di-AMP in both the cellular behaviors of THYD-SCVs, and in the host response to their infection in the CF respiratory tract and elsewhere.

Materials and Methods

Ethics Statement. All experiments involving mice were performed in compliance with guidelines set by the American Association for Laboratory Animal Science (AALAS) and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington. The collection and characterization of all clinical isolates was approved by the Seattle Children's Hospital institutional review board.

Clinical *S. aureus* SCVs from CF secretions. Clinical *S. aureus* isolates were obtained retrospectively during a single-center study of children (n=23) with CF (1). The isolates comprised THYD-SCVs from two different patients and a preceding WT or co-isolated normal colony (NC, non-SCV) isolate from the same patient in each case. Genetic relatedness was determined previously (4) using pulse field gel electrophoresis via standard protocols (23).

***thyA* sequencing in clinical THYD-SCVs and isogenic, co-isolated NC isolates.** *thyA* was amplified by PCR using a forward upstream primer (5'-CCTACCACATCGTAACGTGA-3') and a reverse downstream primer (5'-TGTGCAACTAGAATGGATAAAGTCA-3'). Resulting amplicons were cleaned up with the ExoSap-IT PCR product Cleanup Reagent (Thermo Fisher) and sequenced by Sanger sequencing. Consensus sequences were assembled and analyzed using MacVector software, version 11.0.2

Laboratory *S. aureus* SCVs Construction. Two types of laboratory SCV strains were used in this study, THYD-SCVs with clean deletions in the *thyA* gene and a hemin-dependent SCV (HEMD-SCV) with a clean deletion in *hemB*, which encodes delta-aminolevulinic acid dehydratase, a critical enzyme in the heme biosynthetic operon. Both of these genes have been reported to lead to SCV formation (3, 24). We constructed Δ*thyA* and Δ*hemB* in *S. aureus* strain Newman by allelic exchange using pIMAY as previously described (25). Briefly, primers were designed to amplify up and downstream regions flanking the gene (s) of interest (either *thyA* or *hemB)* to delete the entire gene (Table 1). Genomic DNA from SH1000 was used as template and the resulting PCR products were denatured and allowed to reanneal via the complementary sequences in primers B and C and then amplified using primers A and D, resulting in a fragment consisting of linked sequences upstream and downstream of either *thyA* or *hemB* (Δ*thyA* or Δ*hemB* cassette). The amplimer was cloned into pIMAY between KpnI and NotI restriction sites. The plasmid vector was transformed via electroporation into *E. coli* DH10B (25) and then transformed into electrocompetent *S. aureus* strain RN4220(25). Transduction of *S. aureus* strain Newman was accomplished by first propagating phage ϕ-11 in RN4220 that had been successfully transformed with pIMAY_deletion cassette (Δ*thyA* or Δ*hemB*). ϕ-11 phage will package the pIMAY vector and via subsequent generalized phage transduction the pIMAY_deletion cassette vector was transduced into stain Newman. Deletion of either Δ*thyA* or Δ*hemB* was achieved by allelic exchange. The resulting SCV strains were tested for auxotrophy for either thymidine or hemin; both showed attenuated growth *in vitro* compared with the WT Newman strain in the absence of either thymidine or hemin (Figure 1).

Table 1. Primer sequences 5'-3' for deletion constructs in *S. aureus*

thyA[a] SOE FwdA	ATATGGTACCGAAGCAGTATCGGAGTATATG	underline is KpnI restriction site
thyA SOE RevB	CTATGCAATGACTACATATGCGATAACACCTCATTTTC	
thyA SOE FwdC	GAGGTGTTATCGCATATGTAGTCATTGCATAGTTAGCTAAC	
thyA SOE RevD	ATATGCGGCCGCTATGGCTGGCTGACTTGTC	underline is NotI restriction site
thyA Fwd 1	GTATGGAAACAAATGGCAAACAGAAC	Used to verify deletion construct
thyA Rev 1	GTACCACTTAATCCTGAAGAAAGATG	Used to verify deletion construct
hemB[b] SOE FwdA	ATATGGTACCGAAGCCAGTTGTAGTTATGAC	underline is KpnI restriction site
hemB SOE RevB	CATAAATATAAAACCTTACATTTTTAGCCCCTACTTTC	
hemB SOE FwdC	GTAGGGGCTAAAAATGTAAGGTTTTATATTTATGATTTTCC	
hemB SOE RevD	ATATGCGGCCGCCATATTACCAGCAACAGGTTCTAC	underline is NotI restriction site
hemB Fwd 1	CAGAAATGGATGGTAGTTGTCAG	Used to verify deletion construct
hemB Rev 1	GCACAATGATAACCGACTCTG	Used to verify deletion construct

[a] *thyA* refers to the gene encoding thymidylate synthase in *S. aureus*

[b] *hemB* refers to gene encoding delta-aminolevulinic acid dehydratase, a critical enzyme in the heme biosynthetic operon

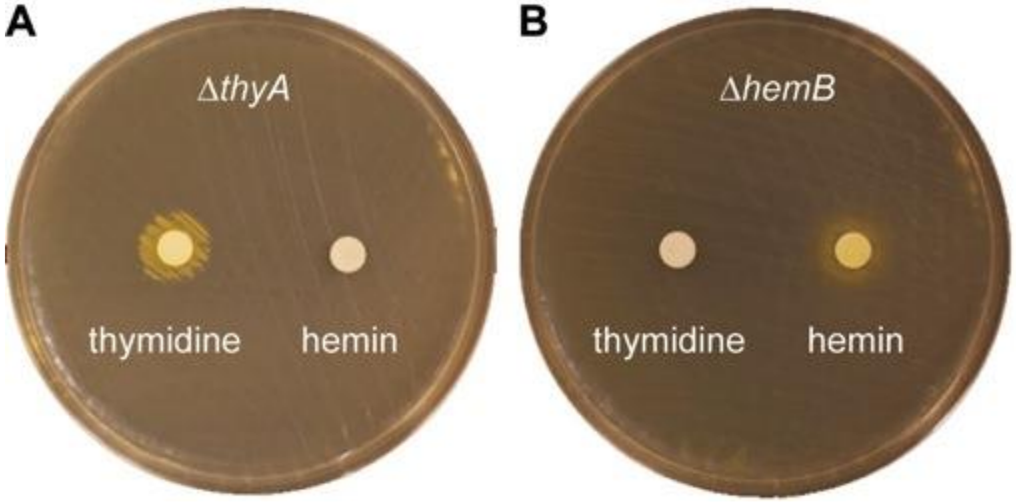


Figure 1. Auxotrophy test for thymidine and hemin dependence in the deletion strains. (A) a lawn of Newman Δ*thyA* plated on Mueller-Hinton agar with enhanced growth around the paper disk impregnated with 5 µg thymidine. (B) A lawn of Newman Δ*hemB* on the same medium with enhanced growth around the paper disk impregnated with 1µg hemin.

Laboratory growth conditions for bacterial strains. *S. aureus* strains were cultured from freezer stocks (TSA with 15% glycerol) and kept at -80° C and grown in brain heart infusion (BHI) broth supplemented with thymidine or hemin at the indicated concentration.

Mice used in this study. Wild-type mice were purchased from Jackson Laboratories. Sting$^{-/-}$ and cGAS$^{-/-}$ mice were generously provided by Daniel Stetson (26).

Cell lines, cultures, and infections. Primary bone marrow derived macrophages (BMDMs) from WT, cGAS$^{-/-}$, and STING$^{-/-}$ mice were generated as described previously (26). Cells were grown in BMM medium which was Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM sodium glutamine, 1 mM sodium pyruvate, 10% heat inactivates FBS, 0.1% 2-Mercaptoethanol and 10% CSF. 1×10^6 cells were plated in 6-well tissue culture plates and incubated at 37 °C overnight. Overnight bacterial cultures were washed twice with sterile phosphate-buffered saline (PBS) and then re-suspended in BMM medium. Macrophages were infected by bacteria slurry optimized for multiplicity of infection. After 1h incubation, the supernatants were removed, washed twice by cold PBS, and replaced by fresh BMM medium containing 200µg/ml gentamicin to kill the extracellular bacteria. At various times post infection, supernatants were collected to study macrophage cell death by lactate dehydrogenase release assays(27) and IFNβ protein expression by luciferase assays (18). Macrophages were collected by incubating in 0.05% Trypsin-EDTA for 5 min. Bacteria were harvested by incubating in 0.1% TritonX-100 solution for 5 min to disrupt the host cells. Appropriate dilutions were plated on BHI agar plates and incubated at 37°C for the enumeration of CFU.

***In vivo* infection and bacterial burden estimation.** 6-8 week old mice were used in infection experiments. The homozygosity of the littermates was confirmed by PCR. Prior to animal experiments, Δ *thyA* and Δ*hemB* strains were grown for 3 h at 37°C with 5 µg/mL of thymidine and 1 µg/ml hemin in BHI broth, respectively, to reach mid-exponential growth. Subsequently, the bacteria were pelleted by centrifugation (2,700×g, 5 min) and washed twice with sterile PBS. Cultures were diluted in PBS and used to infect WT and STING$^{-/-}$ mice via the intranasal route with a final inoculum of $5×10^8$ bacteria. At 24 hours post infection, mice were euthanized and lungs collected. Bacterial burdens were enumerated by plating lung homogenates on BHI plates containing thymidine or hemin to ensure the normal growth of SCVs and then incubated overnight at 37^o C.

Microarray experiments and relative quantitative real-time PCR. Left lungs from mice were sliced into pieces no wider than 0.5 cm and dropped into RNAlater (Thermo Fisher Scientific) for RNA stabilization. Then the samples were removed from RNAlater and homogenized by Bead Mill homogenizer (Fisher Scientific) in RLT buffer (QIAGEN) containing 1% β-mercaptoethanol. RNA from the lung homogenate and macrophages was isolated using RNeasy Mini kit (QIAGEN) and RNAqueous Total RNA Isolation Kit (Thermo Fisher Scientific), respectively. RNA was treated by DNase (Thermo Fisher Scientific), and followed by cDNA synthesis using iScript cDNA synthesis Ktis (Bio-Rad). RT-qPCR was performed using Taqman Gene expression master mix (Applied Biosystems). Primers/probes for mouse *Hprt*, *Ifnb1, Ccl5, Cxcl10* and *Il6* were purchased from Integrated DNA Technologies. mRNA fold changes were normalized to mouse *hprt* using $2^{(-\Delta\Delta Ct)}$ method. All RT-qPCR data are plotted as the mean ± SEM of technical duplicates or triplicates. Immune gene expression profiling was performed using TaqMan Array

Micro Fluidic Cards (Applied Biosystems) containing probe sets for 90 immune genes and 6 endogenous control.

Quantification of intracellular c-di-AMP from bacteria and infected macrophages by LC-MS/MS. Overnight culture of WT, Δ*thyA* and Δ*hemB* strains grown in BHI supplemented with 5 µg/mL of thymidine were washed twice with PBS, then diluted into fresh BHI containing various concentrations of thymidine (1.25, 2.5 and 5 µg/mL) with the initial OD_{600} of 0.05, and grown at 37°C with shaking for 4 h. The bacteria were collected by centrifugation (2,700 × g, 5 min) and used for c-di-AMP extraction or macrophage infection. Macrophages infected with bacteria were collected by incubating in 0.05% Trypsin-EDTA for 5 min. Both macrophages and bacteria were re-suspended in methanol and lysed by sonication. Heavy-labeled c-di-AMP was added to the extract as the internal control. Bacteria lysis was centrifuged to collect the supernatants. The quantification of c-di-AMP by LC-MS was performed as previously described (28).

Statistical Analysis. All data were analyzed using an unpaired Mann-Whitney U test by GraphPad Prism 6 software.

Results

THYD-SCVs carried inactivating mutations in *thyA*. Targeted gene sequencing identified inactivating mutations in *thyA*, the gene for thymidylate synthase, in clinical THYD-SCV isolates from 2 patients. These mutations were absent in the co-isolated NC paired isolates (Figure 2).

A: Patient 1 Multiple sequence alignment of thymidylate synthase

```
WT        1 ATGTTGAATTCATTTGATGCAGCATATCACAGTCTTTGTGAAGAAGTTTTAGAAATAGGA  60
THYD      1 ATGTTGAATTCATTTGATGCAGCATATCACAGTCTTTGTGAAGAAGTTTTAGAAATAGGA  60
```

```
            ************************************************************

WT       61 AATACACGAAATGATCGCACAAATACAGGTACGATTTCGAAATTTGGTCATCAACTTCGC 120
THYD     61 AATACACGAAATGATCGCACAAATACAGGTACGATTTCGAAATTTGGTCATCAACTTCGC 120
            ************************************************************

WT      121 TTTGACTTATCTAAAGGATTTCCACTATTAACGACAAAGAAAGTTTCTTTTAAATTAGTA 180
THYD    121 TTTGACTTATCTAAAGGATTTCCACTATTAACGACAAAGAAAGTTTCTTTTAAATTAGTA 180
            ************************************************************

WT      181 GCAACCGAATTATTATGGTTCATTAAAGGAGATACAAACATCCAATACTTATTAAAATAT 240
THYD    181 GCAACCGAATTATTATGGTTCATTAAAGGAGATACAAACATCCAATACTTATTAAAATAT 240
            ************************************************************

WT      241 AATAATAATATATGGAACGAATGGGCTTTTGAGAATTATATCAAATCAGACGAGTATAAA 300
THYD    241 AATAATAATATATGGAACGAATGGGCTTTTGAGAATTATATCAAATCAGACGAGTATAAA 300
            ************************************************************

WT      301 GGTCCAGATATGACAGATTTCGGGCATCGTGCATTGAGTGATCCTGAATTTAACGAACAA 360
THYD    301 GGTCCAGATATGACAGATTTCGGGCATCGTGCATTGAGTGATCCTGAATTTAACGAACAA 360
            ************************************************************

WT      361 TATAAAGAACAAATGAAACAATTTAAGCAACGTATTCTTGAAGATGATACATTTGCGAAG 420
THYD    361 TATAAAGAACAAATGAAACAATTTAAGCAACGTATTCTTGAAGATGATACATTTGCGAAG 420
            ************************************************************

WT      421 CAATTCGGGGATTTAGGAAATGTTTATGGTAAACAATGGCGAGATTGGGTTGATAAAGAT 480
THYD    421 CAATTCGGGGATTTAGGAAATGTTTATGGTAAACAATGGCGAGATTGGGTTGATAAAGAT 480
            ************************************************************

WT      481 GGTAATCATTTTGATCAACTTAAAACAGTAATTGAACAAATTAAGCATAATCCAGATTCA 540
THYD    481 GGTAATCATTTTGATCAACTTAAAACAGTAATTGAACAAATTAAGCATAATCCAGATTCA 540
            ************************************************************

WT      541 AGGCGACACATCGTATCTGCATGGAATCCAACAGAAATTGATACAATGGCACTTCCGCCT 600
THYD    541 AGGCGACACATCGTATCTGCATGGAATCCAACAGAAATTGATACAATGTCATACCATGTT 600
            ************************************************ **   *    *

WT      601 TGTCATACCATGTTCCAGTTTTATGTCCAAGATGGTAAGTTAAGTTGCCAGTTATACCAA 660
THYD    601 CCAGTTTTATGTCCAAGATGGTAAGTTAAGTTGCCAGTTATACCAACGTAGCGCAGATAT 660
                 *            *  ** **  *           **       **       *

WT      661 CGTAGCGCAGATATCTTTTTAGGTGTGCCATTTAATATCGCAAGCTACGCTTTATTGACA 720
THYD    661 CTTTTTAGGTGTGCCATTTAATATCGCAAGCTACGCTTTATTGACACACCTTATTGCCAA 720
            * *        *  * *** *  *       *     *      *    ***  *    *

WT      721 CACCTTATTGCCAAAGAATGTGGACTTGAAGTGGGTGAATTTGTGCATACATTTGGAGAT 780
THYD    721 AGAATGTGGACTTGAAGTGGGTGAATTTGTGCATACATTTGGAGATGCACATATTTATTC 780
                *     *   *    *  ** **   *        *        **** *  *

WT      781 GCACATATTTATTCAAATCATATTGATGCGATTCAAACACAATTAGCACGTGAAAGCTTC 840
THYD    781 AAATCATATTGATGCGATTCAAACACAATTAGCACGTGAAAGCTTCAATCCTCCAACATT 840
              *     **  *   **   *        *       * *  *   *      * * *

WT      841 AATCCTCCAACATTAAAAATTAACAGTGACAAGTCTATTTTCGACATAAATTATGAAGAT 900
THYD    841 AAAAATTAACAGTGACAAGTCTATTTTCGACATAAATTATGAAGATTTGGAAATTGTTGA 900
            **   *  *   * * ** *  *   *    *     * *      *     **

WT      901 TTGGAAATTGTTGACTATGAATCACATCCAGCAATAAAAGCTCCAATAGCAGTGTAG 957
THYD    901 CTATGAATCACATCCAGCAATAAAAGCTCCAATAGCAGTGTAG-------------- 943
             *   ***      *    *   *    *    *  *  *
```

B: Patient 2 Multiple sequence alignment of thymidylate synthase

```
WT         1 ATGTTGAATTCATTTGATGCAGCATATCACAGTCTTTGTGAAGAGGTTTTAGAAATAGGA  60
```

```
THYD       1 ATGTTGAATTCATTTGATGCAGCATATCACAGTCTTTGTGAAGAGGTTTTAGAAATAGGA  60
             ************************************************************

WT        61 AATACACGAAATGATCGCACAAATACAGGTACGATTTCGAAATTTGTTCATCAACTTCGC 120
THYD      61 AATACACGAAATGATCGCACAAATACAGGTACGATTTCGAAATTTGGTCATCAACTTCGC 120
             ********************************************** *************

WT       121 TTTGACTTATCTAAAGGATTTCCACTATTAACGACAAAGAAAGTTTCTTTTAAATTAGTA 180
THYD     121 TTTGACTTATCTAAAGGATTTCCACTATTAACGACAAAGAAAGTTTCTTTTAAATTAGTA 180
             ************************************************************

WT       181 GCAACCGAATTATTATGGTTCATTAAAGGAGATACAAACATCCAATACTTATTAAAATAT 240
THYD     181 GCAACCGAATTATTATGGTTCATTAAAGGAGATACAAACATCCAATACTTATTAAAATAT 240
             ************************************************************

WT       241 AATAATAATATTTGGAACGAATGGGCTTTTGAAAATTATATAAAATCAGACGAGTATAAC 300
THYD     241 AATAATAATATTTGGAACGAATGGGCTTTTGAAAATTATATAAAATCAGACGAGTATAAC 300
             ************************************************************

WT       301 GGTCCAGATATGACAGATTTCGGGCATCGTGCATTGAGTGATCCTGAATTTAACGAACAA 360
THYD     301 GGTCCAGATATGACAGATTTCGGGCATCGTGCATTGAGTGATCCTGAATTTAACGAACAA 360
             ************************************************************

WT       361 TATAAAGAACAAATGAAACAATTTAAGCAACGTATTCTTGAAGATGATACTTTTGCGAAG 420
THYD     361 TATAAAGAACAAATGAAACAATTTAAGTAACGTATTCTTGAAGATGATACTTTTGCGAAG 420
             *************************** ********************************

WT       421 CAATTCGGTGATTTAGGAAATGTTTATGGTAAACAATGGCGAGATTGGGTTGATAAAGAT 480
THYD     421 CAATTCGGTGATTTAGGAAATGTTTATGGTAAACAATGGCGAGATTGGGTTGATAAAGAT 480
             ************************************************************

WT       481 GGTAATCATTTTGATCAACTTAAAACAGTAATTGAACAAATTAAGCATAATCCAGATTCA 540
THYD     481 GGTAATCATTTTGATCAACTTAAAACAGTAATTGAACAAATTAAGCATAATCCAGATTCA 540
             ************************************************************

WT       541 AGACGACACATCGTATCTGCATGGAATCCAACAGAAATTGATACAATGGCACTTCCGCCT 600
THYD     541 AGACGACACATCGTATCTGCATGGAATCCAACAGAAATTGATACAATGGCACTTCCGCCT 600
             ************************************************************

WT       601 TGTCATACCATGTTCCAGTTTTATGTCCAAGATGGTAAGTTAAGTTGCCAGTTATACCAA 660
THYD     601 TGTCATACCATGTTCCAGTTTTATGTCCAAGATGGTAAGTTAAGTTGCCAGTTATACCAA 660
             ************************************************************

WT       661 CGTAGCGCAGATATCTTTTTAGGTGTGCCATTTAATATCGCAAGCTACGCTTTATTGACA 720
THYD     661 CGTAGCGCAGATATCTTTTTAGGTGTGCCATTTAATATCGCAAGCTACGCTTTATTGACA 720
             ************************************************************

WT       721 CACCTTATTGCCAAAGAATGTGGACTTGAAGTAGGAGAATTTGTGCATACATTTGGAGAT 780
THYD     721 CACCTTATTGCCAAAGAATGTGGACTTGAAGTAGGAGAATTTGTGCATACATTTGGAGAT 780
             ************************************************************

WT       781 GCACATATTTATTCAAATCATATTGATGCGATTCAAACACAATTAGCACGTGAAAGCTTC 840
THYD     781 GCACATATTTATTCAAATCATATTGATGCGATTCAAACACAATTAGCACGTGAAAGCTTC 840
             ************************************************************

WT       841 AATCCTCCAACATTAAAAATTAACAGTGACAAGTCTATTTTCGACATAAATTATGAAGAT 900
THYD     841 AATCCTCCAACATTAAAAATTAACAGTGACAAGTCTATTTTCGACATAAATTATGAAGAT 900
             ************************************************************

WT       901 TTGGAAATTGTTGACTATGAATCACATCCAGCAATAAAAGCTCCAATAGCAGTGTAG 957
THYD     901 TTGGAAATTGTTGACTATGAATCACATCCAGCAATAAAAGCTCCAATAGCAGTGTAG 957
             *********************************************************
```

Figure 2: Nucleotide sequences in thymidylate synthase (*thyA*) in thymidine dependent (THYD) isolates recovered from cystic fibrosis patients. Co-isolated normal-colony/wild-type (WT) and THYD-SCV *S. aureus* isolates showed notable coding differences in the *thyA* gene. The THYD-SCV from patient 1 (A) carried a 14 bp deletion occurred between nucleotides 587 and 588 (highlighted grey) compared to the WT co-isolated normal colony isolate. The THYD-SCV from patient 2 (B) carried two nonsynonymous mutations at nucleotides 106 and 389 compared to the WT co-isolated normal colony isolate.

Δ*thyA* induces higher inflammatory gene expression in macrophages. Genes known to be mutated in clinical SCVs thymidylate synthase (*thyA)* and delta-aminolevulinic acid dehydratase (*hemB)* (29) were targeted for deletion in the *S. aureus* Newman background. As expected, both Δ*thyA* and Δ*hemB* strains grew with small colonies on indicator media and were complemented by disks with thymidine and hemin, respectively (Figure 1). The *hemB* gene was selected as a control for *thyA* because, although strains deleted for *hemB* also exhibit slow growth rates and elevated antibiotic resistance, SCVs with this mutation have not been associated with elevated lung function decline in CF patients.

Macrophages were infected with WT, Δ*thyA*, and Δ*hemB S. aureus* Newman, and bacterial survival and the induction of host cell death were assessed. While there was a decline in recovery of colony forming units (CFU) for all strains over 6 hours of infection, this decline was most marked for the Δ*thyA* strain (Figure 3A). Similarly, all strains generated comparably low levels of macrophage toxicity, as assessed by LDH release (Figure 3B). Given the diminished growth of THYD-SCVs compared to WT *S. aureus* and Δ*hemB* SCVs in macrophages observed by us and by others (30), we hypothesized that the association with worse CF clinical outcomes with Δ*thyA* isolates is not attributable to elevated bacterial burden during infection, but rather to their pro-inflammatory capacity.

We repeated our experiments adjusting starting inocula of each strain to ensure equivalent intracellular CFU of WT, Δ*thyA*, and Δ*hemB S. aureus* Newman (Figure 4) 6 hours post-infection. Inflammatory gene transcriptional response of infected cells was determined by Taqman array. Among the 90 inflammatory genes tested, including surface receptors, signal

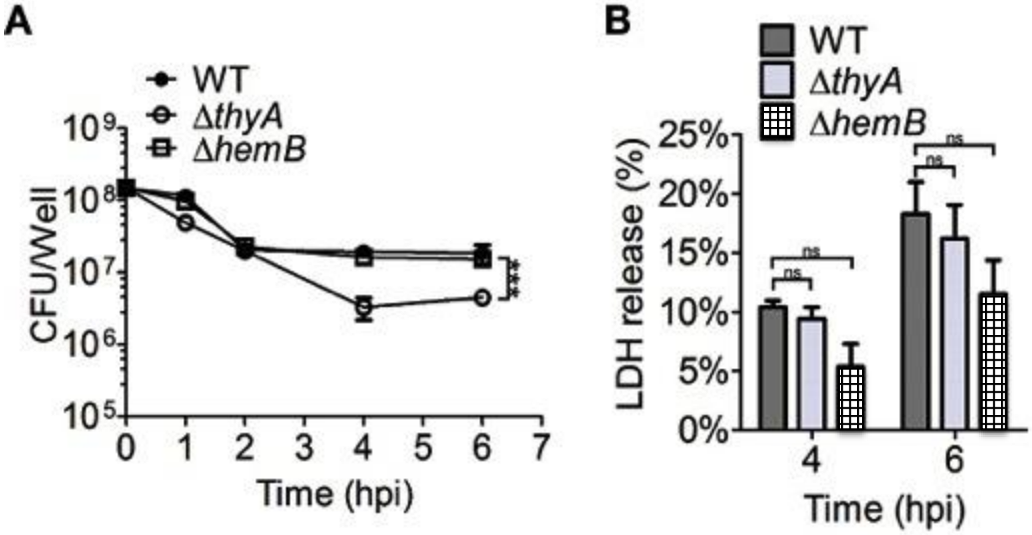


Figure 3 Δ*thyA* strain exhibited decreased survival ability in macrophages compared to *S. aureus* Newman and Δ*hemB* Newman. (A) Bacterial growth curve in iBMDM. (B) LDH release of iBMDM infected with WT, Δ*thyA*, and Δ*hemB* *S. aureus* Newman strains at 4 and 6 hours of post infection (hpi). *P* values were calculated after log transformation of the data using Mann-Whitney U test. Asterisks indicate that differences are statistically significant (***, and $p < 0.001$).

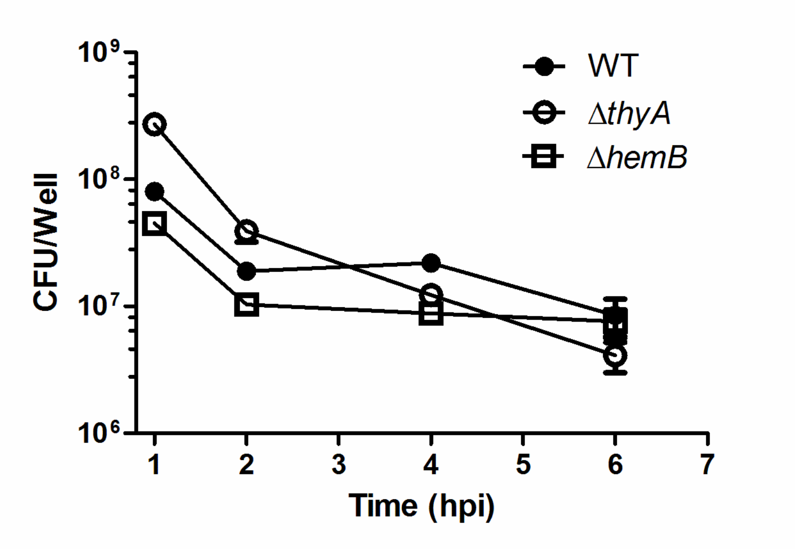


Figure 4. Optimization of multiplicity of infection (MOI). iBMDMs were infected with WT, Δ*thyA*, and Δ*hemB* at different MOI to ensure equivalent resulting intracellular CFU.

transduction proteins, cytokines, and chemokines, and cytokine/chemokine receptors, 44 were induced upon *S. aureus* infection. Among them, a number of inflammatory genes were uniquely elevated by Δ*thyA* relative to both WT and Δ*hemB* strains (Figure 5A). To confirm these results, mRNA levels of *Ccl5, Cxcl10, Cxcl11* and *Il6* were determined by RT-qPCR. In accordance with the Taqman array data, these genes exhibited significantly higher expression relative to WT and Δ*hemB* infected macrophages (Figure 5B, C, D and E). These observations suggest that the Δ*thyA* strain stimulates higher expression of specific immune response genes during infection than do other *S. aureus* strains.

Δ*thyA* activates STING-dependent type I interferon production in macrophages. Many of the genes differentially induced in Δ*thyA*-infected macrophages are IRF3-dependent inflammatory genes, including *Ccl5*, *Cxcl10*, *Cxcl11* and *Il6*. As these genes are co-regulated with IFNβ downstream of IRF3, IFNβ mRNA and protein levels in WT immortalized bone marrow-derived macrophages iBMDMs were tested by RT-qPCR and luciferase bioassay, respectively. As expected, Δ*thyA* induced higher IFNβ expression compared to WT and Δ*hemB* strains (Figure 6A and B). IRF3-dependent induction of type I interferon production in response to microbial pathogens can be triggered by activation of the cGAS-STING signaling pathway (19). 2’3’-cGAMP produced by cGAS, which is triggered by host or pathogen derived cytosolic DNA, and cyclic dinucleotides from bacteria, can activate STING, resulting in IFNβ induction. Wild type, STING$^{-/-}$ and cGAS$^{-/-}$ primary BMDMs were infected with WT, Δ*thyA* and Δ*hemB S. aureus*, and *Ifnb1* transcripts were measured by RT-qPCR relative to uninfected controls. *Ifnb1* mRNA levels were highly elevated in WT but not in STING$^{-/-}$ BMDMs upon *S. aureus* infection (Figure 5C). Moreover, Δ*thyA* induced higher *Ifnb1* transcription in all cell lines, and *Ifnb1*

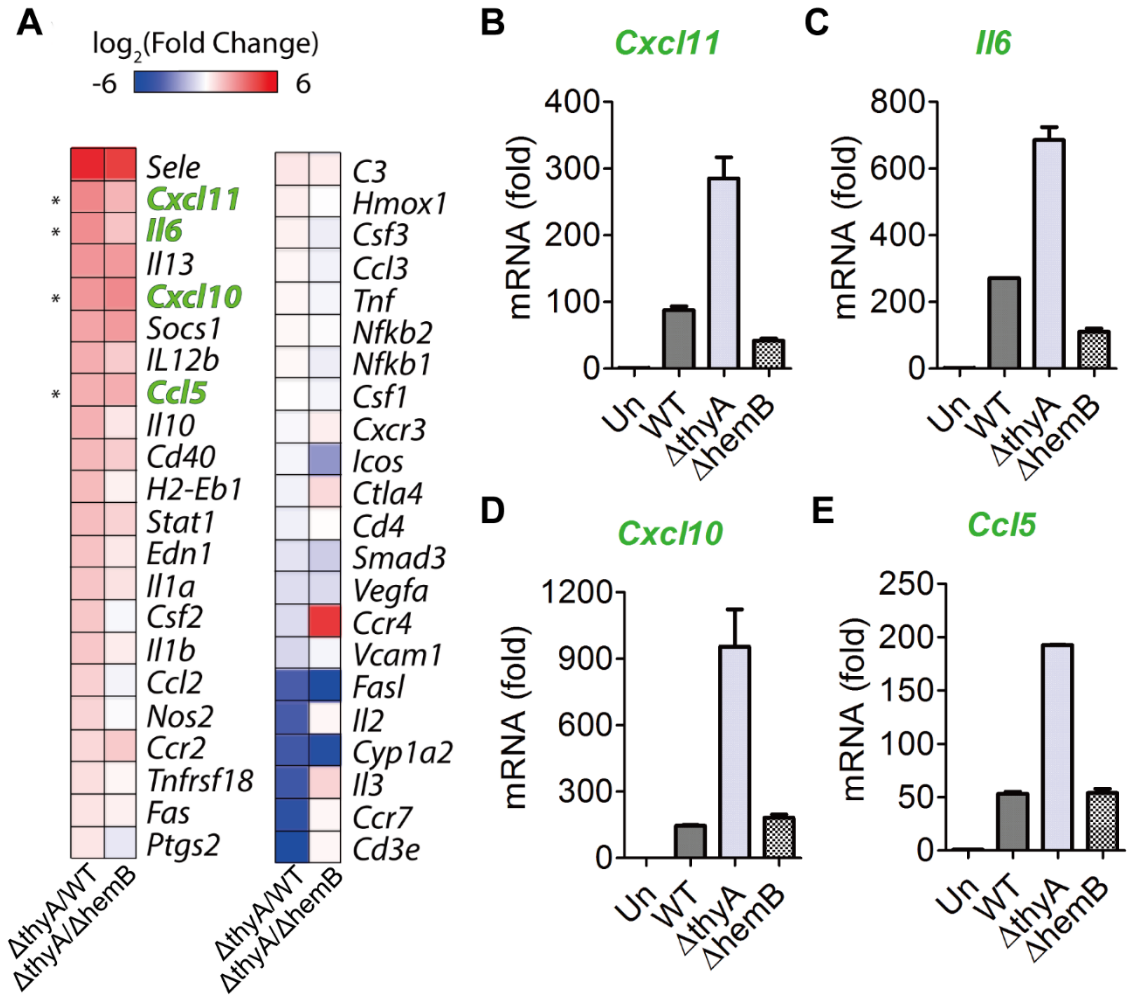


Figure 5. Δ*thyA* induces more inflammation in macrophages compared to *S. aureus* Newman and Δ*hemB* Newman. (A) Heatmap of Taqman gene expression analysis of WT iBMDMs infected with WT, Δ*thyA*, and Δ*hemB* *S. aureus* Newman strains. Values are the log$_2$ ratio of gene induction of Δ*thyA* vs WT and Δ*thyA* vs Δ*hemB*. Genes with green labels are known to be co-regulated with IFNβ downstream of STING. Heatmaps were generated using Morpheus. (B) (C) (D) and (E) Transcriptional changes of genes labeled green in panel A were confirmed by RT-qPCR in iBMDMs.

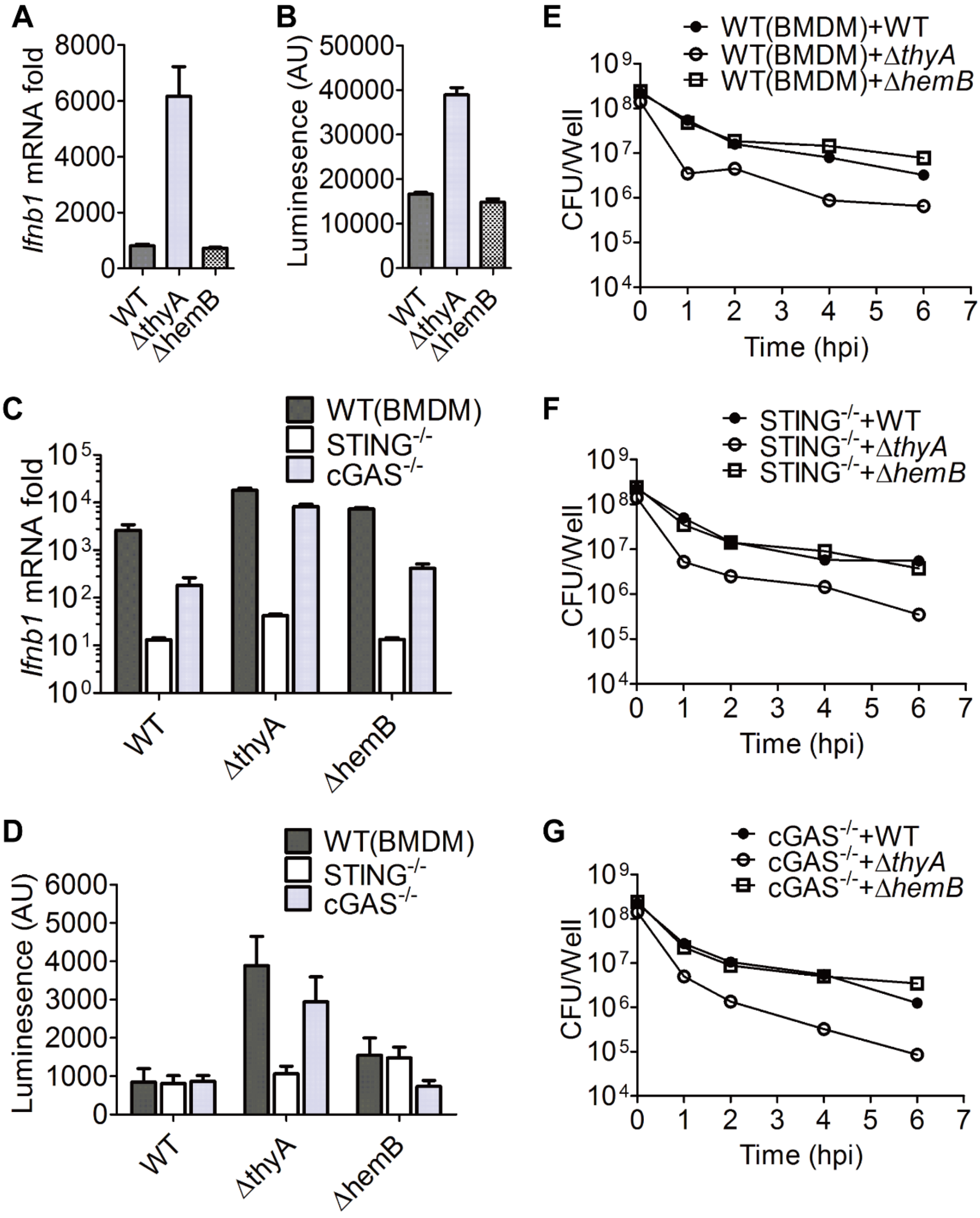

A
Ifnb1 mRNA fold
8000
6000
4000
2000
0
WT
ΔthyA
ΔhemB
B
Luminesence (AU)
50000
40000
30000
20000
10000
0
WT
ΔthyA
ΔhemB
C
WT(BMDM)
STING-/-
cGAS-/-
Ifnb1 mRNA fold
10^5
10^4
10^3
10^2
10^1
10^0
WT
ΔthyA
ΔhemB
D
WT(BMDM)
STING-/-
cGAS-/-
Luminesence (AU)
6000
5000
4000
3000
2000
1000
0
WT
ΔthyA
ΔhemB
E
WT(BMDM)+WT
WT(BMDM)+ΔthyA
WT(BMDM)+ΔhemB
CFU/Well
10^9
10^8
10^7
10^6
10^5
10^4
0 1 2 3 4 5 6 7
Time (hpi)
F
STING-/-+WT
STING-/-+ΔthyA
STING-/-+ΔhemB
CFU/Well
10^9
10^8
10^7
10^6
10^5
10^4
0 1 2 3 4 5 6 7
Time (hpi)
G
cGAS-/-+WT
cGAS-/-+ΔthyA
cGAS-/-+ΔhemB
CFU/Well
10^9
10^8
10^7
10^6
10^5
10^4
0 1 2 3 4 5 6 7
Time (hpi)

Figure 6. Δ*thyA* activates STING-dependent type I IFN response in macrophages. (A) WT iBMDMs were infected with WT, Δ*thyA* and Δ*hemB* for 4 hr, and Ifnb1 mRNA was measured by RT-qPCR relative to uninfected controls. (B) Same as (A), except IFNβ protein was measured by ISRE-luciferase bioassay with 6 hr post-infection. (C) WT, STING$^{-/-}$ and cGAS$^{-/-}$ pBMDMs were infected with WT, Δ*thyA* and Δ*hemB* for 4 hr, and induction of *Ifnb1* transcripts were measured by RT-qPCR relative to uninfected controls. (D) Same as (C), except IFNβ protein was measured by ISRE-luciferase bioassay with 6 hr post-infection. Intracellular growth curve of WT, Δ*thyA* and Δ*hemB* strains in WT (BMDM), STING$^{-/-}$ and cGAS$^{-/-}$ BMDMs. (E) (F) and (G) Intracellular growth curve of WT, Δ*thyA* and Δ*hemB* strains in WT, STING$^{-/-}$ and cGAS$^{-/-}$ BMDMs, respectively.

induction was only slightly diminished in cGAS$^{-/-}$ BMDMs. Similarly, luciferase bioassays revealed that induction of IFNβ production in response to the Δ*thyA* strain was primarily dependent upon STING instead of cGAS (Figure 6D).

We next examined the intracellular survival of WT, Δ*thyA* and Δ*hemB* strains in wild type, STING$^{-/-}$ and cGAS$^{-/-}$ primary BMDMs to determine if elevated activation of cGAS or STING contributes to *S. aureus* survival in tissue culture. WT and Δ*hemB* exhibited similar survival trends, whereas Δ*thyA* exhibited weaker persistence (Figure 6E).

Collectively, these findings indicate that the Δ*thyA* strain induces enhanced STING-dependent inflammation, which does not contribute to bacterial survival in tissue culture. Moreover, induction of Type I interferon production by the Δ*thyA* strain is STING-dependent but largely cGAS/DNA-independent.

Δ*thyA* produces more c-di-AMP to rescue its physiological function. Because of the importance of cyclic dinucleotides as bacterial activators of STING, we investigated the effect of *thyA* deletion on production of these molecules. We hypothesized that THYD-SCVs may exhibit elevated levels of cyclic dinucleotides that trigger STING dependent and cGAS independent Type I interferon during infection. Bacteria have been reported to produce three cyclic dinucleotides, including c-di-GMP, c-di-AMP, and 3’3’-cGAMP. *S. aureus* Newman is not predicted to make either 3’3’-cGAMP or c-di-GMP, as it lacks function enzymes for the synthesis of these second messengers. Therefore, we compared c-di-AMP levels between (i) Δ*thyA* and WT *S. aureus* Newman and (ii) among two sets of clonally-related *S. aureus* normal colony and SCV isolates collected during a single-center study of children with CF (33). By quantitating c-di-AMP levels in these strains, we found that both Δ*thyA S. aureus* Newman and THYD-SCV CF clinical isolates exhibited 10 to 100-fold higher levels of c-di-AMP/CFU relative to their isogenic normal-colony, comparison strains or isolates (Figure 7A).

Mutations in *thyA* disrupt dTMP synthesis from dUMP, resulting in reliance on external thymidine to maintain DNA synthesis (3). We therefore explored the effect of thymidine supplementation on c-di-AMP production by THYD-SCVs. Relative to WT, intracellular c-di-AMP levels of Δ*thyA* were significantly increased upon thymidine starvation (Figure 7C), compared with supplementation with thymidine concentrations that maximized growth (2.5 μg/ml, Figure 7B), indicating that thymidine availability regulates c-di-AMP production when *thyA* is inactivated. Next, c-di-AMP levels in macrophages infected with Δ*thyA* and WT strains were quantified. Consistent with our previous data, macrophages infected with Δ*thyA* contained more c-di-AMP relative to WT infected cells (Figure 7D). We also measured c-di-AMP levels in macrophages infected with Δ*thyA* and WT grown in a range of thymidine concentrations, and in parallel we measured *Ifnb1* mRNA levels using RT-qPCR. Macrophages infected with Δ*thyA* grown in lower concentrations of thymidine contained more c-di-AMP and a corresponding elevated interferon transcriptional response, whereas both c-di-AMP and *Ifnb1* mRNA levels in macrophages infected with WT did not vary when grown in different thymidine concentrations (Figure 7E and F). These results support the hypothesis that *thyA* inactivation leads to significantly higher c-di-AMP production in a thymidine-dependent manner, resulting in an elevated type I interferon response during macrophage infection.

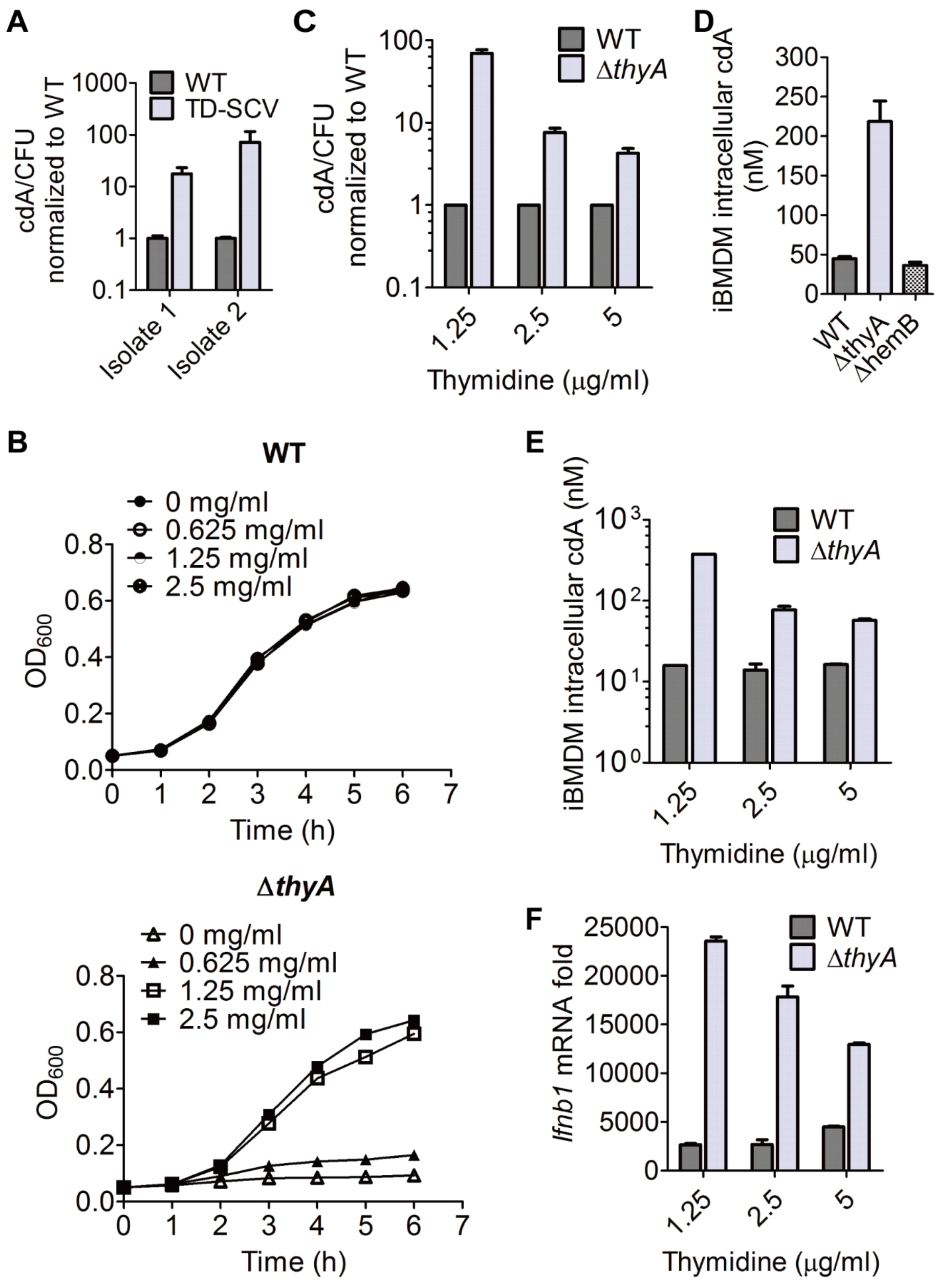

A
cdA/CFU normalized to WT
1000
100
10
1
0.1
WT
TD-SCV
Isolate 1
Isolate 2
C
cdA/CFU normalized to WT
100
10
1
0.1
WT
ΔthyA
1.25
2.5
5
Thymidine (μg/ml)
D
iBMDM intracellular cdA (nM)
300
250
200
150
100
50
0
WT
ΔthyA
ΔhemB
B
WT
0 mg/ml
0.625 mg/ml
1.25 mg/ml
2.5 mg/ml
OD600
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
7
Time (h)
ΔthyA
0 mg/ml
0.625 mg/ml
1.25 mg/ml
2.5 mg/ml
OD600
Time (h)
E
iBMDM intracellular cdA (nM)
10^3
10^2
10^1
10^0
WT
ΔthyA
1.25
2.5
5
Thymidine (μg/ml)
F
Ifnb1 mRNA fold
25000
20000
15000
10000
5000
0
WT
ΔthyA
1.25
2.5
5
Thymidine (μg/ml)

Figure 7. Thymidine controls c-di-AMP levels in a Δ*thyA* strain. (A) LC-MS/MS detection of c-di-AMP levels of clinical *S. aureus* thymidine auxotrophs (TD-SCVs) and their isogenic parental isolates (WT). (B) Growth curve of WT and Δ*thyA* in BHI broth supplemented with various concentrations of thymidine. (C) LC-MS/MS detection of c-di-AMP levels of WT and the Δ*thyA* strain grown in BHI broth supplemented with various concentration of thymidine. (D) C-di-AMP production of WT and Δ*thyA* during infection. WT iBMDMs were infected with WT, Δ*thyA* and Δ*hemB* for 4 hr, and intracellular c-di-AMP levels were determined by LC-MS/MS. (E) WT iBMDMs were infected with WT and Δ*thyA* grown in BHI broth supplemented with various concentration of thymidine for 4 hr, and intracellular c-di-AMP levels were determined by LC-MS/MS. (F) Same as (E), *Ifnb1* mRNA was measured by RT-qPCR relative to uninfected controls.

A Δ*thyA* strain induces more inflammation during lung infection. *S. aureus* SCVs in CF patients predominantly carry mutations in *thyA*, and THYD-SCVs persist longer in the CF lung than do normal-colony *S. aureus* isolates (31). To characterize the host response to SCV infection, wild type and STING$^{-/-}$ mice were intranasally infected with Δ*thyA* and Δ*hemB* strains at an inoculum of 5×10^8 CFU, and lung bacterial burdens were enumerated at 24 hours post infection. As expected, STING$^{-/-}$ mice had lower lung bacterial burdens when compared to wild type mice (Figure 8A). However, there were no significant differences in bacterial burdens within host genotypes infected with Δ*thyA* and Δ*hemB* strains. Therefore, the SCV type did not determine the bacterial burden in the lung.

To examine whether SCVs were associated with increased inflammatory cytokine release, transcripts of *IFNβ* and co-regulated genes were measured from the homogenized lung tissue using RT-qPCR (Figure 8B). In WT mice, the Δ*thyA* strain induced higher *Ifnb1* levels than Δ*hemB*, consistent with our tissue culture findings in macrophages (Figures 5-6). In STING$^{-/-}$ mice, however, the trend was reversed, with Δ*thyA* infection resulting in lower levels of *Ifnb1* transcripts than Δ*hemB*. In comparing WT and STING$^{-/-}$ mice infected with Δ*thyA* and Δ*hemB*, we observed approximately 100-fold lower expression of *Ifnb1* in STING$^{-/-}$ mice infected with Δ*thyA* but similar levels of *Ifnb1* in both host genotypes infected with Δ*hemB*. We observed that *Ifnb1* transcript levels in STING$^{-/-}$ mice infected with Δ*thyA* were significantly reduced compared to those in wild type mice. In contrast, both wild type and STING$^{-/-}$ mice had comparable *Ifnb1* levels after Δ*hemB* infection. These results suggested that the higher level of interferon transcription induced by Δ*thyA* in WT mice required STING. Furthermore, *Cxcl10* and *Il6* induction by Δ*thyA* was impaired in STING-deficient mice, indicating that the STING-

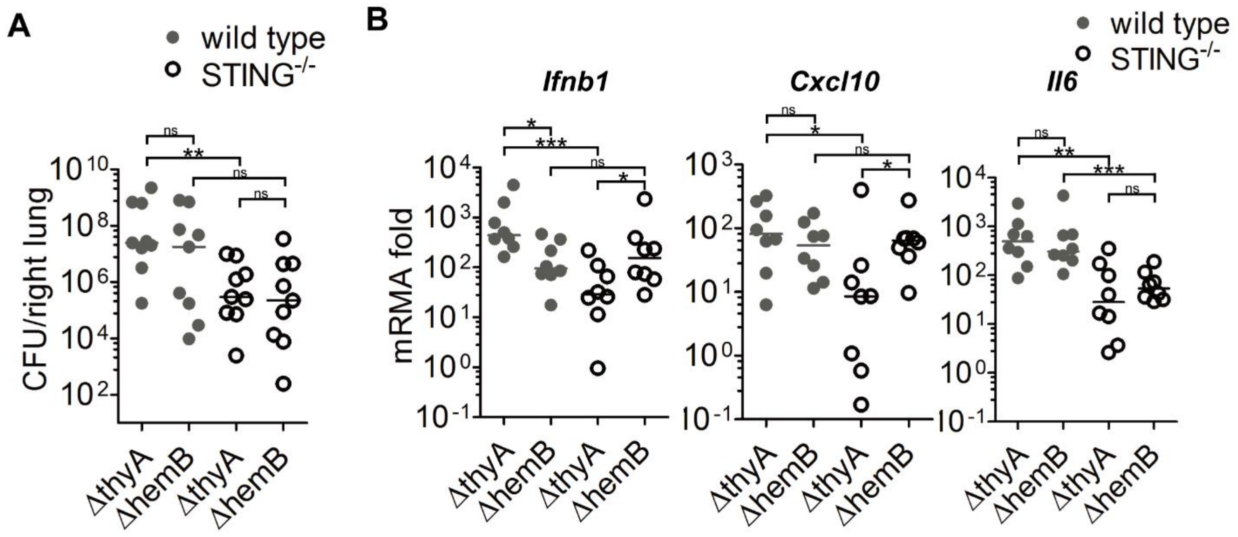


Figure 8. Δ*thyA* drives IFNβ production during lung infection. (A) Bacterial recovery from the right lung of wild type and STING$^{-/-}$ mice infected with Δ*thyA* and Δ*hemB*. (B) *Ifnb1*, *Cxcl10*, and *Il6* mRNA levels in lung were measured by RT-qPCR relative to uninfected controls. *P* values were calculated after log transformation of the data using Mann-Whitney U test. Asterisks indicate that differences are statistically significant (*, $p < 0.05$; **, $p < 0.01$, ***, and $p < 0.001$), and ns indicates no significant difference.

dependent higher pro-inflammatory activity of Δ*thyA* compared with Δ*hemB* and WT strains extends beyond interferon responses.

Discussion

In the current study, we found that an inactivating mutation of thymidylate synthase – *thyA* in *S. aureus,* resulting in a common variant strain associated with higher morbidity than WT strains significantly increases intracellular c-di-AMP levels during host cell infection and results higher type 1 interferon expression through STING activation.

We also found that c-di-AMP levels were inversely associated with exogenous thymidine supplementation. How c-di-AMP levels are linked to thymidine metabolism and utilization remains unclear, but c-di-AMP may be directly affected by thymidine utilization, or it may be indirectly moderated by thymidine starvation. C-di-AMP levels have been linked to metabolic regulation in several bacteria; conversely, metabolic changes are known to alter c-di-AMP levels (32, 33). dTMP is synthesized either from dUMP (deoxyuridylate) or thymidine by ThyA or thymidine kinase, respectively (34). Consequently, disruption of *thyA* results in *S. aureus* reliance upon thymidine kinase following uptake of exogenous thymidine through the NupC nucleoside transport system. Following synthesis of dTMP, thymidylate kinase (TMK) phosphorylates dTMP to dTDP, which is further phosphorylated to dTTP, for DNA synthesis (34). Interestingly, the gene encoding thymidylate kinase (*tmk*) in *S. aureus* is in a conserved operon with the coding gene of a c-di-AMP receptor, PII-like signal transduction protein A (*pstA*) (14, 35-37). The biological function of PstA is not yet known, although the conserved genetic association with *tmk* is intriguing and may indicate a direct link between c-di-AMP levels, pyrimidine metabolism and DNA synthesis.

In addition to a direct link to thymidine utilization, elevated levels of c-di-AMP may be increased indirectly due to altered cellular function within the THYD-SCVs. Cells of the Δ*thyA* mutant are markedly enlarged, exhibiting approximately three times the volume of wild type cells (6, 38). C-di-AMP levels may be altered to support this distorted cellular structure. Bacteria import ions and osmolytes to maintain appropriate cellular turgor and shape in response to fluctuating environmental osmotic pressure. C-di-AMP is a general osmotic stress regulator involved in regulating potassium uptake and compatible solute uptake (10, 15, 39). *S. aureus* is known to alter c-di-AMP production to control cell size and help bacteria to cope with extreme membrane and cell wall stress. In *S. aureus*, higher c-di-AMP levels lead to reduction in the cell size and alteration in the composition of cell wall (14). Furthermore, it has been shown that mutations in *thyA* result in increased cell size in *S. aureus* under low levels of external thymidine (40). Therefore, *S. aureus* may produce more c-di-AMP to compensate for the enlarged cell size conferred by *thyA* mutation.

THYD-SCVs are linked to persistent infection and worsened CF disease progression through an unknown mechanism (31). The current results suggest that THYD-SCVs trigger elevated inflammation during infection, providing a compelling mechanism for these clinical observations. First, we found that excessive c-di-AMP produced by THYD-SCVs promotes type I interferon responses during macrophage and lung infection. Elevated production of interferon during staphylococcal lung infection has been linked to increased pulmonary pathology and bacterial pathogenesis (41, 42). Although we did not find significant STING-dependent differences in lung pathology during acute murine lung infection, it is plausible that longer periods of Type I interferon elevation, as would be expected to occur during chronic THYD-SCV infection could contribute to a relentless decline in lung function. Second, Type I interferon production during viral pulmonary infection increases susceptibility to secondary infection by bacteria, including *S. aureus* and *S. pneumonia* (41, 43, 44). Within the context of CF lung infection, the presence of THYD-SCVs may elevate interferon levels, predisposing patients to secondary bacterial infection or intensified infection by the bacteria already present following antibiotic therapy (45, 46).

While our findings support a role for elevated c-di-AMP and resulting IFNβ production by THYD-SCVs in driving lung disease, the total inflammation observed by pathology tended to be higher among mice infected with THYD-SCVs irrespective of STING presence. The fact that STING was not clearly required for visible inflammation in mice suggests that THYD-SCVs may induce pathology in multiple ways. Among genes we observed to be significantly induced by THYD-SCVs in macrophages, only a subset are known STING targets, while several others may also contribute to altered inflammation. The genes *Il12b*, *Il10*, and *Il1b*, which are typically associated with elevated TLR activation, were also significantly upregulated during THYD-SCV infection. The molecular mechanisms by which these and other host responses are differentially induced by THYD-SCVs are not clear, but our results are consistent with enhanced sensing of the bacterial envelope through TLR2, a predominant sensor of phagocytosed *S. aureus*.

Whether elevated STING activation contributes to declining lung function in CF patients infected with THYD-SCVs remains to be studied. A significant proportion of humans have STING alleles competent for cGAS signaling but that are nonresponsive to bacterial-derived cyclic dinucleotides.

Studies with longitudinal lung function data, coupled with host exome sequencing to identify STING coding variants, may reveal if patients harboring nonresponsive alleles have a better prognosis than those responsive to the elevated bacterial c-di-AMP. These and other studies to define the consequences of both STING-dependent and independent inflammation during *S. aureus* lung infection will be required to further inform the mechanisms of enhanced pathogenesis associated with THYD-SCVs of the CF lung and elsewhere.

References

1. **Wolter DJ**, **Emerson JC**, **McNamara S**, **Buccat AM**, **Qin X**, **Cochrane E**, **Houston LS**, **Rogers GB**, **Marsh P**, **Prehar K**, **Pope CE**, **Blackledge M**, **Déziel E**, **Bruce KD**, **Ramsey BW**, **Gibson RL**, **Burns JL**, **Hoffman LR**. 2013. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with

cystic fibrosis. Clin Infect Dis **57**:384–391.

2. **Zander J**, **Besier S**, **Saum SH**, **Dehghani F**, **Loitsch S**, **Brade V**, **Wichelhaus TA**. 2008. Influence of dTMP on the phenotypic appearance and intracellular persistence of *Staphylococcus aureus*. Infect Immun **76**:1333–1339.

3. **Chatterjee I**, **Kriegeskorte A**, **Fischer A**, **Deiwick S**, **Theimann N**, **Proctor RA**, **Peters G**, **Herrmann M**, **Kahl BC**. 2008. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. J Bacteriol **190**:834–842.

4. **Precit MR**, **Wolter DJ**, **Griffith A**, **Emerson J**, **Burns JL**, **Hoffman LR**. 2016. Optimized In Vitro Antibiotic Susceptibility Testing Method for Small-Colony Variant *Staphylococcus aureus*. Antimicrob Agents Chemother **60**:1725–1735.

5. **Zander J**, **Besier S**, **Faetke S**, **Saum SH**, **Müller V**, **Wichelhaus TA**. 2010. Antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2'-deoxyuridine and rifampicin against *Staphylococcus aureus*. Int J Antimicrob Agents **36**:562–565.

6. **Kriegeskorte A**, **Lorè NI**, **Bragonzi A**, **Riva C**, **Kelkenberg M**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2015. Thymidine-Dependent *Staphylococcus aureus* Small-Colony Variants Are Induced by Trimethoprim-Sulfamethoxazole (SXT) and Have Increased Fitness during SXT Challenge. Antimicrob Agents Chemother **59**:7265–7272.

7. **Corrigan RM**, **Gründling A**. 2013. Cyclic di-AMP: another second messenger enters the fray. Nat Rev Microbiol **11**:513–524.

8. **Witte CE**, **Whiteley AT**, **Burke TP**, **Sauer J-D**, **Portnoy DA**, **Woodward JJ**. 2013. Cyclic di-AMP Is Critical for *Listeria monocytogenes* Growth, Cell Wall Homeostasis, and Establishment of Infection. MBio **4**:e00282–13–e00282–13.

9. **Cho KH**, **Kang SO**. 2013. Streptococcus pyogenes c-di-AMP Phosphodiesterase, GdpP, Influences SpeB Processing and Virulence. PLoS ONE **8**:e69425.

10. **Huynh TN**, **Choi PH**, **Sureka K**, **Ledvina HE**, **Campillo J**, **Tong L**, **Woodward JJ**. 2016. Cyclic di-AMP targets the cystathionine beta-synthase domain of the osmolyte transporter OpuC. Molecular Microbiology **102**:233–243.

11. **Tang Q**, **Luo Y**, **Zheng C**, **Yin K**, **Ali MK**, **Li X**, **He J**. 2015. Functional Analysis of a c-di-AMP-specific Phosphodiesterase MsPDE from Mycobacterium smegmatis. Int J Biol Sci **11**:813–824.

12. **Gries CM**, **Bruger EL**, **Moormeier DE**, **Scherr TD**, **Waters CM**, **Kielian T**. 2016. Cyclic di-AMP Released from *Staphylococcus aureus* Biofilm Induces a Macrophage Type I Interferon Response. Infect Immun **84**:3564–3574.

13. **Dengler V**, **McCallum N**, **Kiefer P**, **Christen P**, **Patrignani A**, **Vorholt JA**, **Berger-Bächi B**, **Senn MM**. 2013. Mutation in the C-Di-AMP Cyclase dacA Affects Fitness and

Resistance of Methicillin Resistant *Staphylococcus aureus*. PLoS ONE **8**:e73512.

14. **Corrigan RM**, **Abbott JC**, **Burhenne H**, **Kaever V**, **Gründling A**. 2011. c-di-AMP Is a New Second Messenger in *Staphylococcus aureus* with a Role in Controlling Cell Size and Envelope Stress. PLoS Pathog **7**:e1002217.

15. **Schuster CF**, **Bellows LE**, **Tosi T**, **Campeotto I**, **Corrigan RM**, **Freemont P**, **Gründling A**. 2016. The second messenger c-di-AMP inhibits the osmolyte uptake system OpuC in *Staphylococcus aureus*. Science Signaling **9**:ra81–ra81.

16. **Barker JR**, **Koestler BJ**, **Carpenter VK**, **Burdette DL**, **Waters CM**, **Vance RE**, **Valdivia RH**. 2013. STING-Dependent Recognition of Cyclic di-AMP Mediates Type I Interferon Responses during Chlamydia trachomatis Infection. MBio **4**:e00018–13–e00018–13.

17. **Yang J**, **Bai Y**, **Zhang Y**, **Gabrielle VD**, **Jin L**, **Bai G**. 2014. Deletion of the cyclic di-AMP phosphodiesterase gene (cnpB) in Mycobacterium tuberculosis leads to reduced virulence in a mouse model of infection. Molecular Microbiology **93**:65–79.

18. **Woodward JJ**, **Iavarone AT**, **Portnoy DA**. 2010. c-di-AMP Secreted by Intracellular *Listeria monocytogenes* Activates a Host Type I Interferon Response. Science **328**:1703–1705.

19. **Andrade WA**, **Firon A**, **Schmidt T**, **Hornung V**, **Fitzgerald KA**, **Kurt-Jones EA**, **Trieu-Cuot P**, **Golenbock DT**, **Kaminski P-A**. 2016. Group B Streptococcus Degrades Cyclic-di-AMP to Modulate STING-Dependent Type I Interferon Production. Cell Host & Microbe **20**:49–59.

20. **Tao J**, **Zhou X**, **Jiang Z**. 2016. cGAS-cGAMP-STING: The three musketeers of cytosolic DNA sensing and signaling. IUBMB Life **68**:858–870.

21. **Škrnjug I**, **Rueckert C**, **Libanova R**, **Lienenklaus S**, **Weiss S**, **Guzmán CA**. 2014. The Mucosal Adjuvant Cyclic di-AMP Exerts Immune Stimulatory Effects on Dendritic Cells and Macrophages. PLoS ONE **9**:e95728.

22. **Yi G**, **Brendel VP**, **Shu C**, **Li P**, **Palanathan S**, **Kao CC**. 2013. Single Nucleotide Polymorphisms of Human STING Can Affect Innate Immune Response to Cyclic Dinucleotides. PLoS ONE **8**:e77846.

23. **McDougal LK**, **Steward CD**, **Killgore GE**, **Chaitram JM**, **McAllister SK**, **Tenover FC**. 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol **41**:5113–5120.

24. **Eiff von C**, **Heilmann C**, **Proctor RA**, **Woltz C**, **Peters G**, **Götz F**. 1997. A site-directed *Staphylococcus aureus* hemB mutant is a small-colony variant which persists intracellularly. J Bacteriol **179**:4706–4712.

25. **Monk IR**, **Foster TJ**. 2012. Genetic manipulation of Staphylococci-breaking through the barrier. Front Cell Infect Microbiol **2**:49.

26. **Gall A**, **Treuting P**, **Elkon KB**, **Loo Y-M**, **Gale M**, **Barber GN**, **Stetson DB**. 2012. Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity **36**:120–131.

27. **Smith SM**, **Wunder MB**, **Norris DA**, **Shellman YG**. 2011. A simple protocol for using a LDH-based cytotoxicity assay to assess the effects of death and growth inhibition at the same time. PLoS ONE **6**:e26908.

28. **Huynh TN**, **Luo S**, **Pensinger D**, **Sauer J-D**, **Tong L**, **Woodward JJ**. 2015. An HD-domain phosphodiesterase mediates cooperative hydrolysis of c-di-AMP to affect bacterial growth and virulence. Proc Natl Acad Sci USA **112**:E747–56.

29. **Proctor RA**, **Kriegeskorte A**, **Kahl BC**, **Becker K**, **Löffler B**, **Peters G**. 2014. *Staphylococcus aureus* Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol **4**:99.

30. **Kriegeskorte A**, **Grubmüller S**, **Huber C**, **Kahl BC**, **Eiff von C**, **Proctor RA**, **Peters G**, **Eisenreich W**, **Becker K**. 2014. *Staphylococcus aureus* small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. Front Cell Infect Microbiol **4**:141.

31. **Goerke C**, **Wolz C**. 2010. Adaptation of *Staphylococcus aureus* to the cystic fibrosis lung. Int J Med Microbiol **300**:520–525.

32. **Sureka K**, **Choi PH**, **Precit M**, **Delince M**, **Pensinger DA**, **Huynh TN**, **Jurado AR**, **Goo YA**, **Sadilek M**, **Iavarone AT**, **Sauer J-D**, **Tong L**, **Woodward JJ**. The Cyclic Dinucleotide c-di-AMP Is an Allosteric Regulator of Metabolic Enzyme Function. Cell **158**:1389–1401.

33. **Block KF**, **Hammond MC**, **Breaker RR**. 2010. Evidence for widespread gene control function by the ydaO riboswitch candidate. J Bacteriol **192**:3983–3989.

34. **Wang L**, **Hames C**, **Schmidl SR**, **Stülke J**. 2010. Upregulation of thymidine kinase activity compensates for loss of thymidylate synthase activity in Mycoplasma pneumoniae. Molecular Microbiology **77**:1502–1511.

35. **Müller M**, **Hopfner K-P**, **Witte G**. 2015. c-di-AMP recognition by *Staphylococcus aureus* PstA. FEBS Lett **589**:45–51.

36. **Choi PH**, **Sureka K**, **Woodward JJ**, **Tong L**. 2015. Molecular basis for the recognition of cyclic-di-AMP by PstA, a PII-like signal transduction protein. MicrobiologyOpen **4**:361–374.

37. **Campeotto I**, **Zhang Y**, **Mladenov MG**, **Freemont PS**, **Gründling A**. 2015. Complex structure and biochemical characterization of the *Staphylococcus aureus* cyclic

diadenylate monophosphate (c-di-AMP)-binding protein PstA, the founding member of a new signal transduction protein family. J Biol Chem **290**:2888–2901.

38. **Kriegeskorte A**, **Block D**, **Drescher M**, **Windmüller N**, **Mellmann A**, **Baum C**, **Neumann C**, **Lorè NI**, **Bragonzi A**, **Liebau E**, **Hertel P**, **Seggewiss J**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2014. Inactivation of thyA in *Staphylococcus aureus* attenuates virulence and has a strong impact on metabolism and virulence gene expression. MBio **5**:e01447–14.

39. **Moscoso JA**, **Schramke H**, **Zhang Y**, **Tosi T**, **Dehbi A**, **Jung K**, **Gründling A**. 2016. Binding of Cyclic Di-AMP to the *Staphylococcus aureus* Sensor Kinase KdpD Occurs via the Universal Stress Protein Domain and Downregulates the Expression of the Kdp Potassium Transporter. J Bacteriol **198**:98–110.

40. **Kahl BC**, **Belling G**, **Reichelt R**, **Herrmann M**, **Proctor RA**, **Peters G**. 2003. Thymidine-dependent small-colony variants of *Staphylococcus aureus* exhibit gross morphological and ultrastructural changes consistent with impaired cell separation. J Clin Microbiol **41**:410–413.

41. **Martin FJ**, **Gomez MI**, **Wetzel DM**, **Memmi G**, **O'Seaghdha M**, **Soong G**, **Schindler C**, **Prince A**. 2009. *Staphylococcus aureus* activates type I IFN signaling in mice and humans through the Xr repeated sequences of protein A. J Clin Invest **119**:1931–1939.

42. **Parker D**, **Planet PJ**, **Soong G**, **Narechania A**, **Prince A**. 2014. Induction of Type I Interferon Signaling Determines the Relative Pathogenicity of *Staphylococcus aureus* Strains. PLoS Pathog **10**:e1003951.

43. **Li W**, **Moltedo B**, **Moran TM**. 2012. Type I interferon induction during influenza virus infection increases susceptibility to secondary Streptococcus pneumoniae infection by negative regulation of γδ T cells. J Virol **86**:12304–12312.

44. **Shahangian A**, **Chow EK**, **Tian X**, **Kang JR**, **Ghaffari A**, **Liu SY**, **Belperio JA**, **Cheng G**, **Deng JC**. 2009. Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. J Clin Invest **119**:1910–1920.

45. **Gorwitz RJ**, **Kruszon-Moran D**. 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001–2004. Journal of Infectious ….

46. **Mika M**, **Korten I**, **Qi W**, **Regamey N**, **Frey U**, **Casaulta C**, **Latzin P**, **Hilty M**. 2016. The nasal microbiota in infants with cystic fibrosis in the first year of life: a prospective cohort study. The Lancet Respiratory Medicine **4**:627–635.

Chapter 4:

Preliminary Examination of Genetic Mechanisms Behind *S. aureus* Thymidine-Dependent and Hemin-Dependent Small-Colony Variant Formation in Cystic Fibrosis Clinical Isolates

Note: The work presented in this chapter was performed by Mimi Precit, Stephen Salipante, Lucas Hoffman, Adam-Walkes, Katherine McLean, Elizabeth Holman, and Daniel Wolter.

Introduction

The cystic fibrosis (CF) lung presents a uniquely hostile environment for bacteria due to neutrophil influx (1), hypoxia (2), competition with co-infecting species (3), and antibiotic exposure (4), all of which can lead to numerous adaptive changes. The genetic adaptations that *S. aureus* CF isolates undergo are not well described, however it is appreciated that certain characteristics, such as small colony variant (SCV) formation are associated with worse lung function outcomes (5).

The most common SCVs recovered from bone and soft tissue infections are those with electron transport chain defects, specifically in hemin (HEM) or menaquinone biosynthetic pathways (6, 7). These defects result in dependencies, or auxotrophies, on extracellular HEM or menadione (MEN) (a synthetic form of the naturally-occurring molecule menaquinone) for adequate *in vitro* growth. In contrast, the most common SCV recovered from CF respiratory secretions are those harboring defects in pyrimidine biosynthesis, resulting in auxotrophy for the nucleoside thymidine (THY) or its derived nucleotide, dTMP (5, 8).

S. aureus SCVs are currently defined as isolates that grow slowly *in vitro* and have small colony morphology on agar media. However, while numerous mechanisms can cause bacteria to glow slowly *in* vitro (9), naturally-occurring SCVs exhibit a relatively limited range of phenotypes like slow growth, atypical pigmentation, and reduced hemolysis (10). Only a few molecular mechanisms leading to small colony formation and resulting auxotrophy have been reported in the literature (8, 11-19) (Table 1), indicating that SCVs emerge during infections for specific reasons. A handful of genetic mechanisms leading to HEM dependent (HEMD) and

Table 1. Defined mechanisms behind SCV formation in *S. aureus*

# Isolates	Source	Auxotrophy	Site of mutation	Reference
8	Osteomyelitis, Abscess, Bacteremia	MEND	*menB, C, E, F*	(11, 12)
1	Prosthetic Hip infection	HEMD	*hemB*	(13)
1	Targeted deletion	MEND	Δ*menD*	(12)
1	Targeted deletion	HEMD	Δ*hemB*	(14)
1	Aminoglycoside selection	HEMD	*hemA, H*	(15)
22	CF Secretions	THYD	*thyA*	(8, 16-18)
1	Targeted deletion	THYD	Δ*thyA*	(17)
1	Blood Stream Infection	Undefined	*relA*	(19)

Abbreviations: menadione-dependent (MEND), hemin-dependent (HEMD), thymidine-dependent (THYD).

MEN dependent (MEND) SCVs have been reported involving mutations in HEM or MEN biosynthetic genes, respectively (11, 12). These observations were supported by studies in which targeted deletions in a small subset of these genes resulted in HEMD- or MEND-SCV phenotypes *in vitro* (14, 20). Both menaquinone and hemin are components of the electron transport chain, and electron transport is required for uptake of aminoglycoside antibiotics; therefore, HEMD and MEND-SCVs are resistant to aminoglycosides, and both SCV types can be selected by aminoglycosides (15, 21). In contrast, THY-dependent (THYD) SCVs, the most common type detected in CF secretions, generally carry mutations in the thymidylate synthase gene *thyA,* which uses folate as a cofactor for the synthesis of dTMP, a critical precursor for de novo DNA biosynthesis (8). The recovery of THYD-SCVs with *thyA* mutations both *in vitro* and *in vivo* has been associated with exposure to trimethoprim-sulfamethoxazole (TMP-SMX) (5, 16, 22), an antibiotic that targets two enzymatic steps in folate metabolism (23). Previous work investigating clinical THYD-SCVs showed that intact copies of the *thyA* gene on a plasmid do not fully restore clinical THYD-SCV growth to wild-type (WT), normal colony (NC) levels, suggesting that additional, underlying mechanisms may also play a role in SCV formation *in vivo* (8, 16). Other, more rare mutations leading to SCV formation have also been identified, for example in *relA*, which plays a key role in the *S. aureus* stringent response (19). Overall, only 10 genes all involved in critical metabolic processes have been shown to play a role in SCV formation in *S. aureus*, and only *thyA* mutations have been described for CF clinical isolates in the literature. A more extensive analysis of SCV causal mutations has yet to be completed on a large collection of clinical CF isolates.

To begin to address this knowledge gap, we performed next-generation whole-genome sequencing (WGS) on a collection of CF *S. aureus* clinical isolates in order to define metabolic genes harboring mutations in *S. aureus* SCVs not present in NC/non-SCV isolates. The work presented here is a preliminary analysis focused on comparing whole-genome data for THYD-SCVs and NC/non-THYD isolates to identify genetic lesions present in THYD-SCVs but absent in non-THYD isolates; for two HEMD-SCVs, comparison to a paired, isogenic NC co-isolate was also carried out. Here I present evidence that mutations in a number of genes involved in critical metabolic processes are present in SCVs and absent in NC isolates and also show that a small number of genes (n=2) do in fact result in SCV formation when engineered in to a clean genetic background.

Materials and Methods

Isolates used in this study. In total 477 clinical CF *S. aureus* isolates were sequenced and analyzed for this initial analysis. 70 THYD-SCVs and 174 NC *S. aureus* isolates were obtained retrospectively from a single-center study of children (n=23) with CF, completed at Seattle Children's Hospital (SCH) (5). The remaining 72 THYD-SCV and 161 NC *S. aureus* isolates were obtained from an on-going multi-center SCV stud from multiple CF centers, including SCH, CF center Birmingham Alabama, CF Center Baylor College of Medicine, Boston Children's Hospital, and Children's Hospital Pittsburg. A single HEMD-SCV and isogenic, paired NC isolate from the same single-center study as the THYD-SCVs (5) were also analyzed. Working stocks of all isolates were stored at -80^{p} C in trypticase soy broth + 15% glycerol.

DNA extraction. *S. aureus* isolates were cultured from freezer stocks aerobically overnight on chocolate agar at 35ºC. DNA was extracted from the resulting culture using the UltraClean® Microbial DNA Isolation Kit manufactured by MoBio (Carlsbad, CA) following the manufacture instructions provided with the kit.

Whole-Genome Sequencing (WGS). Genomic DNA from clinical *S. aureus* isolates (30 to 100 ng) was digested for 60 to 90 min at 37°C in a 10 uL volume using 0.3 uL NEBNext double-stranded DNA (dsDNA) Fragmentase (New Eng- land BioLabs). DNA was end repaired and A-tailed in a 40 uL reaction mixture containing 1X Rapid ligation buffer (Enzymatics Inc., Beverly, MA), 0.1675 mM (each) deoxynucleoside triphosphate (dNTP) (New England BioLabs), 0.1 uL *Escherichia coli* DNA polymerase I (New Eng- land BioLabs), 0.5 uL T4 polynucleotide kinase (New England BioLabs), and 0.02 uL *Taq* DNA polymerase (New England BioLabs) and was incubated at 37°C for 30 min and 72°C for 20 min. Annealed Y-adaptors (0.2 uM) (5'-[PO4]GATCGGAAGAGCGGTTCAGCAGGAATGCCGAG-3') and (5'-ACACTCTTTCCCT ACACGACGCTCTTCCGATCT-3') were added and ligated at 25°C for 20 min using T4 DNA ligase in Rapid ligation buffer (Enzymatics Inc.). Following purification with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA), PCR amplification was performed with KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wil- mington, MA) using primer PRECAP_FWD_AMP_COMMON (5'AATGATACGGCGACCACCGAGATCTACACTCTT TCCCTACACGACGC-3') and sample specific index primers (5'-CAAGCAGAAGA CGGCATACGAGAT*XXXXXXXX*CGGTCTCGGCATTCCTGCTGAACCG-3'), where *XXXX XXXX* indicates the 8-bp index). Cycling conditions were 95°C for 3 min, 10 cycles of 98°C for 20 s, 65°C for 15 s, and 72°C for 1 min, and 1 cycle of 72°C for 5 min. PCR products were purified using AMPure beads, pooled in equimolar amounts, and sequenced on a MiSeq se- quencer (Illumina, San Diego, California) using 150-bp paired-end reads with a custom index primer(5’-AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCG-3’). Oligonucleotides were synthesized by IDT.

Analyses of *S. aureus* whole genomes. Sequences were assembled using ABySS 2.0 (24) with large kmers enabled at kmer sizes 21, 35, 48, 61, 74, 87, 100, 113, 126, 139 and 150. The best assembly was determined by comparing the E-size for each of the assemblies. That assembly was then blasted against NCBI to determine the best reference for that isolate. For each group of isolates a best reference for the group was chosen by isolate majority. All of the isolates in the group were then aligned to the chosen reference using bwa-mem (v0.7.12) (25) and Samtools (v1.1) (26). Samtools was used for variant calling of SNPs and small insertions and deletions (indels), and Pindel was used for detection of large indels and structural rearrangement (27). A comparison of all isolates in the group was performed. Isolates were determined not to be clonal if there was a distance of greater than 10000 variants (2339 largest difference left in group, 13500 smallest distance that was split) and were split into separate groups. To compare across groups we clustered all 166 references found using cd-hit-v4.6.1 (28) and mapped all annotated variants to these clusters. Additional analysis was done comparing isolates with particular auxotrophies (i.e. THY or HEM dependence). We used a Fisher exact test to compare isolates with and without a particular auxotrophy looking at all clusters and the synonymous vs. non-synonymous variation. A second path of analysis involved determining a majority reference across all isolates (*Staphylococcus aureus* subsp. JH1, complete genome, CP000736). All isolates were also aligned to this reference. We imputed missing data with groups then combined groups and plan to look at using R package bugwas (29) to do genome-wide association study (GWAS) analysis.

Targeted gene deletions for *in vitro S. aureus* SCV Construction. Based on WGS analysis, to confirm that expected auxotrophic phenotypes were due to genetic lesions present in SCVs but not isogenic NC co-isolates, we constructed Δ*thyA* (encodes thymidylate synthase) *and* Δ*hemA* (encodes glutamyl-tRNA reductase) strains in the *S. aureus* Newman background by allelic exchange using pIMAY as previously described (30). Briefly, primers were designed to amplify upstream and downstream regions flanking the gene(s) of interest (either *thyA*, or *hemA,)* to delete the entire gene (Table 2). Genomic DNA from SH1000 was used as template and the resulting PCR products were denatured and allowed to reanneal via the complementary sequences in primers B and C and then amplified using primers A and D, resulting in a fragment consisting of linked sequences upstream and downstream of the gene. The amplimer was cloned into pIMAY between KpnI and NotI restriction sites. The plasmid vector was transformed via electroporation into *E. coli* DH10B and then transformed into electrocompetent *S. aureus* strain RN4220 (30). Transduction of *S. aureus* strain Newman was accomplished by first propagating phage ϕ-11 in RN4220 that had been successfully transformed with pIMAY_deletion cassette. ϕ-11 phage will package the pIMAY vector and via subsequent generalized phage transduction the pIMAY_deletion cassette vector was transduced into stain Newman.

Table 2. Primers 5’-3’ used for *thyA* and *hemA* deletion constructs

thyA[a] SOE Fwd A	ATATGGTACCGAAGCAGTATCGGAGTATATG	underline is KpnI restriction site
thyA SOE Rev B	CTATGCAATGACTACATATGCGATAACACCTCATTTTC	
thyA SOE Fwd C	GAGGTGTTATCGCATATGTAGTCATTGCATAGTTAGCTAAC	
thyA SOE Rev D	ATATGCGGCCGCTATGGCTGGCTGACTTGTC	underline is NotI restriction site
thyA Fwd 1	GTATGGAAACAAATGGCAAACAGAAC	Used to verify deletion construct
thyA Rev 1	GTACCACTTAATCCTGAAGAAAGATG	Used to verify deletion construct
hemA[b] SOE Fwd A	ATATGGTACCGTCTGAAGTTGCACTGAGCGG	underline is KpnI restriction site
hemA SOE Rev B	TTAATATGCTTATTCCATTTGAAACGCCCCCATATATAC	
hemA SOE Fwd C	GGGCGTTTCAAATGGAATAAGCATATTAATATGGTGATAA	
hemA SOE Rev D	ATATGCGGCCGCGTCAATGAATATACGTTCACCAAC	underline is NotI restriction site
hemA Fwd 1	CAGAAATGGATGGTAGTTGTCAG	Used to verify deletion construct
hemA Rev 1	GCACAATGATAACCGACTCTG	Used to verify deletion construct

[a] *thyA* – thymidylate synthase gene

[b] *hemA* – glutamyl tRNA reductase

Results

Genetic differences in metabolic genes between SCVs and NC isolates. Gene products involved in *S. aureus* metabolism with nonsynonymous mutations present in THYD-SCVs but absent in NC isolates, with a p-value ≤0.05 as determined by the Fischer exact test, are summarized in Table 3. The most common gene mutated, found in ~77% of THYD-SCVs, was the thymidylate synthase *thyA* gene. Mutations in this gene are a known genetic mechanism behind THYD-SCV formation (8, 16, 17) and provides preliminary proof of principal for our WGS analysis pipeline. Other genes identified to harbor mutations involved in nucleotide metabolism include pyrimidine-nucleoside phosphorylase *pdp,* pyrimidine nucleoside transporter *nupC*, phosphoribosylaminoimidazolecarboxamide formyltransferase, and inosine-5'-monophosphate dehydrogenase (Table 3). A handful of other metabolic genes from various classes were also found to be mutated in THYD-SCVs (Table 3). The full list of mutations present in THYD-SCVs determined by our WGS analysis is available for reference in Appendix A. When comparing the HEMD-SCVs with the respective paired, isogenic NC isolate two mutations were uncovered: a deletion resulting in a frameshift mutation in *hemA* glutamyl-tRNA reductase and an insertion mutation resulting in a framshift mutation in a heme-acquisition protein *isdA* (Table 4).

Targeted Deletions. To confirm that expected auxotrophic phenotypes were due to the genetic lesions present in SCVs but not isogenic NC co-isolates we performed targeted deletions in either in *thyA,* or *hemA*. Resulting isolates were tested for auxotrophy for either THY or HEM, respectively and showed attenuated growth *in vitro* compared with wild-type Newman strain in the absence of media supplementation (Figure 1).

Table 3. Genes with mutations in THYD-SCVs not present in NC isolates

# Isolates of 142 THYD-SCVs	% THYD-SCVs with mutation in a given gene product	Gene Product with Mutation	Class	Fisher P-value
9	6.338028169	thiamine biosynthesis protein ThiI	Thiamine metabolism	0.04884
18	12.67605634	sialic acid synthase	Sialic Acid Metabolism	6.85E-08
9	6.338028169	pyrimidine nucleoside transporter NupC	Pyrimidine Salvage	0.02005
109	76.76056338	thymidylate synthase	Pyrimidine Metabolism	2.20E-16
17	11.97183099	Pyrimidine-nucleoside phosphorylase	Pyrimidine Metabolism	2.70E-05
5	3.521126761	phosphopentomutase	Pyrimidine & purine salvage pathway	0.002515
9	6.338028169	phosphoribosylaminoimidazolecarboxamide formyltransferase	Purine Metabolism	0.004418
10	7.042253521	inosine-5'-monophosphate dehydrogenase	Purine Metabolism	0.03364
9	6.338028169	polysaccharide biosynthesis protein	Polysaccharide Metabolism	0.0001639
13	9.154929577	isopentenyl pyrophosphate isomerase	Isoprenoid Metabolism	0.0003372
8	5.633802817	4-diphosphocytidyl-2C-methyl-D-erythritol kinase	Isoprene Metabolism	0.004626
5	3.521126761	imidazolonepropionase	Histidine Metabolism	0.0294
8	5.633802817	ferrochelatase	Heme Biosynthesis	0.03853
10	7.042253521	folylpolyglutamate synthase	Folate Metabolism	0.004076
5	3.521126761	dihydropteroate synthase	Folate Metabolism	0.01124
20	14.08450704	2-oxoglutarate dehydrogenase E1 component	Central Metabolism: TCA Cycle	0.001258
9	6.338028169	deoxyribose-phosphate aldolase	Central Metabolism: Pentose Phosphate Pathway	0.04884
10	7.042253521	Acetyl-CoA acetyltransferase	Central Metabolism	0.04274
7	4.929577465	phosphopantothenoylcysteine decarboxylase	Central Metabolism	0.004557
7	4.929577465	aminoacyltransferase	Central Metabolism	0.01114
5	3.521126761	CoA-transferase	Central Metabolism	0.01124

5	3.521126761	acetyl-CoA acetyltransferase	Central Metabolism	0.0294
8	5.633802817	acetyl-CoA acetyltransferase	Central Metabolism	0.03853
10	7.042253521	mannose-6-phosphate isomerase	Carbohydrate Metabolism	0.004076
6	4.225352113	carbamate kinase	Arginine-aminase metabolism	0.003971
3	2.112676056	diaminopimelate decarboxylase	Amino Acid Biosynthesis	0.02717
10	7.042253521	pyruvate oxidase	Acetate Metabolism	0.0007484

Note: Cells with Bolded cell perimeters highlight genes involved in nucleotide metabolism.

Table 4. Mutations present in HEMD-SCVs but absent in isogenic, co-isolated NC isolates

Isolate #	Auxotrophy	Gene	Mutation	Amino Acid	Protein
0102-11	hemin	*hemA*	Deletion	Frameshift	Glutamyl-tRNA reductase
0114-46	hemin	*isdA*	Insertion	Frameshift	Heme-iron binding protein

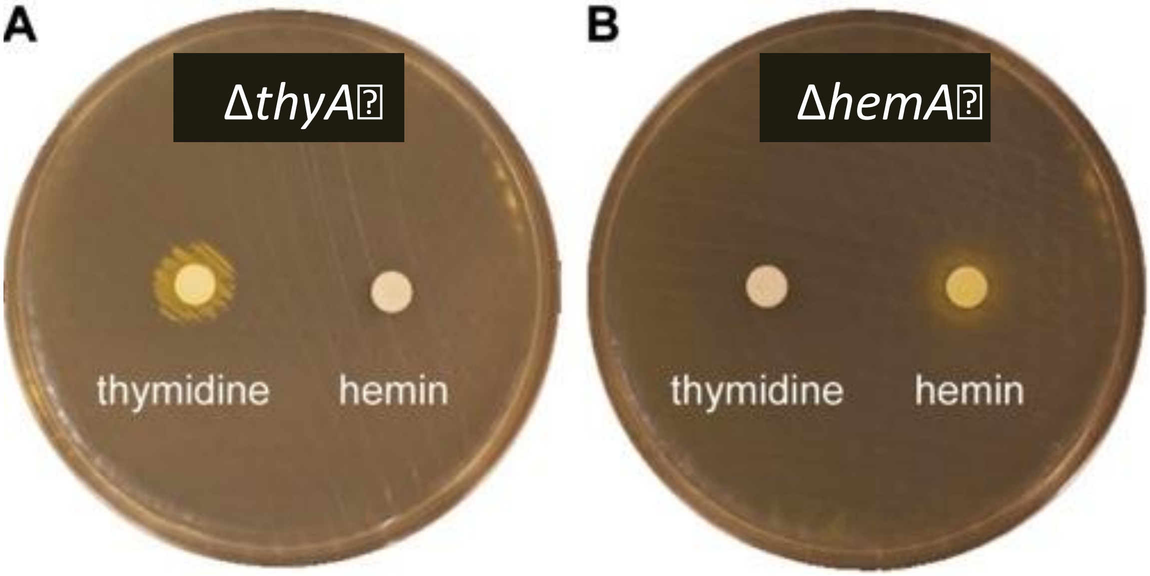


Figure 1. Demonstration of auxotrophy for thymidine and hemin in respective deletion strains. (A) A lawn of *S. aureus* strain Newman Δ*thyA* grown on Mueller-Hinton Agar (MHA) exhibits enhanced growth around the paper disk impregnated with 5 µg thymidine. (B) A lawn of Newman Δ*hemA* grown on MHA exhibits enhanced growth around the paper disk impregnated with 1µg hemin.

Discussion

Numerous research studies have demonstrated the power of bacterial WGS in understanding the genetic changes bacteria undergo as they evolve and adapt to different environments (31-33). Moreover, it is appreciated that certain phenotypic variants of *S. aureus* that arise during persistent *S. aureus* CF lung infections, such as SCVs have important clinical implications given that SCV recovery is associated with increased patient morbidity (5). Despite this, only a few studies have examined the adaptive changes undergone by *S. aureus* during chronic infections (34, 35), and therefore little is known about how *S. aureus* adapts during CF respiratory infections and only a limited number of genes have been described to contribute to SCV formation (Table 1).

Here, using a newly developed WGS pipeline in collaboration with Dr. Stephen Salipante we have completed a preliminary analysis of the genetic changes present in SCVs but absent in co-isolated NC isolates in effort to examine the mechanisms behind SCV formation. In THYD-SCVs, we identified genes involved in pyrimidine metabolism and pyrimidine salvage, with the majority of our SCV isolates harboring mutations in thymidylate synthase *thyA*. This finding was expected given previous literature describing *thyA* mutations leading to THYD-SCV formation (8, 16, 22) and provides an internal control that validates the sequencing pipeline. Additionally, I was able to engineer a clean deletion in *thyA* in *S. aureus* Newman as others have done (16, 17) to confirm the phenotype.

A myriad of other metabolic genes that have not been previously reported in SCV literature and play roles in central metabolism were also found to be mutated in THYD-SCVs. Some examples include, gene products that play a role in the tricarboxylic acid (TCA) cycle, acetyl-CoA metabolism, acetate metabolism, polysaccharide metabolism, and folic acid metabolism. Intriguingly, previous studies examining transcriptional analysis and ^{13}C-isotopologue profiling in THYD-SCVs have reported altered TCA cycle function, acetate metabolism, and amino acid metabolism (36, 37). It has also been shown that complementation with an intact *thyA* gene on a plasmid in clinical THYD-SCV does not fully restore growth to NC levels (8, 16), suggesting that possibly additional mutations, maybe some we have identified here, play a role in small colony formation. Although these are exciting initial observations, extensive further work needs to be completed in order to characterize if the genes we have identified in any of these pathways play a role in SCV formation by performing functional studies using targeted genetic approaches *in vitro.*

In the two HEMD-SCVs, we were able to show that two genes *hemA* and *isdA* not present in the NC isolates were mutated. To our knowledge neither of these genes has been reported in the literature as possible genetic mechanisms behind SCV formation in clinical CF *S. aureus* isolates. Mutations in *hemA*, which encodes glutamyl-tRNA reductase the first step in the heme-biosynthesis pathway, have been found in SCVs following exposure to aminoglycosides *in vitro* (15) but not in clinical SCV isolates. I was able to recapitulate the HEMD phenotype *in vitro* with a targeted deletion of *hemA* in *S. aureus* Newman. In contrast, the heme-acquisition gene, *isdA* represents a completely novel mutation likely responsible for the SCV phenotype in that isolate, however further work must be completed in order to fully characterize if *isdA*, as well as other likely causal mutations, play a role in SCV formation.

The WGS analysis pipeline data we have generated from our analytical platform has the potential to provide extensive genetic information for *S. aureus* CF clinical isolates that can be used for a variety of future analyses expanding from just the examination of mutations responsible for SCV formation. Our work with the Salipante lab developing this platform, and performing this preliminary analysis presented in this work has provided the foundation for future studies to examine genes including, but not limited to those, involved in antibiotic resistance, persistence, and clinical outcomes in patients, with the ultimate goal to understand how *S. aureus* adapts during CF lung infections and develop ways to optimize both treatment and patient outcomes.

References

1. **Cohen TS**, **Prince A**. 2012. Cystic fibrosis: a mucosal immunodeficiency syndrome. Nature Medicine **18**:509–519.

2. **Worlitzsch D**, **Tarran R**, **Ulrich M**, **Schwab U**, **Cekici A**. 2002. Reduced oxygen concentrations in airway mucus contribute to the early and late pathogenesis of *Pseudomonas aeruginosa* cystic fibrosis airway infection. J Clin Invest.

3. **Hogardt M**, **Heesemann J**. 2013. Microevolution of. *Pseudomonas aeruginosa*.

4. **Goerke C**, **Wolz C**. 2010. Adaptation of *Staphylococcus aureus* to the cystic fibrosis lung. Int J Med Microbiol **300**:520–525.

5. **Wolter DJ**, **Emerson JC**, **McNamara S**, **Buccat AM**, **Qin X**, **Cochrane E**, **Houston LS**, **Rogers GB**, **Marsh P**, **Prehar K**, **Pope CE**, **Blackledge M**, **Déziel E**, **Bruce KD**, **Ramsey BW**, **Gibson RL**, **Burns JL**, **Hoffman LR**. 2013. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis **57**:384–391.

6. **Eiff von C**, **Bettin D**, **Proctor RA**, **Rolauffs B**, **Lindner N**, **Winkelmann W**, **Peters G**. 1997. Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. Clin Infect Dis **25**:1250–1251.

7. **Proctor RA**, **van Langevelde P**, **Kristjansson M**, **Maslow JN**, **Arbeit RD**. 1995. Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. Clin Infect Dis **20**:95–102.

8. **Besier S**, **Ludwig A**, **Ohlsen K**, **Brade V**, **Wichelhaus TA**. 2007. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. Int J Med Microbiol **297**:217–225.

9. **Proctor RA**, **Kriegeskorte A**, **Kahl BC**, **Becker K**, **Löffler B**, **Peters G**. 2014. *Staphylococcus aureus* Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol **4**:99.

10. **Kahl BC**, **Becker K**, **Löffler B**. 2016. Clinical Significance and Pathogenesis of Staphylococcal Small Colony Variants in Persistent Infections. Clin Microbiol Rev **29**:401–427.

11. **Lannergård J**, **Eiff von C**, **Sander G**, **Cordes T**, **Seggewiss J**, **Peters G**, **Proctor RA**, **Becker K**, **Hughes D**. 2008. Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother **52**:4017–4022.

12. **Dean MA**, **Olsen RJ**, **Long SW**, **Rosato AE**, **Musser JM**. 2014. Identification of point mutations in clinical *Staphylococcus aureus* strains that produce small-colony variants auxotrophic for menadione. Infect Immun **82**:1600–1605.

13. **Roggenkamp A**, **Haas A**, **Eder W**, **Kreuzer E**, **Hoffmann H**. 2004. Metabolically deficient methicillin-resistant *Staphylococcus aureus* as cause of chronic post-thoracotomy sternal wound infection. Eur J Clin Microbiol Infect Dis **23**:419–421.

14. **Eiff von C**, **Heilmann C**, **Proctor RA**, **Woltz C**, **Peters G**, **Götz F**. 1997. A site-directed *Staphylococcus aureus* hemB mutant is a small-colony variant which persists intracellularly. J Bacteriol **179**:4706–4712.

15. **Schaaff F**, **Bierbaum G**, **Baumert N**, **Bartmann P**, **Sahl H-G**. 2003. Mutations are involved in emergence of aminoglycoside-induced small colony variants of *Staphylococcus aureus*. Int J Med Microbiol **293**:427–435.

16. **Chatterjee I**, **Kriegeskorte A**, **Fischer A**, **Deiwick S**, **Theimann N**, **Proctor RA**, **Peters G**, **Herrmann M**, **Kahl BC**. 2008. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. J Bacteriol **190**:834–842.

17. **Kriegeskorte A**, **Block D**, **Drescher M**, **Windmüller N**, **Mellmann A**, **Baum C**, **Neumann C**, **Lorè NI**, **Bragonzi A**, **Liebau E**, **Hertel P**, **Seggewiss J**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2014. Inactivation of thyA in *Staphylococcus aureus* attenuates virulence and has a strong impact on metabolism and virulence gene expression. MBio **5**:e01447–14.

18. **Besier S**, **Smaczny C**, **Mallinckrodt von C**, **Krahl A**, **Ackermann H**, **Brade V**, **Wichelhaus TA**. 2007. Prevalence and clinical significance of *Staphylococcus aureus* small-colony variants in cystic fibrosis lung disease. J Clin Microbiol **45**:168–172.

19. **Gao W**, **Chua K**, **Davies JK**, **Newton HJ**, **Seemann T**, **Harrison PF**, **Holmes NE**, **Rhee H-W**, **Hong J-I**, **Hartland EL**, **Stinear TP**, **Howden BP**. 2010. Two novel point mutations in clinical *Staphylococcus aureus* reduce linezolid susceptibility and switch on the stringent response to promote persistent infection. PLoS Pathog **6**:e1000944.

20. **Eiff von C**, **McNamara P**, **Becker K**, **Bates D**, **Lei X-H**, **Ziman M**, **Bochner BR**, **Peters G**, **Proctor RA**. 2006. Phenotype microarray profiling of *Staphylococcus aureus* menD and hemB mutants with the small-colony-variant phenotype. J Bacteriol **188**:687–693.

21. **Hoffman LR**, **Déziel E**, **D'Argenio DA**, **Lépine F**, **Emerson J**, **McNamara S**, **Gibson RL**, **Ramsey BW**, **Miller SI**. 2006. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of Pseudomonas aeruginosa. Proc Natl Acad Sci USA **103**:19890–19895.

22. **Kriegeskorte A**, **Lorè NI**, **Bragonzi A**, **Riva C**, **Kelkenberg M**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2015. Thymidine-Dependent *Staphylococcus aureus* Small-Colony Variants Are Induced by Trimethoprim-Sulfamethoxazole (SXT) and Have Increased Fitness during SXT Challenge. Antimicrob Agents Chemother **59**:7265–7272.

23. **Zander J**, **Besier S**, **Faetke S**, **Saum SH**, **Müller V**, **Wichelhaus TA**. 2010. Antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2'-deoxyuridine and rifampicin against *Staphylococcus aureus*. Int J Antimicrob Agents **36**:562–565.

24. **Jackman SD**, **Vandervalk BP**, **Mohamadi H**, **Chu J**, **Yeo S**, **Hammond SA**, **Jahesh G**,

Khan H, **Coombe L**, **Warren RL**, **Birol I**. 2017. ABySS 2.0: resource-efficient assembly of large genomes using a Bloom filter. Genome Res **27**:768–777.

25. **Li H**, **Durbin R**. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics.

26. **Ruan J**, **Homer N**, **Marth G**, **Abecasis G**, **Durbin R**. 2009. The sequence alignment/map format and SAMtools. ….

27. **Ye K**, **Schulz MH**, **Long Q**, **Apweiler R**, **Ning Z**. 2009. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. Bioinformatics.

28. **Huang Y**, **Niu B**, **Gao Y**, **Fu L**, **Li W**. 2010. CD-HIT Suite: a web server for clustering and comparing biological sequences. Bioinformatics.

29. **Earle SG**, **Wu C-H**, **Charlesworth J**, **Stoesser N**, **Gordon NC**, **Walker TM**, **Spencer CCA**, **Iqbal Z**, **Clifton DA**, **Hopkins KL**, **Woodford N**, **Smith EG**, **Ismail N**, **Llewelyn MJ**, **Peto TE**, **Crook DW**, **McVean G**, **Walker AS**, **Wilson DJ**. 2016. Identifying lineage effects when controlling for population structure improves power in bacterial association studies. Nature microbiology **1**:16041.

30. **Monk IR**, **Foster TJ**. 2012. Genetic manipulation of Staphylococci-breaking through the barrier. Front Cell Infect Microbiol **2**:49.

31. **Hegreness M**, **Kishony R**. 2007. Analysis of genetic systems using experimental evolution and whole-genome sequencing. Genome Biology 2007 8:1 **8**:201.

32. **Barrick JE**, **Lenski RE**. 2013. Genome dynamics during experimental evolution. Nature Reviews Genetics **14**:827–839.

33. **Pepin KM**, **Wichman HA**. 2008. Experimental evolution and genome sequencing reveal variation in levels of clonal interference in large populations of bacteriophage φX174. BMC Evolutionary Biology 2008 8:1 **8**:85.

34. **Cullen L**, **McClean SN**. 2015. Bacterial Adaptation during Chronic Respiratory Infections. Pathogens **4**:66–89.

35. **McAdam PR**, **Holmes A**, **Templeton KE**, **Fitzgerald JR**. 2011. Adaptive Evolution of *Staphylococcus aureus* during Chronic Endobronchial Infection of a Cystic Fibrosis Patient. PLoS ONE **6**:e24301.

36. **Chatterjee I**, **Herrmann M**, **Proctor RA**, **Peters G**, **Kahl BC**. 2007. Enhanced post-stationary-phase survival of a clinical thymidine-dependent small-colony variant of *Staphylococcus aureus* results from lack of a functional tricarboxylic acid cycle. J Bacteriol **189**:2936–2940.

37. **Kriegeskorte A**, **Grubmüller S**, **Huber C**, **Kahl BC**, **Eiff von C**, **Proctor RA**, **Peters G**,

Eisenreich W, **Becker K**. 2014. *Staphylococcus aureus* small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. Front Cell Infect Microbiol **4**:141.

Chapter 5:

Preliminary Investigation of *in vitro* Colony Size Distribution of *Staphylococcus aureus* Cystic Fibrosis Isolates

Introduction

The earliest published descriptions of *Staphylococcus aureus* small-colony variants (SCVs) from clinical specimens reported that small colonies were approximately 1/10 the size of wild-type (WT), normal colony (NC) *S. aureus* when compared on nutrient or blood agar (1-3) For many years, this somewhat arbitrary description has been the gold standard characterization for defining an SCV from *in vitro* growth. However, SCVs exhibit diverse phenotypes, including in colony sizes, even among clinical isolates from within individual patients (4-6). For example, it is common for *S. aureus* isolates collected from one patient with cystic fibrosis (CF) to display a range of colony sizes, often exhibiting progressively smaller colonies over time (X. Qin, J. Burns. B. Kahl personal communication). This phenotypic diversity among *S. aureus* isolates has not been rigorously or quantitatively described. Therefore, the specificity of the common definition of an SCV, and thus the accuracy of categorizing isolates in different laboratories, is limited at best. Furthermore, while the recovery of thymidine-dependent (THYD) SCVs from CF patients is associated with elevated patient morbidity (4), it is unknown if other SCV types or isolates with specific colony sizes also impact clinical outcomes in CF patients. Therefore, defining a rigorous definition of SCVs, based on the distribution of *in vitro* phenotypes of clinical isolates, is important for studying the associations between clinical outcomes and *S. aureus* colony size, the molecular mechanisms behind SCV formation, and optimal treatments of *S. aureus* SCVs.

In this work I report preliminary, descriptive statistical analyses on recently generated colony size measurement data in effort to ultimately develop a statistics-based definition of *S. aureus* SCVs based on the colony size distribution of a large collection of *S. aureus* isolates recovered from children with CF chronically infected with *S. aureus*. Growth experiments and analyses were completed for 851 *S. aureus* isolates on three different agar media known to be quite variable in their ability to support *S. aureus* growth (7, 8). Although primarily descriptive, these initial statistical analyses of colony size as a measure of *in vitro* growth provides a central framework for future studies to build off to associate colony size with important factors such as clinical outcome, antibiotic resistance, or genetic adaptation.

Materials and Methods

CF Patients. A total of 66 patients chronically infected with *S. aureus*, defined as being culture positive for at least 2 years, from multiple CF centers, including Seattle Children's Hospital (SCH), CF center Birmingham Alabama, CF Center Baylor College of Medicine, Boston Children's Hospital, and Children's Hospital Pittsburg were represented in this study. Complete patient demographic data for this study is presented in Appendix B.

Isolates used in this study. A total of clinical 851 isolates from the 66 patients, with an average of 13 *S. aureus* isolates per patient (ranging from a low of 3 to a high of 22), were acquired retrospectively from three separate sources: 319 isolates were obtained from a single center study of children (n=24) with CF at SCH (4), 290 isolates were obtained from an ongoing multi-center CF study described above, and the remaining 239 were obtained from the SCH *S. aureus* isolate core repository directed by Dr. Rafael Hernandez. Additionally an *in vitro* selected menadione-dependent SCV and parent *S. aureus* strain Newman (9) were also part of the isolate collection. *S. aureus* strain NTCC 8325 was used as a quality control strain for measuring colony size (methodology below). Additionally a single *in vitro* selected menadione-dependent (MEND) SCV and the parental strain *S. aureus* Newman from a previous study were also used (9). Working stocks of all isolates were stored at -80^{p} C in trypticase soy broth + 15% glycerol.

Media used in this study. Growth media used in this study included trypticase soy agar with 5% sheep blood (BAP, Remel, Lenexa, KS), Mueller-Hinton agar (MHA, Oxoid, Basingstoke, Hampshire, UK), and chocolate agar (Remel). These media were chosen using two criteria: 1) All three of these media are used by clinical microbiology laboratories for isolation (BAP and chocolate agar) and diagnostics (MHA) of *S. aureus*, and 2) previous work found that *S. aureus* SCVs auxotrophic for hemin, menadione, or thymidine exhibit variable colony size and morphology on these different media (7, 8), with the same isolate often growing differently on each media, providing multiple measures of colony sizes for clinical *S. aureus* CF isolates.

Image-based colony size determination. All *S. aureus* isolates were grown aerobically on chocolate agar overnight at 35^{o}C. Resulting cultures were suspended into 5mL PBS to an OD equivalent to 0.5 McFarland standard, ~1x10^8 cfu/mL. Dilutions resulting in 30-50 CFU for each strain, suspended in 100 uL of PBS were plated on to three media BAP, MHA, and chocolate agar. Plates were incubated aerobically overnight at 35^{o}C. Photographs of each plate with a sterile, 6.4 mm diameter paper disk size reference were taken after incubation. Colony sizes were measured with image-analysis software (Image J version 1.49u). Briefly, images were converted to 8-bit grey scale and the image threshold manipulated so that colonies were fully outlined. Using the ROI manager tool colony size diameter was measured using the measurement tool as minimum ferret diameter for ~20 colonies on each plate, the sterile disk was used as a control to verify correct threshold balance was achieved since it had a known diameter of 6.4mm. Average colony size was then calculated for each isolate on each media. *S. aureus* NTCC 8325 was used as a quality control for each run to verify there were no notable differences between lots of media and consistency between runs.

Auxotrophy testing. Isolates were plated in duplicate from freezer stocks as lawn cultures on MHA, which does not support the growth of thymidine-, hemin-, or menadione-dependent SCVs. Three separate sterile filter paper disks, one each impregnated with 10ug thymdine, 1 ug hemin, or 1ug menadione, were placed on the agar surface prior to incubation. One plate of each isolate was incubated aerobically while the other was incubated in 5% CO_2 ,both overnight at 35°C to test for CO_2 auxotrophy.

Descriptive Statistics. Each medium was analyzed separately by D'Agostino & Pearson omnibus (10), Shapiro-Wilk (11), and Kolmogorov-Smirnov normality tests (12) using Graphpad Prism 6 software to determine if the colony size data on each medium was well modeled by a normal distribution. Frequency distribution of average colony sizes for all isolates were also analyzed using Graphpad Prism 6 software to visualize histograms of the colony size values for each media. Data sets that did not pass normality tests (chocolate and BAP) were log-transformed with Graphpad Prism 6 to normalize the data. Descriptive statistics, including mean and standard deviations (these were back-transformed from values calculated based off the log transformation for chocolate and BAP) were calculated.

Median and interquartile ranges to measure colony size data spread on each medium. Median and interquartile ranges for colony sizes on each medium were calculated and plotted using Graphpad Prism 6. This analysis was completed in order to visualize individual data points and the spread of colony sizes for each medium. Additional measurements and plots for THYD-SCVs were also completed in order to visualize the distribution of colony sizes amongst the most common SCV type recovered from CF secretions.

Colony sizes of *S. aureus* isolates from single patients. Three representative patients supplying 11, 19, and 16 *S. aureus* isolates respectively, over the span of two years were selected at random to show if any trends between a patient and colony size could be observed or if notable changes in colony size could be observed over time.

Defining slow growing isolates without common auxotrophies. Descriptive statistics based on values obtained on MHA, i.e. mean and standard deviation (σ), were used to determine cut offs to define SCVs that did not display THY dependence. For the purpose of this study, only MHA was used to develop slower grower/SCV cut offs since the data were normally distributed on this media. The first cut off/category: designated the slow growers, were isolates that fell between 1σ and 2σ below the mean. The second category: designated the very slow growers, were isolates that fell more than 2σ below the mean. A third category: designated above normal, were any isolates that that fell above 1σ from the mean.

Results

Auxotrophy types identified in the isolate collection. Both thymidine-dependent (THYD) and hemin-dependent (HEMD) SCVs were found in our clinical isolate collection (Table 1). Approximately 19% isolates showed a definite auxotrophy. Of the SCVs with defined

Table 1. Breakdown of auxotrophy types found in the isolate set

Auxotrophy[a]	**# of Isolates**	**% of total**	**Average Colony Size MHA (mm)**	**Average Colony Size Chocolate (mm)**	**Average Colony Size BAP (mm)**
Thymidine	156	18.3	NO GROWTH	2.2	.87
Hemin	6	0.7	.35	2.1	1.24
Menadione	1	0.1	.44	1.46	.70

[a] Isolates with undefined auxotrophies behind SCV/slow growth are discussed later and data is presented in Table 7.

auxotrophies, ~ 96% were THYD and ~4% were HEMD. These findings align with previous literature showing that THYD-SCVs are the most common SCV type found in CF secretions, while

HEMD/MEND SCVs are less often detected (4, 13-15). Additionally, a number of slow growing isolates without identifiable auxotrophies or with undefined mechanisms resulting in small colony formation were found (discussed in more detail later). No CO_2 dependent isolates were recovered from any of our samples.

Tests for Normality. Individual normality tests on each three colony size data sets for all isolates showed that colony sizes followed a normal distribution on MHA but not on either chocolate or BAP (Table 2). It should be noted that THYD-SCVs are not represented in the MHA colony size data since they do not grow on this medium. A second normality test was completed for BAP after plotting all of the colony size values as a frequency distribution removing colony size data for THYD-SCVs (rationale and histogram data described in more detail in the next section below). Even after-removing THYD-SCVs from the data set, it still did not follow a normal distribution (Table 2). In order to normalize the data, values for chocolate and BAP were log-transformed (discussed in detail below).

Frequency distribution analysis and corresponding descriptive statistics of colony sizes. Histograms were constructed to visualize the distribution frequency of colony sizes on each medium in order to investigate what features of the data sets for BAP and chocolate were responsible for the non-normal distributions. Colony sizes on MHA, as expected, showed a visually normal distribution (Figure 1A). Descriptive statistics including mean, range, and standard deviation (σ) are summarized in Table 3A. Again, with MHA, it should be noted that

Table 2. Normality Tests and generated values completed for each growth medium

	D'Agostino & Pearson omnibus normality test	Shapiro-Wilk normality test	Kolmogorov-Smirnov normality test

MHA[e]	K2[a]	1.105	W[b]	0.9957	KS distance[c]	0.0254
	P value	0.5754	P value	0.0544	P value	> 0.1000
	Passed normality test (alpha=0.05)	Yes	Passed normality test (alpha=0.05)	Yes	Passed normality test (alpha=0.05)	Yes
	P value summary	ns	P value summary	ns	P value summary	ns
Chocolate	K2	36.18	W	0.9736	KS distance	0.09458
	P value	< 0.0001	P value	< 0.0001	P value	< 0.0001
	Passed normality test (alpha=0.05)	No	Passed normality test (alpha=0.05)	No	Passed normality test (alpha=0.05)	No
	P value summary	****	P value summary	****	P value summary	****
BAP	K2	33.27	W	0.9731	KS distance	0.07573
	P value	< 0.0001	P value	< 0.0001	P value	< 0.0001
	Passed normality test (alpha=0.05)	No	Passed normality test (alpha=0.05)	No	Passed normality test (alpha=0.05)	No
	P value summary	****	P value summary	****	P value summary	****
BAP w/o THYD SCVs[d]	K2	21.84	W	0.9858	KS distance	0.05298
	P value	< 0.0001	P value	< 0.0001	P value	< 0.0001
	Passed normality test (alpha=0.05)	No	Passed normality test (alpha=0.05)	No	Passed normality test (alpha=0.05)	No
	P value summary	****	P value summary	****	P value summary	****

[a] K2 is the statistic generated by the D'Agostino & Pearson normality test

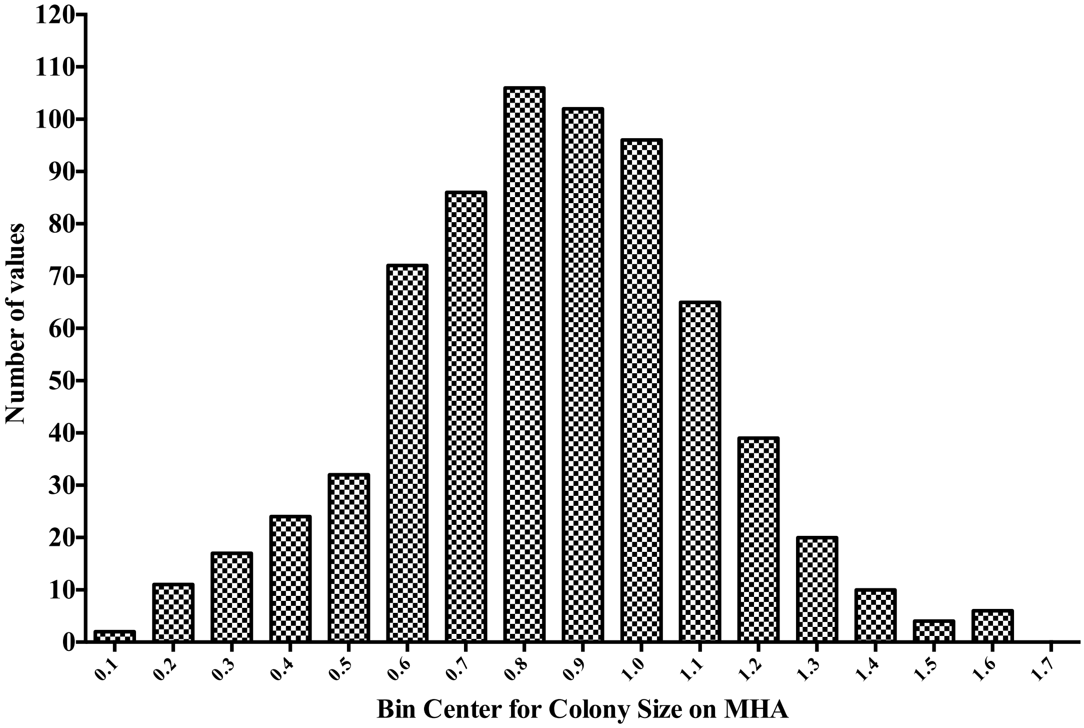
[b] W is the statistic generated by the Shapiro-Wilk normality test

[c] KS distance is the statistic generated by the Kolomogorov-Smirnov normality test

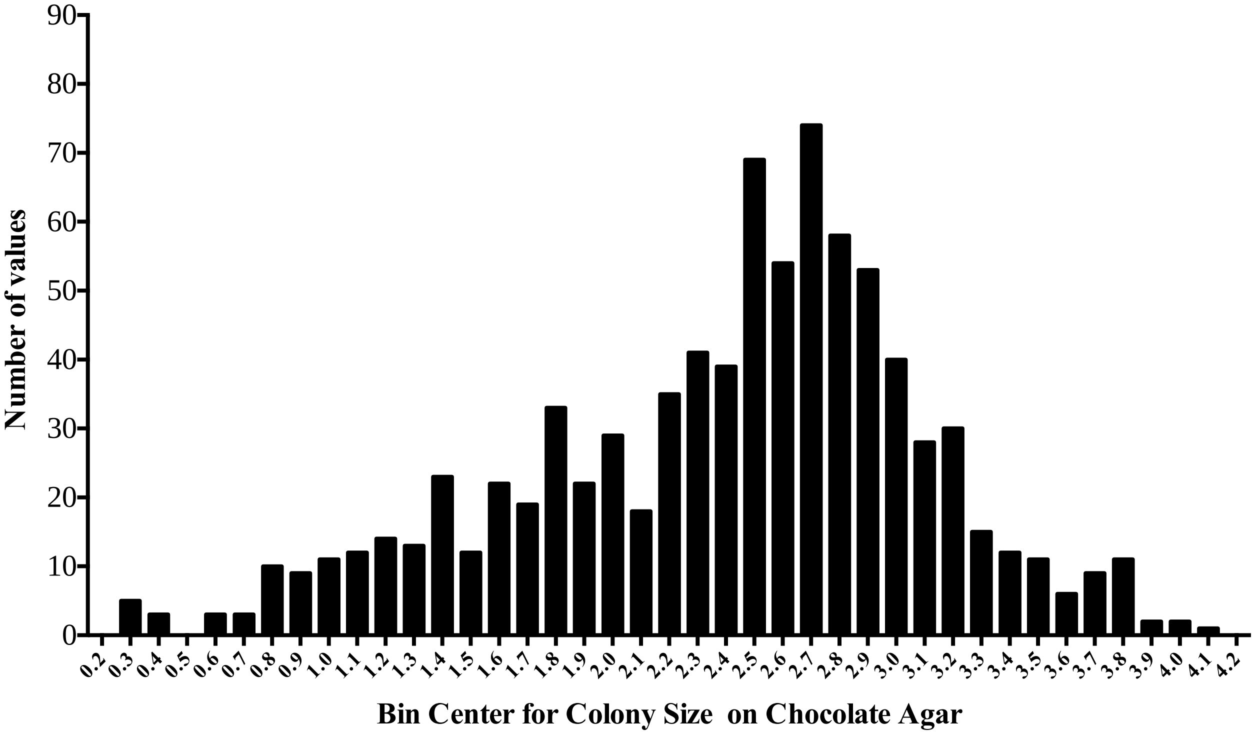
[d] BAP was examined re-tested without thymidine-dependent (THYD) SCVs due to their abundance and ability to scew the distribution of the data

[e] MHA was the only media to show normal distribution

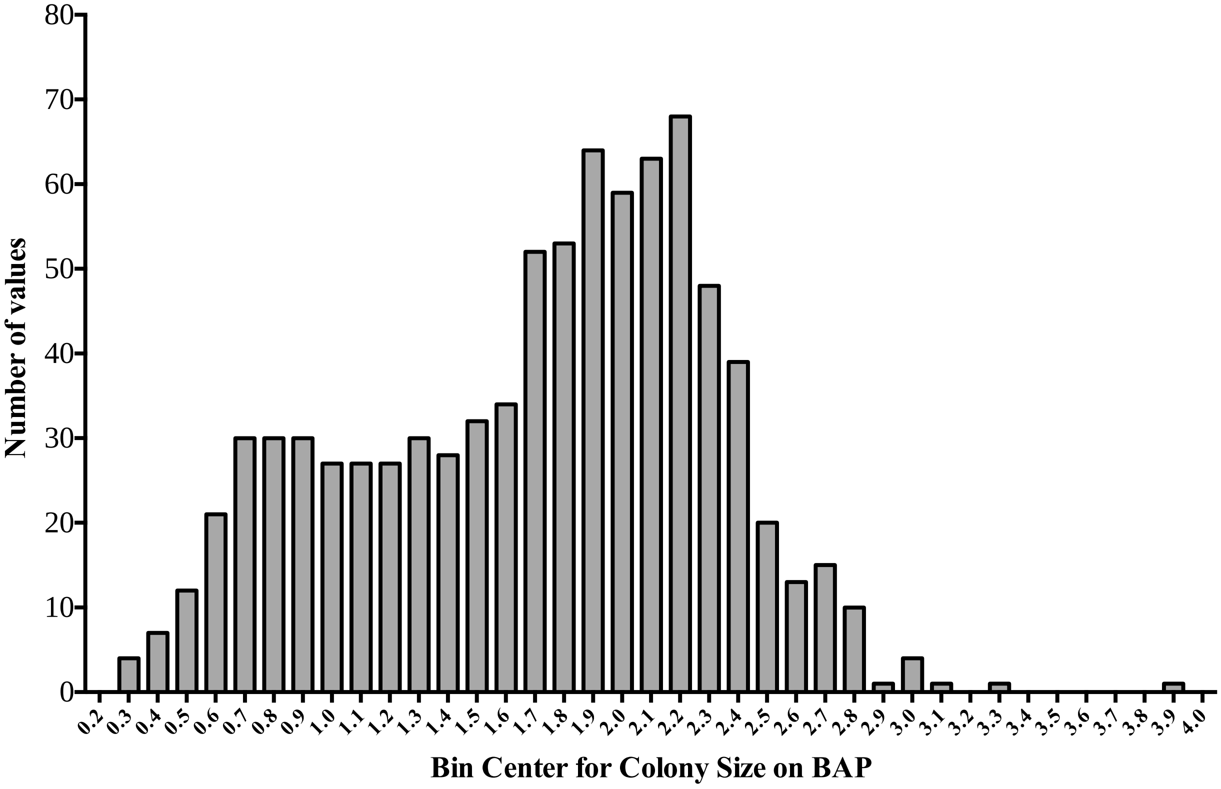
A


Number of values
120
110
100
90
80
70
60
50
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Bin Center for Colony Size on MHA

B


Number of values
90
80
70
60
50
40
30
20
10
0
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
Bin Center for Colony Size on Chocolate Agar

C


Number of values
80
70
60
50
40
30
20
10
0
0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3 2.4 2.5 2.6 2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0
Bin Center for Colony Size on BAP

D

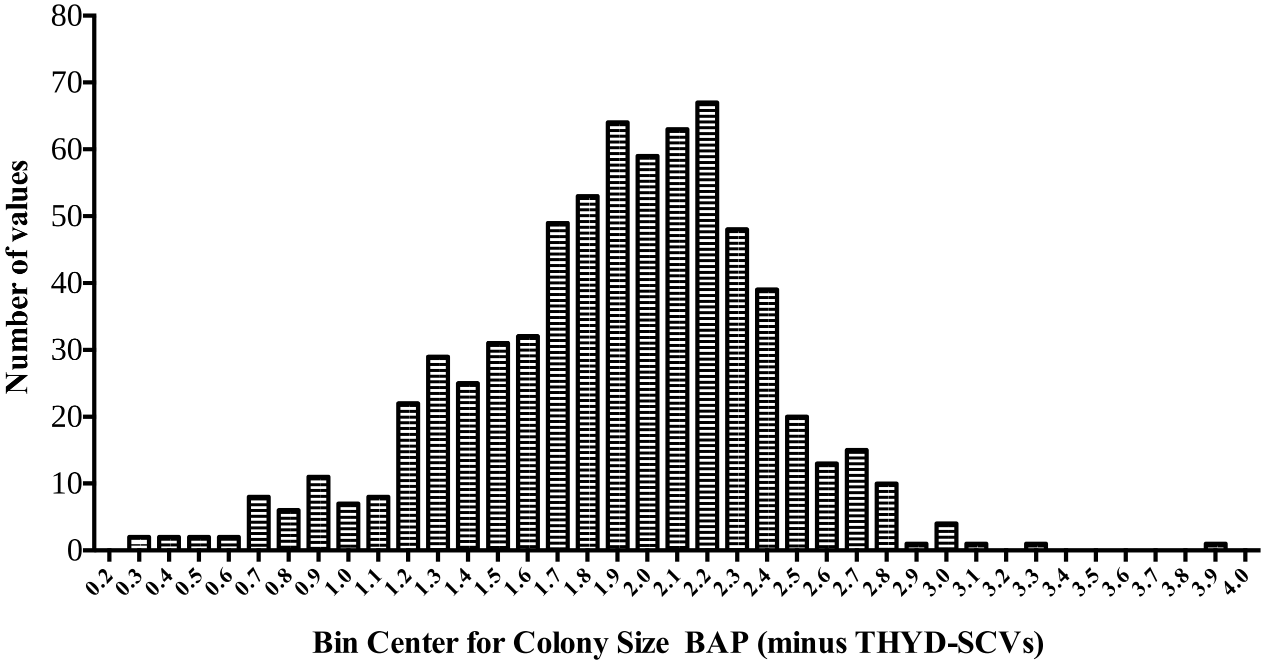

Number of values
80
70
60
50
40
30
20
10
0
0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3 2.4 2.5 2.6 2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0
Bin Center for Colony Size BAP (minus THYD-SCVs)

Figure 1. Frequency distribution of colony sizes of the isolate set on three growth media. (A) Colony size distribution for isolates that grew on MHA, THYD-SCVs that do not grow on this media are not represented in this distribution. (B) Colony size distribution on chocolate agar for all isolates in the collection. (C) Colony size distribution on blood agar plate (BAP) for all isolates, and (D) with THYD-SCVs removed from the dataset to show that the peak centered around 0.8 – 0.9 mm (C) was due to this auxotrophy group.

Table 3A. Descriptive statistics of colony sizes on MHA (normally distributed)

	Media
Descriptive Statistics[a]	MHA
Mean Colony Size (mm)	0.84
Range (mm)	.1070 – 1.62
Standard Deviation (σ)	0.27
Mean - 1 σ	0.58
Mean - 2 σ	0.31

Note: These measurements were used in later analyses to define preliminary cut offs based on colony size

Table 3B. Descriptive statistics of colony sizes on media not modeled by a normal distributed

	Media		
Descriptive Statistics	Chocolate	BAP	BAP without THYD SCVs
Log-transformed mean colony size[a]	0.35	0.20	0.26
Back-transformed mean colony size[b]	2.25	1.57	1.81
Log-transformed standard deviation (σ)	0.17	0.19	0.14
Back-transformed σ	1.48	1.56	1.37
Mean - 1 σ	0.78	0.01	0.44
Mean - 2 σ	-0.70	-1.54	-0.93

[a] Log Transformed data was completed to normalize the data

[b] Data was Back-transformed to provide meaningful values

the 156 THYD-SCVs did not grow on this medium and were excluded from this analysis, leaving 695 (851 minus the 156 THYD-SCVs) isolates represented in the MHA histogram. Values on chocolate agar did show a somewhat central peak, however long tails as well intermittent peaks at certain colony sizes were observed (Figure 1B). The BAP distribution showed a second peak of values centered on 0.8 – 0.9 mm near one tail of the frequency distribution (Figure 1C). Because THYD-SCVs were present a relative high frequency of ~20% in our population, and grew with an average diameter of .87mm, we investigated whether the observed distribution shift was due to the THYD-SCVs. Upon removing these isolates from the histogram analysis, colony size frequency distribution values for the remaining non-THYD isolates 695 isolates) on BAP media showed a more central peak of values, however long tails in both directions were observed and another peak between 1.2-1.5 mm remained. Log-transformed as well as back-transformed descriptive statistics for chocolate, BAP, and BAP without THYD-SCVs are shown in Table 3B.

Median and interquartile range as a measure of spread. Scatter plots of average colony size data for each isolate on the three media with error bars showing median and interquartile ranges are shown in Figure 2. These plots show that colony sizes, and therefore isolate growth, is more variable on BAP and chocolate agar when compared to MHA. Compared to MHA, both BAP and chocolate are very rich media, with chocolate being the most nutritious, which could explain this variability. Additionally BAP is plotted without THYD-SCVs (Figure 2) the median increases and the interquartile range decreases (Table 4), however the range of colony sizes remains similar to BAP alone. In order to visualize the variability of just the THYD-SCVs, plots of these isolates on BAP and chocolate were graphed (MHA was not assessed since THYD-

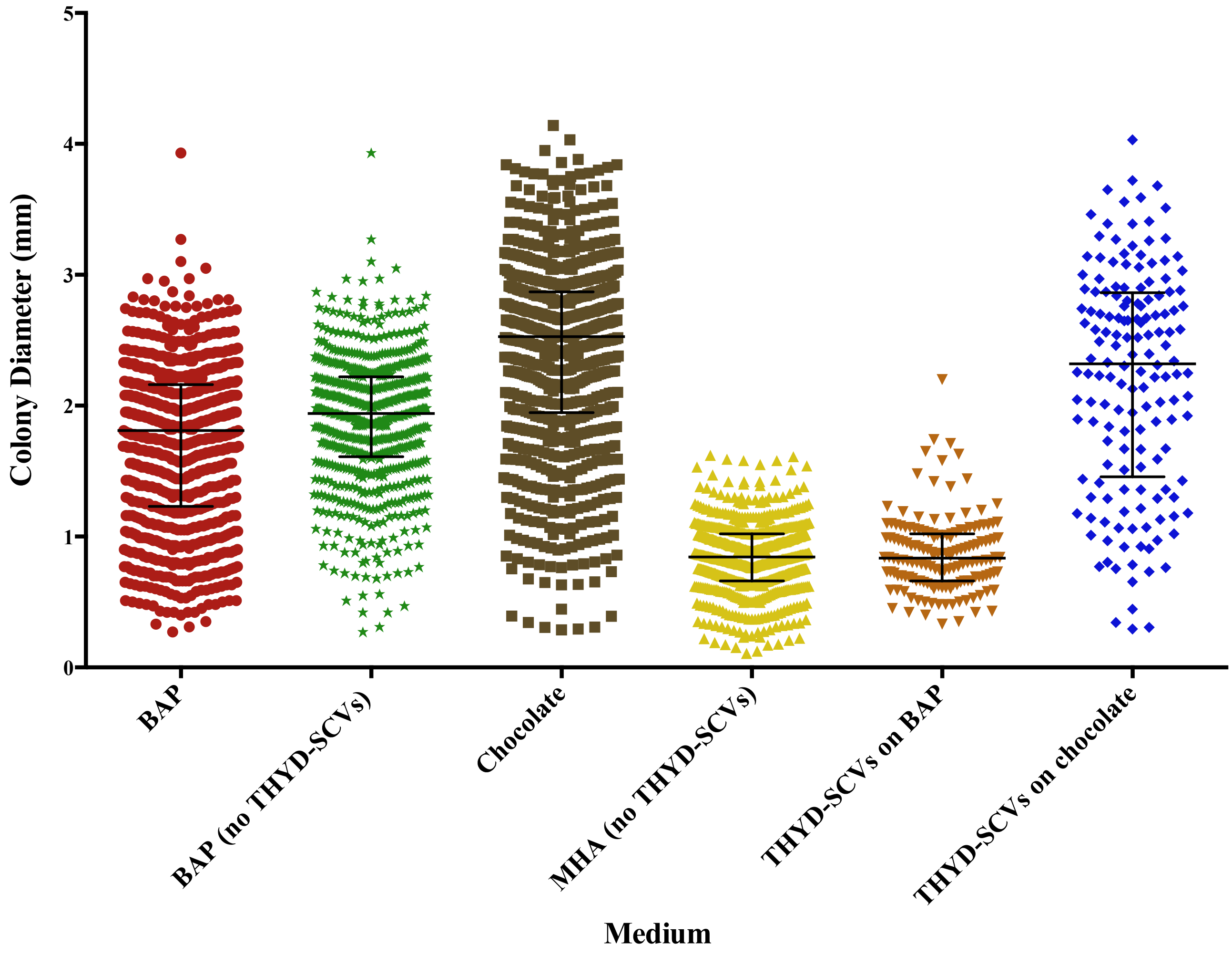


Figure 2. Scatter-dot plots showing spread of colony sizes. Each data point represents the average colony size of a given isolate on media specified on the x-axis. Error bars show the median and interquartile range of the data set in order to visualize the spread of the values for the isolates on a given medium. Thymidine-dependent (THYD) SCVs were assessed separately on both blood agar (BAP) and chocolate agar (plots furthest to the right).

Table 4. Median and interquartile ranges for average colony sizes

	Medium					
Descriptive Statistics	BAP[a]	BAP (no THYD[b]-SCVs)	Chocolate agar	MHA[c]	THYD-SCVs BAP	THYD-SCVs Chocolate
Number of values analyzed	851	851	851	695	156	156
Minimum value	0.27	0.27	0.29	0.11	0.33	0.29
25% Percentile	1.23	1.61	1.95	0.66	0.66	1.46
Median	1.81	1.94	2.53	0.84	0.84	2.32
75% Percentile	2.16	2.22	2.87	1.02	1.02	2.86
Interquartile Range (measure of spread)	0.93	0.61	0.92	0.36	0.36	1.41
Maximum	3.93	3.93	4.14	1.62	2.20	4.03

[a] Blood agar plate (BAP)

[b] Thymidine-dependent (THYD)

[c] Mueller-Hinton Agar (MHA) only 695 isolates were analyzed since THYD-SCVs do not grow on this medium

SCVs do not grow on this medium). These plots demonstrate that THYD-SCVs show less colony size variability on BAP than on chocolate (Figure 2). Extracellularly available THY is at higher concentrations in chocolate compared to BAP, therefore on chocolate THYD-SCVs should be growing under fully complemented growth conditions; this finding that chocolate varies in its ability to complement THYD-SCV growth, suggests that the mechanism behind THYD in many of isolates is not the only driver of slow growth and small colony formation.

Colony sizes of *S. aureus* isolates from single patients. Isolates recovered from three representative patients from our collection, supplying an average of 15 longitudinally collected *S. aureus* clinical isolates over the span of two years, were selected at random to show if any trends between patient and colony size could be observed as well as if colony sizes changed over time. Through this initial observational analysis, variable colony sizes on the three media are observed for all patients (Figure 3). Patients 1 and 2 showed more THYD-SCVs (shown as zero values for MHA in Figure 3) than patient 3 and patient 2 had an interesting isolate (2L) that grew slowly on all three of the media tested (Figure 3). The data suggest that recovered phenotypes don’t follow a noticeable trend towards slower growth over time, however for patient 3 THYD-SCVs were recovered at later time points than for patients 1 and 2 (Figure 3). Patient age at the time of each culture is shown in Table 5.

SCV/Slow growing isolates with undefined auxotrophies. Mean and standard deviation (σ) calculations obtained on MHA (Table 6A), were used to determine cut offs to define SCV/slow growing isolates (Table 6B). For the purpose of this study, only MHA was used to develop SCV/slow grower cut off values since the data were normally distributed on this media. Using

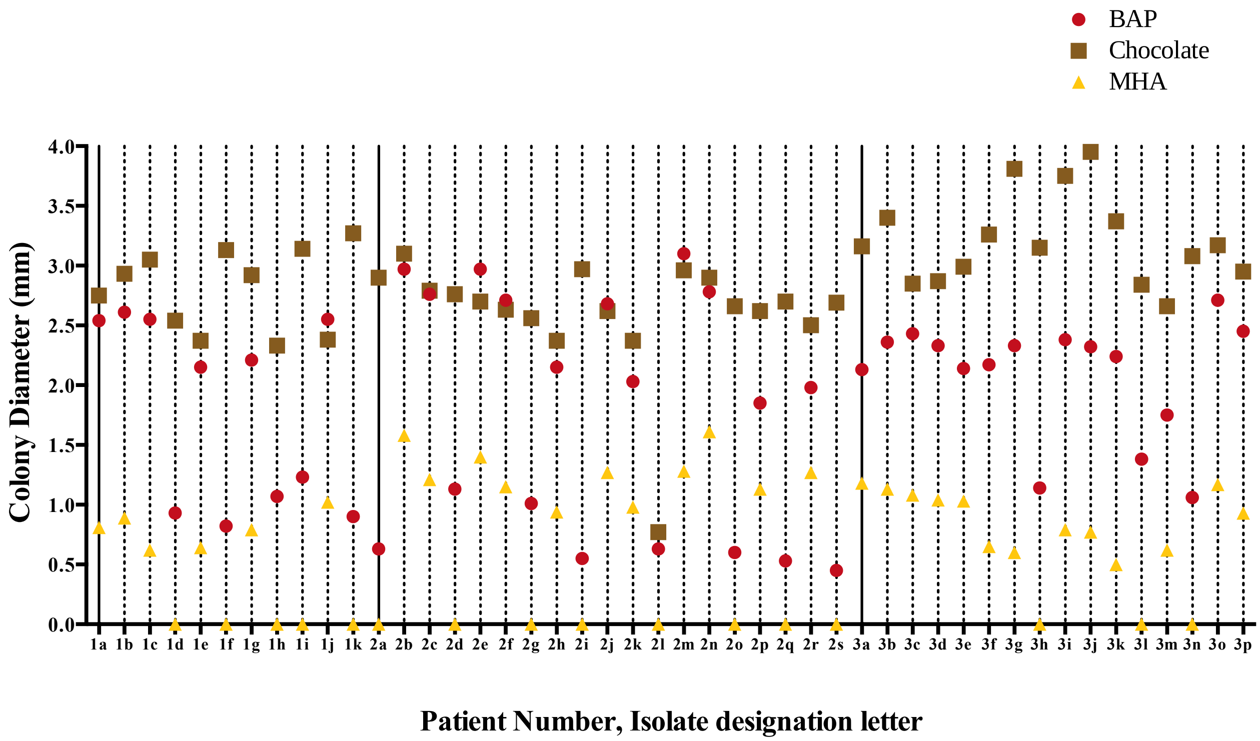


Figure 3. Average colony size for three representative patients on three growth media. Average colony diameters for each isolate from three separate patients on blood agar (BAP), chocolate, and Mueller-Hinton agar (MHA) are shown above. Patients are designated on the x-axis by number (1, 2, or 3) and longitudinal isolates are designated alphabetically i.e. ‘a’ was collected before ‘b’, ‘b’ before ‘c’… etc. on the x-axis. A breakdown of the age of the patient during culture of each isolate is shown in Table 5. Zero values measured on MHA are thymidine-dependent (THYD) SCVs that do not grow on this media. Dotted vertical lines are provided to help align the average colony sizes to a certain patient/isolate combination and solid vertical lines represent the first isolate from that patient to help designate where a certain patient’s isolates begin.

Table 5. Patient age corresponding to *S. aureus* cultures graphed in Figure 3

Patient #, Isolate Designation letter	Patient Age (years)
1a	13.31
1b	13.56
1c	13.81
1d	14.08
1e	14.35
1f	14.56
1g	14.9
1h	14.9
1i	15.07
1j	15.3
1k	15.3
2a	13.38
2b	13.38
2c	13.65
2d	13.81
2e	13.94
2f	13.94
2g	13.94
2h	14.17
2i	14.17
2j	14.38
2k	14.59
2l	14.59
2m	14.87
2n	14.87
2o	14.87
2p	15.09
2q	15.09
2r	15.3
2s	15.3
3a	15.14
3b	15.17
3c	15.17
3d	15.42
3e	15.52
3f	15.67
3g	15.73
3h	15.86
3i	15.94
3j	16.21
3k	16.4
3l	16.4
3m	16.51
3n	16.51
3o	16.8
3p	16.97

Note: Areas are shaded in order to visually group a patients isolates together

Table 6A Descriptive statistics for Mueller-Hinton agar (MHA) to establish cut off values based on mean and standard deviation

Statistic [a]	Value (mm)
Mean + 2σ	1.38
Mean + 1σ	1.11
Mean	0.84
Standard deviation (σ)	0.27
Mean - 1σ	0.57
Mean - 2σ	0.30

[a] Values were calculated based off the 694 isolates that grew on this media and excludes THYD-SCVs that do not grow on MHA

Table 6B Colony size categorization cut offs based on distribution of colony sizes on MHA

Preliminary Categorization	Cut off Values (mm)
Above normal	>.1.11
Normal	≥.57-1.11
Slow Grower Ranges	.3-.57
Very Slow Grower Ranges (SCVs)	≤.3

the values outlined in Table 6B, we identified a total of 91 slow growing isolates (Table 7), of these 18 isolates grew ≤0.3mm in diameter and represent a meaningful phenotype that fell 2σ below the mean, designated “very slow growers”. 66 isolates fell between more than 1σ but less than 2σ below the mean and also represent a statistically noteworthy portion of the population that also grows slowly, designated “slow growers” (Tale 7). Lastly, it should be noted that all HEMD/MEND (n=7) SCVs fell more than 1σ below the mean and were categorized within one of these two “slow-grower” categories. Two HEMD isolates as “very slow growers” with the remaining four, and the MEND isolate, as “slow growers” (Table 7).

Discussion

To my knowledge, this work is the first large-scale analysis of its kind examining naturally occurring *in vitro* colony size distributions of *S. aureus* clinical CF isolates. Although preliminary and primarily descriptive, the statistical as well as observational analyses presented here provide a critical framework for which to ultimately develop both a methodology for categorization of clinical isolates based on colony size and a quantitative definition of *S. aureus* SCVs. The range of *in vitro* colony size phenotypes we observed on BAP, chocolate, and MHA shows colony size depends substantially on concentrations of specific nutrients, and therefore presents a significant challenge when attempting to categorize isolates based off colony size.

For the purpose of this work, colony size distribution values measured on MHA were used to define preliminary cut off values to assign above normal, normal, slow grower, and very slow grower categories. Potentially using MHA as a recommended SCV-indicator medium in our mission to provide a quantitative definition for SCVs has both advantages and limitations.

Figure 7. Isolate categorization based on colony size phenotype as determined by descriptive statistics for Mueller-Hinton Agar

Colony Size phenotype Categorization	# of HEMD/MEND[a] isolates	# of isolates w/o[b] defined auxotrophies (Out of 694)	Percentage of total population in each category
Very Slow Growing	2	18	2.88%
Slow Growing	5	66	10.23%
Normal	0	508	73.19%
Above Normal	0	94	13.54%

[a] Hemin-dependent (HEMD), Menadione-dependent (MEND)

[b] Without (w/o)

Advantages include: 1) in our case, the data were normally distributed and therefore basic descriptive statistics could be used to assign colony size categories, 2) MHA is a good discriminatory reference medium in that SCVs with more common auxotrophies i.e. THYD, HEMD, and MEND either don't grow (in the case of THYD-SCVs) or grow statistically significantly slower on this medium (in the case of HEMD/MEND SCVs) based on our analyses. Therefore when either assaying for no growth or slow growth based on statistical measures we were able to capture known SCVs with our approach, 3) When thinking long-term MHA is readily available in clinical microbiology laboratories and could possibly prove to be a useful growth medium for a *S. aureus* SCV screening assay from clinical samples. Limitations include: 1) The data analyzed here are preliminary and collecting additional colony size phenotype data from more clinical *S. aureus* isolates, even extending out from CF secretions, may later alter the distribution data so that it is no longer normally distributed potentially making the above categorization analysis obsolete. 2) Our data suggest that MHA fails to capture the range of complemented phenotypes that BAP and chocolate agar are able to display (Figure 2) and therefore potentially noteworthy phenotypic nuances such as variably in complementation of growth or unusual colony morphologies amongst isolates may go overlooked MHA were used alone. 3) MHA does not support the growth of THYD-SCVs and therefore presents limitations when attempting to culture SCVs and could only be used after an initial culture on a richer medium.

In reality, any approach used when attempting to assign categories based on colony size has both advantages as well as limitations and will prove to be an iterative process depending on the question one is trying to answer. Our findings presented in this work, if nothing else, underscore the importance of culturing and studying likely SCVs on both a rich (BAP or chocolate) and relatively less rich (MHA) medium for maximum accuracy and sensitivity. We also present data, from a purely observational trend, that suggests colony size of an isolate grown on one medium does not necessarily predict its colony size on another medium (Figure 3), therefore the use of a combination of media captures the most information for a given isolate.

The process in which we define an SCV will be iterative, and will depend greatly on either the research question or whether we are attempting to develop a diagnostic methodology optimized for integration into a clinical microbiology laboratory. The analyses presented here are critical initial steps for future projects in the Hoffman laboratory. Such projects include correlating colony size with clinical outcomes in patients, and this approach would require a stringent, well-defined set of characteristics derived from colony size distribution that can be easily obtained and interpreted by a clinical laboratory to maximize patient care and treatment. Second, we hope to use this analysis to correlate colony size with genomic changes in effort to examine the mechanisms behind slow growth *in vitro*, this type of study however may benefit from casting a wider net and defining less-stringent parameters in order to maximize the chances of discovering a novel mutation that may contribute to the SCV or slow grower formation in *S. aureus* clinical isolates.

References

1. **Proctor RA**, **Balwit JM**, **Vesga O**. 1994. Variant subpopulations of as cause of persistent and recurrent infections. Infect Agents Dis **3**:302–312.

2. **Proctor RA**, **van Langevelde P**, **Kristjansson M**, **Maslow JN**, **Arbeit RD**. 1995. Persistent and relapsing infections associated with small-colony variants of . Clin Infect Dis **20**:95–102.

3. **HALE JH**. 1947. Studies on *Staphylococcus* mutation; characteristics of the G (gonidial) variant and factors concerned in its production. Br J Exp Pathol **28**:202–210.

4. **Wolter DJ**, **Emerson JC**, **McNamara S**, **Buccat AM**, **Qin X**, **Cochrane E**, **Houston LS**, **Rogers GB**, **Marsh P**, **Prehar K**, **Pope CE**, **Blackledge M**, **Déziel E**, **Bruce KD**, **Ramsey BW**, **Gibson RL**, **Burns JL**, **Hoffman LR**. 2013. small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis **57**:384–391.

5. **Besier S**, **Ludwig A**, **Ohlsen K**, **Brade V**, **Wichelhaus TA**. 2007. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of . Int J Med Microbiol **297**:217–225.

6. **Chatterjee I**, **Kriegeskorte A**, **Fischer A**, **Deiwick S**, **Theimann N**, **Proctor RA**, **Peters G**, **Herrmann M**, **Kahl BC**. 2008. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of . J Bacteriol **190**:834–842.

7. **Precit MR**, **Wolter DJ**, **Griffith A**, **Emerson J**, **Burns JL**, **Hoffman LR**. 2016. Optimized In Vitro Antibiotic Susceptibility Testing Method for Small-Colony Variant . Antimicrob Agents Chemother **60**:1725–1735.

8. **Kahl BC**, **Becker K**, **Löffler B**. 2016. Clinical Significance and Pathogenesis of Staphylococcal Small Colony Variants in Persistent Infections. Clin Microbiol Rev **29**:401–427.

9. **Hoffman LR**, **Déziel E**, **D'Argenio DA**, **Lépine F**, **Emerson J**, **McNamara S**, **Gibson RL**, **Ramsey BW**, **Miller SI**. 2006. Selection for small-colony variants due to growth in the presence of Pseudomonas aeruginosa. Proc Natl Acad Sci USA **103**:19890–19895.

10. **D'AGOSTINO R**, **Pearson ES**. 1973. Tests for departure from normality. Empirical results for the distributions of b2 and√ b1. Biometrika.

11. **Shapiro SS**, **Wilk MB**. 1965. An analysis of variance test for normality (complete samples). Biometrika.

12. **Ghasemi A**, **Zahediasl S**. 2012. Normality Tests for Statistical Analysis: A Guide for Non-Statisticians. International Journal of Endocrinology and Metabolism **10**:486–489.

13. **Kahl B**, **Herrmann M**, **Everding AS**, **Koch HG**, **Becker K**, **Harms E**, **Proctor RA**, **Peters G**. 1998. Persistent infection with small colony variant strains of in patients with

cystic fibrosis. J Infect Dis **177**:1023–1029.

14. **Gilligan PH**, **Gage PA**, **Welch DF**, **Muszynski MJ**, **Wait KR**. 1987. Prevalence of thymidine-dependent in patients with cystic fibrosis. J Clin Microbiol **25**:1258–1261.

15. **Besier S**, **Zander J**, **Kahl BC**, **Kraiczy P**, **Brade V**, **Wichelhaus TA**. 2008. The thymidine-dependent small-colony-variant phenotype is associated with hypermutability and antibiotic resistance in clinical isolates. Antimicrob Agents Chemother **52**:2183–2189.

Chapter 6:

Discussions and Future Directions

Overview

History and clinical significance of small colony variant *Staphylococcus aureus*

Atypical, slow-growing small colony variant (SCV) bacteria have been reported in the literature beginning in the early 1900's (1, 2), with SCV *Staphylococcus aureus* being the best studied. Nevertheless, both phenotypic and genetic/genomic characterizations of SCV *S. aureus* remains a relatively unexplored field of study that has only gathered attention from researchers in the past ~20 years. It is appreciated that *S. aureus* SCVs are linked to a variety of chronic, recurrent, antibiotic-refractory infections like those seen in the lungs of patients with genetic disorder cystic fibrosis (CF) (3-7). Additionally, the impaired growth exhibited by SCVs *in vitro* renders SCV detection and diagnostics challenging, and at times impossible, when using traditional clinical microbiological approaches.

The most common types of SCVs recovered from clinical samples have defects in critical metabolic pathways resulting in auxotrophies for specific nutrients such as hemin (HEM), menadione (MEN), or thymidine (THY) (8). SCVs with specific auxotrophies are known to be selected both *in vitro* and *in vivo* following exposure to antimicrobial compounds. Aminoglycoside antibiotics (9, 10) and exoproducts secreted by the co-infecting species *Pseudomonas aeruginosa* (11, 12) can select for HEMD and MEND-SCVs. Furthermore, folate-pathway inhibitors can select for thymidine-dependent (THYD) SCVs (13, 14). THYD-SCVS are the most common type cultured from CF secretions and their recovery from the CF lung is independently associated with poor clinical outcomes (13). This suggests that SCVs may be an important pathogen in CF, or a maker for advanced lung disease. However only a limited number of studies have investigated phenotypic and genomic characteristics of clinical SCVs.

Summary of work presented in this thesis

The goal of the work presented in this thesis was to address some of knowledge gaps introduced above through a combination of novel phenotypic and genetic approaches to further our understanding of these atypical *S. aureus* variants. In Chapter 2, I defined *S. aureus* SCV antibiotic resistance profiles and presented the development of a novel, optimized antibiotic susceptibility diagnostic test for SCVs. Chapter 3 focused on host-pathogen interaction studies examining the pro-inflammatory features of THYD-SCVs, which investigates the role SCVs play during host infection and begins to address why THYD-SCVs may be associated with poor clinical outcomes. In Chapter 4, I summarized a preliminary analysis of next generation whole-genome sequencing (WGS) data for clinical *S. aureus* isolates in effort to define genetic mechanisms responsible for SCV formation in clinical CF isolates. Lastly, in Chapter 5 I presented a novel large-scale phenotypic screen examining the distribution of colony sizes for a collection of clinical *S. aureus* CF clinical isolates to provide a descriptive framework analysis of the range of phenotypes they display *in vitro*.

Each of these individual chapters as a body of work addresses a critical subject concerning *S. aureus* SCVs, for which unique approaches and meaningful conclusions that further our knowledge of SCVs are presented.

Discussions and Future Directions

Chapter 2: An Optimized *in vitro* Antibiotic Susceptibility Testing Method for Small Colony Variant *Staphylococcus aureus*

When I started my graduate work, *S. aureus* SCVs were known to be associated with persistent, relapsing infections (3, 15-17) and poor clinical outcomes in patients (13). Despite this, due to their unusual appearance and poor growth *in vitro*, no standardized methods for antibiotic susceptibility testing (AST) had been developed and therefore no treatment guidelines were available for clinical laboratories and physicians to direct treatment of SCV-related infections. The development, employment, and susceptibility data generated by the AST method (18) discussed in detail in Chapter 2 has since gone on to inform clinical practice at Seattle Children's Hospital by providing empirical treatment options for CF patients infected with *S. aureus* SCVs. The impact this work has had on clinical practice speaks to the importance of addressing issues surrounding SCVs. Furthermore, this work provides rationale for future studies focused on validating our methodology with a clinical trial investigating if directing SCV treatment based on results obtained from AST positively influences patient outcomes. Future studies scrutinizing the legitimacy of the AST methodology have the potential to greatly impact how SCV related infections are managed at both the clinical microbiology laboratory as well as provider level in the healthcare system.

Chapter 3: Thymidine-dependent *Staphylococcus aureus* Small Colony Variants Produce Excessive cyclic-di-AMP that Modulates STING-dependent Type I Interferon Production

The work presented in Chapter 3 was completed in collaboration with a fellow graduate student Qing Tang and Dr. Joshua Woodward and begins to address the very perplexing question of why thymidine-dependent SCVS (THYD-SCVs), the most common type recovered from CF lung secretions (3-5, 13), are associated with advanced lung disease in CF patients (13). Our findings show that the small signaling molecule – cyclic-di-AMP (CDA) is abundantly expressed in THYD-SCVs and plays a pro-inflammatory role during infection. Previous studies investigating SCV pathogenesis have primarily focused on intracellular persistence of SCVs (10, 19, 20) and the changes in SCV metabolism associated with persistence infections (21). However the pro-inflammatory role we report is a novel mechanism likely contributing to SCV pathogenesis and lung disease. Additionally, the work presented here provokes further questions regarding the mechanisms behind increased CDA abundance in THYD-SCVs. It is known that an essential gene involved in pyrimidine metabolism, thymidine kinase – *tdk*, resides in an operon with a CDA binding protein *pstA*, an uncharacterized PII like protein (22). Future studies examining the details behind these interesting features are warranted to further characterize SCV CDAs potential role in pyrimidine metabolism and SCV physiology and how it relates to infections. Additionally, sputum focused studies measuring CDA levels and inflammatory markers in CF sputum coupled with analyzing associations with clinical outcomes would provide further evidence of the pro-inflammatory role of THYD-SCVs.

Chapter 4: Preliminary Examination of Genetic Mechanisms behind *S. aureus* Thymidine-Dependent and Hemin-Dependent Small-Colony Variant Formation in Cystic Fibrosis Clinical Isolates

Previous work investigating the genetic mechanisms behind SCV formation have been primarily targeted approaches defining genetic changes in SCVs selected *in vitro* following antimicrobial exposure (9, 11, 23, 24) with only a limited number of studies examining the genetic changes in clinical SCV isolates; which predominantly targeted specific genes involved in either HEM, MEN, or THY biosynthesis (25-28). Next-generation whole-genome sequencing (WGS) is a powerful and high-throughput tool to study the genetic adaptations behind meaningful *in vitro* phenotypes. In Chapter 4, we employed such methodology in collaboration with Dr. Steven Salipante and identified a myriad of potentially important genetic lesions in metabolic pathways involved in pyrimidine biosynthesis as well as many in central metabolism present in clinical THYD-SCVs but absent in NC *S. aureus* isolates. Chapter 4 solely focuses on metabolic genes however preliminary data gazing has showed that a number of cell wall as well as stress response genes involved in nitric oxide production were also mutated in THYD-SCVs (Appendix A). The sheer amount of genomic data this study generated can be used to answer a variety of clinically relevant questions regarding *S. aureus* adaptation in the CF lung environment.

Previous groups have shown that SCVs with mutations in the same gene responsible for THYD, thymidylate synthase – *thyA*, recovered from the same patient display dissimilar colony morphologies and growth patterns during *in vitro* studies (5, 16). Additionally in Chapter 5 (discussed below) I present data showing that not all THYD-SCVs grow at the same rates on agar based media even when plenty of external THY is present. Taken together, these findings suggest that the mutations resulting in THYD may not be the whole story. Future studies will be focused on more accurately defining genes that contribute to SCV formation over the course of infection in the CF lung, and investigating what genes are involved in small colony formation of clinical *S.*

aureus isolates given that a range of colony size phenotypes are observed *in vitro* (discussed in chapter 5).

Chapter 5: Preliminary Investigation of *in vitro* Colony Size Distribution of *Staphylococcus aureus* Cystic Fibrosis Isolates

Although openly discussed in the literature as SCVs, the current classification of what defines an SCV is fairly arbitrary. Most reports state that SCVs are defined by their slow growth *in vitro* and are approximately 1/10 the size of normal colony *S. aureus* isolates, or display common auxotrophies such as THY, HEM, or MEN dependence. Previous studies as well as data shown in Chapter 5 reveal that even an SCV defined with the above criteria will grow entirely different depending on nutrient content in the media that can mask the SCV phenotype (2, 18). Additionally, SCVs even within the same auxotrophic group will grow at various rates on different media and even on the same media (29). The analyses presented in Chapter 5 are the first steps of a long road in the mission to develop a rigorous, quantitative method for defining an SCV based on the distribution of colony sizes of clinical *S. aureus* CF isolates employing a combination of growth media and descriptive statistics. For the purpose of identifying SCVs lacking the classic auxotrophies in this analysis we used distribution data collected from MHA, given that is was modeled by a normal distribution and precise cut off values could be established using basic descriptive statistics such as mean and standard deviation.

For future studies, the definition of an SCV would likely present a dynamic, iterative process that may be adjusted based on what types of either clinically driven or basic research questions regarding the SCV phenotype are being investigated. For example, the WGS approach presented in Chapter 4 and the colony size distribution data presented in Chapter 5 could be combined into a genome-wide association study to examine the mechanisms responsible for colony size formation in clinical *S. aureus* clinical isolates and eventually expanded to also include antibiotic resistance or even virulence genes. Defining an SCV for this type of study may benefit from being less stringent in order to diversify and maximize your genetic hits, which can be validated through targeted genetic approaches. In contrast, a diagnostic definition of an SCV that could be used by a clinical laboratory would require a strict, readily interpretable definition in order to accurately, and precisely identify SCVs present within a clinical sample and then report them to help direct treatment. Preliminary findings and analysis presented in this chapter suggest that a study possibly incorporating Mueller-Hinton Agar (MHA) in the work-up of clinical *S. aureus* cultures, in addition to the rich medium already used, and using it to phenotypically screen for no growth or slow growth may be a useful way to identify clinical SCVs.

Final remarks

As a body of work, taken together the chapters presented in this thesis notably advance the field of *S. aureus* SCVs by addressing a range of both clinically relevant and basic research topics including antibiotic resistance, diagnostic development, physiology, pathogenesis, phenotypic and genomic analyses. Still, many questions remain to be answered and the work presented here will undoubtedly aid in the development of future studies further exploring the role *S. aureus* SCVs play in disease.

References

1. **Kahl BC**, **Becker K**, **Löffler B**. 2016. Clinical Significance and Pathogenesis of Staphylococcal Small Colony Variants in Persistent Infections. Clin Microbiol Rev

29:401–427.

2. **Proctor RA**, **Kriegeskorte A**, **Kahl BC**, **Becker K**, **Löffler B**, **Peters G**. 2014. *Staphylococcus aureus* Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol **4**:99.

3. **Kahl B**, **Herrmann M**, **Everding AS**, **Koch HG**, **Becker K**, **Harms E**, **Proctor RA**, **Peters G**. 1998. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. J Infect Dis **177**:1023–1029.

4. **Sadowska B**, **Bonar A**, **Eiff von C**, **Proctor RA**, **Chmiela M**, **Rudnicka W**, **Różalska B**. 2002. Characteristics of *Staphylococcus aureus*, isolated from airways of cystic fibrosis patients, and their small colony variants. FEMS Immunol Med Microbiol **32**:191–197.

5. **Besier S**, **Smaczny C**, **Mallinckrodt von C**, **Krahl A**, **Ackermann H**, **Brade V**, **Wichelhaus TA**. 2007. Prevalence and clinical significance of *Staphylococcus aureus* small-colony variants in cystic fibrosis lung disease. J Clin Microbiol **45**:168–172.

6. **Yagci S**, **Hascelik G**, **Dogru D**, **Ozcelik U**, **Sener B**. 2013. Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. Clin Microbiol Infect **19**:77–84.

7. **Besier S**, **Zander J**, **Siegel E**, **Saum SH**, **Hunfeld K-P**, **Ehrhart A**, **Brade V**, **Wichelhaus TA**. 2008. Thymidine-dependent *Staphylococcus aureus* small-colony variants: human pathogens that are relevant not only in cases of cystic fibrosis lung disease. J Clin Microbiol **46**:3829–3832.

8. **Proctor RA**, **Eiff von C**, **Kahl BC**, **Becker K**, **McNamara P**, **Herrmann M**, **Peters G**. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol **4**:295–305.

9. **Schaaff F**, **Bierbaum G**, **Baumert N**, **Bartmann P**, **Sahl H-G**. 2003. Mutations are involved in emergence of aminoglycoside-induced small colony variants of *Staphylococcus aureus*. Int J Med Microbiol **293**:427–435.

10. **Eiff von C**, **Heilmann C**, **Proctor RA**, **Woltz C**, **Peters G**, **Götz F**. 1997. A site-directed *Staphylococcus aureus* hemB mutant is a small-colony variant which persists intracellularly. J Bacteriol **179**:4706–4712.

11. **Hoffman LR**, **Déziel E**, **D'Argenio DA**, **Lépine F**, **Emerson J**, **McNamara S**, **Gibson RL**, **Ramsey BW**, **Miller SI**. 2006. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of Pseudomonas aeruginosa. Proc Natl Acad Sci USA **103**:19890–19895.

12. **Biswas L**, **Biswas R**, **Schlag M**, **Bertram R**, **Götz F**. 2009. Small-colony variant selection as a survival strategy for *Staphylococcus aureus* in the presence of Pseudomonas aeruginosa. Appl Environ Microbiol **75**:6910–6912.

13. **Wolter DJ**, **Emerson JC**, **McNamara S**, **Buccat AM**, **Qin X**, **Cochrane E**, **Houston LS**, **Rogers GB**, **Marsh P**, **Prehar K**, **Pope CE**, **Blackledge M**, **Déziel E**, **Bruce KD**, **Ramsey BW**, **Gibson RL**, **Burns JL**, **Hoffman LR**. 2013. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis **57**:384–391.

14. **Kriegeskorte A**, **Block D**, **Drescher M**, **Windmüller N**, **Mellmann A**, **Baum C**, **Neumann C**, **Lorè NI**, **Bragonzi A**, **Liebau E**, **Hertel P**, **Seggewiss J**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2014. Inactivation of thyA in *Staphylococcus aureus* attenuates virulence and has a strong impact on metabolism and virulence gene expression. MBio **5**:e01447–14.

15. **Hirschhausen N**, **Block D**, **Bianconi I**, **Bragonzi A**, **Birtel J**, **Lee JC**, **Dübbers A**, **Küster P**, **Kahl J**, **Peters G**, **Kahl BC**. Extended *Staphylococcus aureus* persistence in cystic fibrosis is associated with bacterial adaptation. Int J Med Microbiol **303**:685–692.

16. **Proctor RA**, **van Langevelde P**, **Kristjansson M**, **Maslow JN**, **Arbeit RD**. 1995. Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. Clin Infect Dis **20**:95–102.

17. **Proctor RA**, **Balwit JM**, **Vesga O**. 1994. Variant subpopulations of *Staphylococcus aureus* as cause of persistent and recurrent infections. Infect Agents Dis **3**:302–312.

18. **Precit MR**, **Wolter DJ**, **Griffith A**, **Emerson J**, **Burns JL**, **Hoffman LR**. 2016. Optimized In Vitro Antibiotic Susceptibility Testing Method for Small-Colony Variant *Staphylococcus aureus*. Antimicrob Agents Chemother **60**:1725–1735.

19. **Eiff von C**, **Becker K**, **Metze D**, **Lubritz G**, **Hockmann J**, **Schwarz T**, **Peters G**. 2001. Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease. Clin Infect Dis **32**:1643–1647.

20. **Zander J**, **Besier S**, **Saum SH**, **Dehghani F**, **Loitsch S**, **Brade V**, **Wichelhaus TA**. 2008. Influence of dTMP on the phenotypic appearance and intracellular persistence of *Staphylococcus aureus*. Infect Immun **76**:1333–1339.

21. **Kriegeskorte A**, **Grubmüller S**, **Huber C**, **Kahl BC**, **Eiff von C**, **Proctor RA**, **Peters G**, **Eisenreich W**, **Becker K**. 2014. *Staphylococcus aureus* small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. Front Cell Infect Microbiol **4**:4494.

22. **Choi PH**, **Sureka K**, **Woodward JJ**, **Tong L**. 2015. Molecular basis for the recognition of cyclic- di- AMP by PstA, a PII- like signal transduction protein. MicrobiologyOpen **4**:361–374.

23. **Kriegeskorte A**, **Lorè NI**, **Bragonzi A**, **Riva C**, **Kelkenberg M**, **Becker K**, **Proctor RA**, **Peters G**, **Kahl BC**. 2015. Thymidine-Dependent *Staphylococcus aureus* Small-Colony Variants Are Induced by Trimethoprim-Sulfamethoxazole (SXT) and Have Increased

Fitness during SXT Challenge. Antimicrob Agents Chemother **59**:7265–7272.

24. **Cullen L**, **McClean SN**. 2015. Bacterial Adaptation during Chronic Respiratory Infections. Pathogens **4**:66–89.

25. **Dean MA**, **Olsen RJ**, **Long SW**, **Rosato AE**, **Musser JM**. 2014. Identification of point mutations in clinical *Staphylococcus aureus* strains that produce small-colony variants auxotrophic for menadione. Infect Immun **82**:1600–1605.

26. **Chatterjee I**, **Kriegeskorte A**, **Fischer A**, **Deiwick S**, **Theimann N**, **Proctor RA**, **Peters G**, **Herrmann M**, **Kahl BC**. 2008. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. J Bacteriol **190**:834–842.

27. **Lannergård J**, **Eiff von C**, **Sander G**, **Cordes T**, **Seggewiss J**, **Peters G**, **Proctor RA**, **Becker K**, **Hughes D**. 2008. Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother **52**:4017–4022.

28. **Roggenkamp A**, **Haas A**, **Eder W**, **Kreuzer E**, **Hoffmann H**. 2004. Metabolically deficient methicillin-resistant *Staphylococcus aureus* as cause of chronic post-thoracotomy sternal wound infection. Eur J Clin Microbiol Infect Dis **23**:419–421.

29. **Besier S**, **Ludwig A**, **Ohlsen K**, **Brade V**, **Wichelhaus TA**. 2007. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. Int J Med Microbiol **297**:217–225.

Appendices:

Appendix A: All genes, and variations of a gene name, with mutations present in *Staphylococcus aureus* thymidine-dependent small colony variants but absent in normal colony *S. aureus*

Appendix B: Demographic data for patients represented in the *Staphylococcus aureus* colony size distribution study

Appendix A

KEY:

Reference Number, isolate count, total count, percent mutated, groups mutated, fisher P-val, cluster names, Site of mutation

1 109 142 76.76056338 34 2.20E-16 >Cluster 1941 thymidylate synthase(62) Thymidylate synthase(17) "thymidylate synthase, putative(1)"

2 18 142 12.67605634 4 6.85E-08 >Cluster 331 sialic acid synthase(30) hypothetical protein(13) ferric iron reductase FhuF-like transporter family protein(6) "aerobactin biosynthesis protein, IucA/IucC family(4)"Aerobactin siderophore biosynthesisprotein(3) aerobactinbiosynthesisIucA/IucCfamily protein(3) iucA(2) iucA / IucC family protein(2) putative siderophore biosynthesis protein near heme transporter HtsABC(2) IucA/IucC family protein(2) conserved hypothetical protein(2) aerobactin biosynthesis protein(2) siderophore biosynthesis protein near heme transporter HtsABC(1) possible Iuc family aerobactin synthesis protein(1) "putative siderophore biosynthesis protein near heme transporter HtsABC, Siderophore synthetase superfamily, group B(1)" "siderophore biosynthesis protein, IucA/IucC family(1)" Siderophoresynthase(1) "putativesiderophore biosynthesis protein near heme transporter HtsABC / Siderophore synthetase superfamily, group B(1)" "IucA-IucC family aerobactin biosynthesis protein, putative(1)" "Uncharacterized siderophore biosynthesis protein near heme transporter HtsABC @ Siderophore synthetase superfamily, group B(1)" Siderophore biosynthesis protein(1)

3 14 142 9.85915493 3 7.44E-07 >Cluster 3153 lantibiotic ABC transporter ATP-binding protein(29) ABC transporter ATP-binding protein(22) ABC transporter family protein(8) "ABC superfamily ATP binding cassette transporter, ABC protein(5)" "ABC transporter, ATP-binding protein(4)" putative antibiotic transport ATP-binding protein(3) ABC transporter(2)

hypotheticalprotein(1) conservedhypotheticalprotein(1) probable ABC transporter(1) ABC transporter related(1) AAA domain protein(1) MW2433(1)

4 13 142 9.154929577 2 2.19E-06 >Cluster 5843 PTS lactose transporter subunit IIB(32) putative PTS transport system protein(7) "PTS system, Lactose/Cellobiose specific IIB subunit(7)" hypothetical protein(6) conserved hypotheticalprotein(4) "phosphotransferasesystem,lactose/ cellobiose-specific IIB subunit(4)" PTS family porter component

IIB(3) PTStransporter(3) putativephosphotransferase transportsystemIIBcomponent(3)PTSsystemcomponent(2) pTSfamily porter component IIB(2) probable PTS system component(1) "PTS transport system protein, putative(1)" "PTS sysytem, mannitol- specific enzyme II, B component(1)" phosphotransferase system lactose/cellobiose-specific IIB subunit(1)Hypothetical protein(1) FIG01108524: hypothetical protein(1) "Pts system, iib component(1)"

5 14 142 9.85915493 3 4.18E-06 >Cluster 1749 pectate lyase(32) hypothetical protein(15) conserved hypotheticalprotein(8) cellwallsurfaceanchorfamilyprotein(8) pectate lyase superfamily protein(7) parallel beta-helix repeat- containing protein(3) Plasmin and fibronectin-binding protein A precursor(2) parallel beta-helix repeat protein(2) parallel beta-helix repeat(1) Parallel beta-helix repeat(1) Hypothetical protein(1)

6 12 142 8.450704225 2 6.47E-06 >Cluster 506 ABC transporter substrate-binding protein(25) "Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA(7)" peptide/nickel transport system substrate-binding protein(6) hypothetical

protein(4) "bacterial extracellular solute-binding s, 5 Middle family protein(4)" "bacterial extracellular solute-binding, 5 Middle family protein(4)"oligopeptide ABC transporter substrate- binding protein(4) transport system extracellular binding lipoprotein(4) "oligopeptide ABC transporter, oligopeptide-binding protein(3)" "extracellular solute-binding protein, family 5(2)"oligopeptide ABC transporter oligopeptide-binding protein(1) extracellular solute-binding protein family 5(1) "bacterial extracellular solute-binding proteins, family 5 Middle family protein(1)" "Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein oppA(1)" "Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA (TC 3.A. 1.5.1)(1)" "aBC transporter, substrate-binding, family 5 domain protein(1)" Oligopeptide-binding protein oppA(1) "oligopeptide ABC transporter, substrate-binding protein, putative(1)" "oligopeptide ABC superfamily ATP binding cassette transporter, binding protein(1)" "oligopeptide ABC transporter, substrate-binding lipoprotein oppA, putative(1)" "ABC transporter, substrate-binding protein, family 5(1)" MW0873(1)

7 9 142 6.338028169 2 2.28E-05 >Cluster 4556 ferritin(53) Ferritin-like protein 2(8) Ferritin(8)ferritin-like domain protein(4) "ferritin, putative(2)" ferritinsfamilyprotein(1) hypotheticalprotein(1) MW1834(1)ferritin-like protein(1) Ferritin Dps family protein(1)

8 17 142 11.97183099 6 2.70E-05 >Cluster 1103 thymidine phosphorylase(27) pyrimidine-nucleoside phosphorylase(18) Pyrimidine-nucleoside phosphorylase(16) putative pyrimidine-nucleoside phosphorylase(10) pyrimidine nucleoside phosphorylase(5) Thymidine phosphorylase(1) pyrimidine nucleoside phosphorylase C-terminal domain protein(1) pyrimidine-nucleoside phosphorylase (pynp)(1) "glycosyl transferase family, a/b domain protein(1)"pyrimidine-nucleoside phosphorylase(PYNP)(1) "pyrimidinenucleosidephosphorylase, putative(1)"

9 10 142 7.042253521 2 5.60E-05 >Cluster 3308 MerR family transcriptional regulator(34) "transcriptional regulator, MerR family(12)" MerR family regulatory protein(6) merR regulatory family protein(5) MerR family HTH-type transcriptional regulator(3) TipAS antibiotic-recognition domain protein(2) probable regulatory protein(1) merR family regulatory family protein(1) "antibiotic resistance transcriptional regulator, putative(1)" HTH-type transcriptional activator tipA(1)MW2312(1) TipAS antibiotic-recognition domain family(1) conserved hypotheticalprotein(1) hypotheticalprotein(1)

10 11 142 7.746478873 4 8.82E-05 >Cluster 1813 spermidine/putrescine ABC transporter substrate-binding

protein(31) protein(8) protein(8) binding protein PotD(6)" "spermidine/putrescine ABC superfamily ATP binding cassette transporter, binding protein(5)" Spermidine/ putrescine-binding periplasmic protein(3) "spermidine/putrescine ABC transporter, spermidine(3)" "extracellular solute-binding protein, family 1(2)" Spermidine/putrescine-binding protein(2) spermidine putrescine-binding periplasmic protein(2) "ABC transporter, periplasmic spermidine putrescine-binding protein potD(1)" spermidine/putrescine-binding periplasmic protein precursor(1)

potD(1) "spermidine/putrescine ABC transporter, substrate- binding lipoprotein, putative(1)" "spermidine/putrescine ABC transporter, binding protein(1)" "ABC transporter, periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)(1)" spermidine/putrescine-binding protein precursor homolog(1) extracellular solute-binding protein family 1(1) "ABC transporter, solute-binding protein(1)" "spermidine/putrescine ABC transporter, spermidine/putrescine-binding protein, putative(1)"

11 18 142 12.67605634 2 9.92E-05 >Cluster 2569 hypothetical protein(16) membrane protein(16) membranebacterial extracellular solute-binding familyABC transporter extracellular binding"ABC transporter, periplasmic spermidine putrescine-protein oxaA(6) putative membrane protein(6) "Inner membrane protein translocase component YidC, short form OxaI-like(5)" OxaI/YidC membrane insertion protein(5) Membrane protein insertase YidC(4) membrane protein insertase YidC(3) 60Kd inner membrane family protein(2) lipoprotein precursor(2) Membrane protein insertase(2) preprotein translocase subunit(2) 60 kDa inner membrane protein YIDC(1) "membrane protein insertase, YidC/Oxa1 family(1)" protein translocase component YidC(1) 60 kDa inner membrane insertion protein(1) Membrane protein oxaA(1) putative membrane protein OxaA(1) 60Kd inner membrane protein insertase YidC(1) membrane protein oxaA precursor(1) hypothetical membrane protein(1) membrane-embedded lipoprotein precursor(1) conserved hypothetical protein(1) "Inner membrane protein translocase component YidC, short form OxaI-like protein(1)"membrane protein OxaA(1) 12 12 142 8.450704225 2 0.0001141 >Cluster 3040 hypothetical protein(45) yycH family protein(8) putative exported protein(7) conserved hypothetical protein(6) regulatory protein YycI(4) YycI protein(3) Hypothetical protein(2) YycI like protein(1) hypothetical protein SA_21(1)Two-component system regulatory protein(1)hypothetical protein YycI(1) YycI(1) YycH protein(1)

13 15 142 10.56338028 4 0.0001466 >Cluster 3362 transcriptional regulator(34) Transcriptional regulator in cluster with Zn-dependent hydrolase(7) "bacterial regulatory s, gntR family protein(4)" GntR family transcriptional regulator(4) UTRA domain transcriptional regulator(3) conserved hypothetical protein(3) UTRA domain protein(3) HTH-type transcriptional regulator(3) "transcriptional regulator, GntR family protein(2)" UbiC transcription regulator-associated domainprotein(2) hypotheticalprotein(2) "bacterial regulatory, gntR family protein(2)" GntR family regulatory protein(2) possible GntR family transcriptional regulator(1) "transcriptional regulator, GntR family, putative(1)" MW1160(1) Hypotheticalprotein(1) "bacterial regulatory proteins, gntR family protein(1)" probable GntR family transcriptional regulator(1) Transcriptional regulator(1) "Transcriptional regulator, GntR(1)" transcription regulator GntR family(1) "Transcriptional regulator, GntR family protein(1)" 14 18 142 12.67605634 3 0.0001593 >Cluster 1629 iron-sulfur cluster-binding protein(21) epoxyqueuosine reductase(17) Epoxyqueuosine (oQ) reductase QueG(7) iron-sulfur cluster binding protein(4) conserved hypothetical protein(4) putative iron-sulphur protein(3) putative iron-sulfur protein(3) Epoxyqueuosine reductase(3) putative iron-sulfur cluster-binding protein(3) Iron-sulfur cluster-binding protein(2) tRNA epoxyqueuosine(34) reductase QueG(2) "iron-sulfur cluster binding protein, putative(2)" phenylalanyl-tRNA synthetase alpha chain(2)phenylalanyl-tRNA synthetase subunit alpha(2)probable iron-sulfur binding protein(1) "iron-sulfur cluster-binding protein, putative(1)" putative iron-sulfur cluster binding protein(1) putative iron sulfur binding protein(1) iron-sulfur (Fe-S) cluster-binding protein(1)

15 9 142 6.338028169 2 0.0001639 >Cluster 576 polysaccharide biosynthesis protein(44) putative polysaccharide biosynthesis protein(12) matE family protein(5) Polysaccharide biosynthesis protein(4) "Membrane protein involved in the export of O-antigen, teichoic acid lipoteichoic acids(4)" mviN-like family protein(3) "polysaccharide biosynthesis protein, putative(2)" Export protein for polysaccharide and teichoic acid(1) probable polysaccharide transport protein(1) spore cortex protein homolog(1) "spore cortex protein; Membrane protein involved in the export of O-antigen, teichoic acid lipoteichoic acids(1)" O-antigen export related membrane protein(1)MW1697(1)

16 22 142 15.49295775 4 0.0002781 >Cluster 546 teichoic acid biosynthesis protein(30) Putative polyribitolphosphotransferase(12) hypothetical protein(6) Poly(glycerophosphate) glycerophosphotransferase family protein(6) CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase(5) teichoic acid biosynthesis protein B(4) putative polyribitolphosphotransferase(3) putative teichoic acid biosynthesis protein(2) conserved hypothetical protein(2) CDP- glycerol:poly(Glycerophosphate) glycerophosphotransferase(2) CDP-Glycerol:Poly(glycerophosphate) glycerophosphotransferase family protein(2) glycosyl/glycerophosphatetransferase(1) putative CDP-Glycerol:Poly(glycerophosphate) glycerophosphotransferase(1) MW0233(1) CDP-ribitolribitolphosphotransferase(1) "teichoic acid biosynthesis protein, putative(1)"

17 13 142 9.154929577 2 0.0003372 >Cluster 707 malate:quinone oxidoreductase(35) putative malate:quinone oxidoreductase 1(14) Malate:quinone oxidoreductase(9) malatedehydrogenase(7) malate dehydrogenase (acceptor)(3) malate:quinone oxidoreductase 1(2) malate dehydrogenase (quinone)(1) malate:quinone- oxidoreductase(1) Malate quinone oxidoreductase 1(1) malate:quinone-oxidoreductase 1(1) Probable malate:quinone oxidoreductase 1(1) malate--quinone oxidoreductase(1) malate- quinoneoxidoreductase1(1) hypotheticalprotein(1) MW2286(1) Malate:quinone oxidoreductase 1(1)

18 13 142 9.154929577 2 0.0003372 >Cluster 1892 isopentenyl pyrophosphate isomerase(25) isopentenyl-diphosphate delta-isomerase(16) "isopentenyl- diphosphatedelta-isomerase,type2(11)" type2isopentenyl- diphosphate Delta-isomerase(9) "Isopentenyl-diphosphate delta-isomerase, FMN-dependent(8)" Isopentenyl-diphosphate delta- isomerase(7) isopentenyl diphosphate isomerase(2) conserved hypotheticalprotein(1) "Isopentenyl-diphosphatedelta-isomerase,FMN- dependent(1)"

19 8 142 5.633802817 3 0.000478 >Cluster 5993 hypothetical protein(20) phage protein(1) conserved hypotheticalprotein(1) hypotheticalphageprotein(1)

20 9 142 6.338028169 2 0.0006498 >Cluster 2790 PTS glucose transporter subunit IIABC(28) "PTS system, glucose subfamily, IIA component domain protein(8)"putative PTS transport system protein(7) "PTS system, IIA component(5)" PTS systemglucose-specificIIAcomponent(4) "PTSsystem,N- acetylglucosamine-specific IIA component(3)" putative PTS system glucose-specific EIICBA component(3) PTS system

glucose/glucoside porter IIABC component(2)"PTS system, IIA component, putative(1)" "PTS system, beta-glucoside-specific IIB component / PTS system, beta-glucoside-specific IIC component / PTS system, beta- gluco>(1)" "PTS system, glucose subfamily, IIA component(1)" "PTS system, N-acetylglucosamine-specific IIA component / PTS system, N-acetylglucosamine-specific IIB component / PTS system, >(1)" beta-glucoside-specific IIB component(1) "PTS system, beta-glucoside-specific IIABC component(1)" PTS family glucose/glucoside (glc) porter component IIABC(1) "PTS system, N-acetylglucosamine-specific IIA component / PTS system, N-acetylglucosamine-specific IIB component / PTS system, ... at 87.50%(1)" "PTS system, glucose subfamily, IIA subunit(1)" "PTS system, N-acetylglucosamine-specific IIA component / PTS system, N-acetylglucosamine-specific IIB component / PTS system, ... at 99.87%(1)" "PTS system, N-acetylglucosamine- specific IIA component / PTS system,N-acetylglucosamine-specific IIB component / PTS system, N>(1)" "Pts system, beta-glucoside-specific iiabc component(1)" conserved hypothetical protein(1) probable PTS system transport protein(1) hypothetical protein(1) MW0241(1) "PTS system, beta-glucoside-specific IIB component(1)" 142 11.26760563 3 metal-dependent hydrolase(31) putative membrane protein(7)

21 16 >Cluster 2171 protein(18) hypothetical protein(6) yfhP(4) Hypothetical protein(3) membrane- bound metal-dependent hydrolase(3) Putative membrane spanning 0.0006641 hypothetical conserved protein(2) protein(1) putative(1)" possible metal-dependent hydrolase(1) YfhP "membrane-bound metal-dependent hydrolase, putative membrane-bound metal-dependent hydrolase(1) probable membrane protein(1) SAV1869(1) Hypothetical protein

22 10 >Cluster 501 enzyme(12) binding domain protein(4)" Pyruvate oxidase(4) "thiamine pyrophosphate enzyme, central domain protein(3)" "Pyruvate oxidase (ubiquinone, cytochrome)(2)" "Pyruvate oxidase, CidC / Pyruvate oxidase (2)" "Pyruvate oxidase, CidC / Pyruvate oxidase (ubiquinone, cytochrome)(2)" Pyruvate oxidase (2) Pyruvate dehydrogenase (quinone)(1) "pyruvate oxidase, putative(1)" hypotheticalprotein(1) thiaminepyrophosphateproteindomainprotein TPP-binding(1) oxidase(1) protein(1)"MW2460(1) putative pyruvate "thiamine pyrophosphate enzyme, TPP binding domain 142 7.042253521 pyruvate oxidase(43) "thiamine pyrophosphate enzyme, C-terminal TPP

23 6 142 4.225352113 2 0.0007706 >Cluster 5620 protein spoVG(10) Protein of unknown function identified by role in sporulation (SpoVG)(6) regulatory protein SpoVG(5) Putative septation protein SpoVG(4) putative septation protein SpoVG(3) Putative septation protein(2) septation protein SpoVG(2) SpoVG superfamily(1) stage V sporulation protein G homolog(1) SpoVGfamilyprotein(1) putativeseptationprotein spoVG(StageVsporulationproteinG)(1) conservedhypothetical protein(1) hypotheticalprotein(1) StageVsporulationprotein G(1) spoVG(1) sporulation protein SpoVG(1) stage V sporulation protein G homologue(1)

24 12 142 8.450704225 4 0.0007874 >Cluster 1184 arsenical pump membrane protein(27) arsenic transporter(14) arsenical pump membrane protein 2(8) Arsenic efflux pump protein(8) arsenite/antimonite efflux pump membrane family protein(6) Arsenical pump membrane protein(5) arsenic efflux pump protein(2) arsenical pump membrane protein (Arsenic efflux pump protein)(2) aesenical pump membrane protein(2) MW1714(1) arsenical pump membrane protein subfamily(1) arsenical pump membrane family protein(1) aesenical pump membrane protein homolog(1) aresenical pump membrane protein(1) "ArsB arsenite-antimonite efflux family transporter, membrane subunit(1)"

25 17 142 11.97183099 4 0.0009195 >Cluster 5196 hypothetical protein(44) "Transcriptional activator rinB, phage associated(8)" phage protein(8) conserved hypotheticalprotein(7) hypotheticalbacteriophageprotein(2) 3 0.0007484 thiamine pyrophosphate stage V sporulation protein G(36) putative septation hypothetical phage protein(1) conserved hypothetical phage protein(1) putative phage protein(1)Phage protein(1)

26 14 142 9.85915493 3 0.001021 >Cluster 774 beta-lactamase(24) serine hydrolase(10) Beta- lactamase(8) putative exported protein(6) beta-lactamase family protein(5) Protein flp(4) serine hydrolase FLP(3) fmtA-like beta-lactam resistance protein(3) protein flp(3) hypothetical protein(2) flp protein(2) FmtA-like protein(2) fmtA-like protein(1) conserved hypothetical protein(1) Beta- lactamase family protein(1) FmtA protein(1) fmtA-like protein Flp(1) MW2365(1) probabledrugresistance-involvedmembrane protein(1)

27 14 142 9.85915493 3 0.001021 >Cluster 1475 multidrug MFS transporter(30) Macrolide-efflux protein(8) major facilitator transporter(6) putative membrane protein(5) majorFacilitatorSuperfamilyprotein(4) transmembrane secretion effector family protein(3) major facilitator superfamily transporter(3) macrolide MFS transporter(3) "transporter, putative(2)" major facilitator superfamily MFS_1(2) conserved hypothetical protein(2) hypotheticalprotein(2) majorfacilitatorsuperfamilytransporter MFS_1(2) transporter(1) "transporter, major facilitator family protein(1)" protein(1)putative(1)"putative(1)"

28 20 126 2-oxoglutarate dehydrogenase E1 component(32) 2- oxoglutarate dehydrogenase(18) 2-oxoglutarate dehydrogenase subunit E1(9) "oxoglutarate dehydrogenase (succinyl-transferring), E1 component(9)" oxoglutarate dehydrogenase (succinyl-transferring) E1(5) 2-oxoglutarate dehydrogenase E1 component protein(2) MW2106(1) probable membrane transport "major facilitator superfamily protein (MFS), "major facilitator superfamily transporter, MFS family major facilitator transporter(1) 142 14.08450704 7 0.001258 >Clusteroxoglutarateputative(1)"oxoglutaratedehydrogenase E1 component domain protein(1)dehydrogenase(2) "2-oxoglutarate dehydrogenase E1, "2-oxoglutarate dehydrogenase, E1 subunit(1)" "2-dehydrogenase, E1 component(1)" 2-oxoglutarate

29 21 142 14.78873239 6 0.001437 >Cluster 6374 hypothetical protein(52) phage protein(17) Hypotheticalprotein(5) conservedhypotheticalprotein(5)proteinof unknown function DUF1381(3) hypothetical phage protein(3) conserved hypothetical phage protein(2) hypothetical bacteriophage protein(2) conserved domain protein(2) hypothetical phage- related protein(1) "hypothetical protein SA bacteriophages 11,Mu50B(1)"homolog (1)"protein(1)

30 16 484 siderophore synthetase(31) hypothetical protein(12) ferric iron reductase FhuF-like transporter family protein(6) "aerobactin biosynthesis protein, IucA/IucC family(5)" conserved hypothetical protein(4) Aerobactin siderophore biosynthesis protein(3) IucA/IucC family protein(3)iucA(2) putative siderophore biosynthesis protein near heme transporter HtsABC(2) iucA / IucC family protein(2) "siderophore biosynthesis protein, IucA/IucC family(2)" possible Iuc family aerobactin synthesis protein(1)"putative siderophore biosynthesis protein near heme transporter HtsABC, Siderophore synthetase superfamily, group B(1)" siderophore biosynthesis protein near heme transporter HtsABC(1) aerobactin biosynthesis protein(1) "putative siderophore biosynthesis protein near heme transporter HtsABC / Siderophore synthetase superfamily,

group B(1)" Amino-acid citrate synthetase(1) "Uncharacterized siderophore biosynthesis protein near heme transporter HtsABC @ Siderophore synthetase superfamily, group B(1)" Siderophore biosynthesis protein(1) aerobactin biosynthesisIucA/IucCfamilyprotein(1) "IucA-IucCfamilyaerobactin biosynthesis protein, putative(1)"

31 8 142 5.633802817 2 0.001734 >Cluster 1782 nitric oxide synthase(30)Nitric oxide synthase oxygenase(16)nitric oxide synthase oxygenase(10) "nitric-oxide synthase, oxygenase subunit(6)" putative oxygenase(6) Nitric- oxide synthase(2) nitric-oxide synthase(2) "nitric oxide synthase, oxygenase domain protein(1)" nitric-oxide synthase homolog(1) "nitric oxide synthase, oxygenase domain, putative(1)" "Nitric oxide synthase, oxygenase domain protein(1)"MW1855(1) probable nitric oxide synthase(1) "nitric- oxide synthase, oxygenase subunit, putative(1)" "nitric oxide synthase, oxygenase domain(1)"

32 8 142 5.633802817 2 0.001734 >Cluster 4672 hypothetical protein(44) membrane protein(12) conserved hypotheticalprotein(8) bacterialmembraneflankeddomainprotein(5)bacterial PH domain protein(4) Hypothetical protein(3) hypothetical membrane protein(1) Hypothetical protein SAV2074(1) "membrane protein, putative(1)"

33 9 142 6.338028169 2 0.001878 >Cluster 1481 nitrate transporter NarT(31) Nitrate/nitritePhage protein(1) "Hypothetical protein, SAV0877 transcriptional activator RinB(1) phage-like142 11.26760563 4 0.001673 >Clustertransporter(9) nitrite transport protein(8) nitrite extrusion protein(7) putative nitrate transporter NarT(6) "MFS family major facilitator transporter, nitrate:nitrite antiporter(5)" nitrate transporter narT(4) putative nitrate transporter narT(4)nitrite extrusion protein (Nitrite facilitator)(2) Nitrite extrusion protein(1) major facilitator superfamily MFS_1(1)"nitriteextrusionprotein,putative(1)" conserved hypothetical protein(1)

34 9 142 6.338028169 3 4593 terminase(8) hypothetical protein(4) small subunit(2) terminase small subunit(2) protein(2) "phage terminase, small subunit(1)" COG3728 phage terminase small subunit(1) phage protein(1)

35 13 142 9.154929577 3 0.002019 >Cluster 1623 RNA methyltransferase(31)hypothetical protein(12) putative N6-adenine-specific DNA methylase(11) conserved hypothetical protein(8) site-specific DNA-methyltransferase (adenine- specific)(5) methyltransferase domain protein(3) site- specific DNA-methyltransferase(2) RNA methylase family UPF0020 family protein(1) Methyltransferase(1) putative RNA methylase(1) FIG001721: Predicted N6-adenine-specific DNA methylase(1) protein YpsC(1) Predicted N6-adenine-specific DNA methylase(1) THUMP domain protein(1) "N6-adenine-specific DNA methylase, putative(1)"

36 13 142 9.154929577 3 0.002019 >Cluster 3092 histidine kinase(18) accessory gene regulator protein A(10) two-component system response regulator(9)autoinducer sensor protein response regulator protein(8) accessory gene regulator A(6) Accessory gene regulator protein A(5) accessory regulator protein A(4) Accessory protein regulator protein A(4) DNA-binding response regulator(3) Response regulator of the competence regulon ComE(2) accessory regulator A(2) response regulator(1)staphylococcal accessory gene regulator A(1) Accessory gene regulator protein A AGRA(1) AgrA(1) agrA protein(1) Accessory generegulatorA(responseregulator)(1) Accessorygeneregulator A(1) accessory protein regulator protein A(1) response regulator receiver(1)

37 5 142 3.521126761 4 0.002515 >Cluster 1480 phosphopentomutase(55) Phosphopentomutase(15) putative phosphopentomutase(9) "phosphopentomutase, putative(1)"0.001878 >Cluster phage terminase Phage

38 12 142 8.450704225 2 0.002745 >Cluster 2929 ABC transporter permease(31) disA bacterial checkpoint controller nucleotide-binding family protein(7) putative membrane protein(7) hypotheticalprotein(5) conservedhypothetical protein(5) DisA protein(4) checkpoint controller nucleotide-binding protein(4) TIGR00159 family protein(3) "ABC transporter, permease protein YbbP clustered with maltose/maltodextrin transporter / Tlr1762 protein(2)" DisA checkpoint controller nucleotide-binding protein(2) "Hypothetical protein YbbP, contains nucleotide-binding domain of DisA bacterial checkpoint controller(2)"protein of unknown function DUF147(1) Diadenylate cyclase spyDAC(1) hypothetical protein with TIGR00159 domain(1) Putative membrane spanning protein(1) probable membrane protein(1)DNA-binding protein(1) hypothetical membrane protein(1) conserved hypothetoical protein(1) Diadenylate cyclase spyDAC; Bacterial checkpoint controller DisA with nucleotide-binding domain(1)

39 13 142 9.154929577 3 0.003221 >Cluster 2148 LytR family transcriptional regulator(31) Peptide methioninesulfoxide reductase regulator MsrR(8)putative membrane protein(5)Regulatory protein MsrR(4) function transcriptional attenuator, LytR/CpsA family(3)" peptide methionine sulfoxide reductase regulator(3) peptide methionine sulfoxide reductase regulator MsrR(3) cell envelope transcriptional attenuator(2) "transcriptional regulator, putative(2)" peptide methionine sulfoxide reductase transcriptional regulator MsrR(1) Protein MsrR(1) Regulatory protein msrR(1) transcriptional attenuator MsrR(1) cell envelope- related function transcriptional attenuator common domain protein(1)Transcriptional regulator(1) "Transcriptional regulator, LytRfamilyprotein(1)" cellenvelope-likefunctiontranscriptional attenuator common domain protein(1) hypothetical protein(1)

40 6 142 4.225352113 2 0.003971 >Cluster 1690 hypothetical protein(25) membrane protein(18) putative membrane protein(8) major Facilitator Superfamily protein(4) major facilitator superfamily transporter(3) major facilitator superfamily protein(3) conserved hypotheticalprotein(3) "transporter, major facilitator family protein(2)" Putative uncharacterized protein MW0861(1)Hypothetical protein(1) Putative membrane spanning protein(1) Putative uncharacterized protein(1) putative major facilitator superfamily protein(1) hypothetical membrane protein(1)

41 6 142 4.225352113 2 0.003971 >Cluster 2219 carbamate kinase(59) Carbamate kinase(10) Carbamate kinase 2(6) carbamate kinase 2(4) amino acid kinase familyregulatory protein msrR(6)regulatory protein MsrR(4)"cell envelope-relatedprotein(2) carbamate kinase ArcC2(1)hypothetical protein(1)

42 6 142 4.225352113 3 0.003971 >Cluster 2884 glycerol transporter(21) glycerol uptake facilitator protein(10) Glycerol uptake facilitator

protein(10) aquaporin(9) putative glycerol uptake facilitator protein(7) MIP family major intrinsic protein channel protein(5) glycerol uptake facilitator(5) MIP family channel protein(4) MIP channel s family protein(4) MIP channel family protein(3) glyceroluptake facilitator protein GlpF(1)protein(1)

43 10 142 7.042253521 1251 folylpolyglutamate synthase(25) folylpolyglutamate synthase/dihydrofolate synthase(16) bifunctional FolC family protein(8) putative folylpolyglutamate synthase(7) Dihydrofolate synthase / Folylpolyglutamate synthase(5) Dihydrofolate synthase(4)folylpolyglutamate synthase/ dihydrofolate synthase(3)Folylpolyglutamate synthase(3) FolCprotein(2) putative(1)" putative(1)" glutamatesynthetase) (fpgs) (tetrahydrofolate synthase) (tetrahydrofolylpolyglutam>(1) tetrahydrofolate synthase(1) folylpolyglutamate synthase/dihydrofolate synthase protein(1) bifunctional protein FolC(1) FolC bifunctional protein(1)conserved hypothetical2 0.004076 >Cluster bifunctional"folylpolyglutamate synthase,"folylpolyglutamate synthase/dihydrofolate synthase,folylpolyglutamate synthase (folylpoly-gamma-

44 10 142 7.042253521 2 0.004076 >Cluster 1683 hypothetical protein(34) Salicylate hydroxylase(11) putative monooxygenase(9)FAD binding domain protein(6) FAD- dependent oxidoreductase(4) monooxygenase(3) monooxygenase family protein(3) aromatic-ring hydroxylase-like protein(3) squalene epoxidase family protein(2) conserved hypothetical protein(2) MW2225(1) monooxygenase FAD-binding(1) flavin containing amine oxidoreductase family protein(1) "UDP- glucose/GDP-mannose dehydrogenase family, NAD binding domain protein(1)"

45 10 142 7.042253521 2 0.004076 >Cluster 2352 mannose-6-phosphate isomerase(51) "mannose-6-phosphate isomerase,classI(16)" Mannose-6-phosphateisomerase(13)

46 10 142 7.042253521 3 0.004076 >Cluster 6213 hypothetical protein(20) phage protein(5) conservedhypotheticalprotein(3) "hypotheticalproteinSAbacteriophages11Mu50B(1)"protein(1)Hypotheticalprotein(1) hypotheticalbacteriophage "Hypothetical protein, SAV0880 homolog (1)"142 6.338028169 2 0.004418 >Cluster

47 9 803 phosphoribosylaminoimidazolecarboxamide formyltransferase(18)bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMPcyclohydrolase(10) bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/inosine monophosphate cyclohydrolase(10) phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase(10) IMP cyclohydrolase / Phosphoribosylaminoimidazolecarboxamide formyltransferase(6) Bifunctional purine biosynthesis protein PurH(6) IMP cyclohydrolase(4) bifunctional purine biosynthesis protein PurH(3) putative bifunctional purine biosynthesis protein (3) bifunctional purine biosynthesis protein(2) putative bifunctional purine biosynthesis protein includes phosphoribosylaminoimidazolecarboxamide formyltransferase and IMP c>(1) bifunctional purine biosynthesis protein PurH (1) bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase / IMP cyclohydrolase PurH(1) bifunctional purine biosynthesis protein with phosphoribosylaminoimidazolecarboxamide formyltransferase and IMP cyclohydrolase(1) putative bifunctional purine biosynthesis protein(1) bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase(1)Putative bifunctional purine biosynthesis protein(1)Phosphoribosylaminoimidazolecarboxamide formyltransferase(1)

48 9 142 6.338028169 2 0.004418 >Cluster 3022 hypothetical protein(41) Nitric oxide reductase activation protein NorQ(8) sigma-54 interaction domain protein(6) conserved hypotheticalprotein(4) ATPase(4) CbbQ/NirQ/NorQ/GpvNfamily protein(3) nitric-oxide reductase(3)"ATPase associated with various cellular activities, AAA_5(2)" nitric-oxide reductase-like protein(1) probable nitric oxide reductase protein(1)Putative ATPase(1) ATPase family associated with various cellular activities (AAA)(1) MW1299(1) ATPase associated with various cellular activities AAA_5(1) ATPase family associated with various cellular activities (AAA) family protein(1) AAA domain family protein(1) ATPase family associated with various cellular activities protein(1)

49 7 142 4.929577465 2 0.004557 >Cluster 1447 phosphopantothenoylcysteine decarboxylase(32) bifunctional phosphopantothenoylcysteine decarboxylase/ phosphopantothenate--cysteine ligase(7) phosphopantothenoylcysteinedecarboxylase/phosphopantothenate--cysteine ligase(6) phosphopantothenoylcysteine decarboxylase / phosphopantothenate-- cysteine ligase(6) putative flavoprotein(6) coenzyme A biosynthesis bifunctional protein CoaBC(6)Phosphopantothenoylcysteine decarboxylase / Phosphopantothenoylcysteine synthetase(6) Phosphopantothenoylcysteine decarboxylase(3) bifunctional phosphopantothenoylcysteine decarboxylase/phosphopantothenate-cysteine ligase(2) pantothenate metabolism flavoprotein homolog(1) MW1094(1) pantothenate metabolism flavoprotein(1) bifunctional phosphopantothenoylcysteine decarboxylase/ phosphopantothenate--cysteine ligase CoaBC(1) bifunctional phosphopantothenoylcysteine decarboxylase/phosphopantothenate cysteine ligase(1)

50 8 142 5.633802817 2 0.004626 >Cluster 307 potassium-transporting ATPase subunit B(3)"K+-transporting ATPase, B subunit(2)" potassium ABC transporter ATPase(1) P- ATPase superfamily P-type ATPase potassium (K+) transporter subunit B(1) potassium-transporting ATPase B chain homologue(1) potassium- transporting ATPase B chain(1) Potassium-transporting ATPase B chain(1)

51 8 142 5.633802817 3 0.004626 >Cluster 2660 HAD family hydrolase(31) "HAD hydrolase, IIB family protein(7)" putative haloacid dehalogenase-like hydrolase(7) conserved hypothetical protein(5) Hydrolase (HAD superfamily)(4) haloacid dehalogenase-like hydrolase(4) HAD hydrolase(3)hypothetical phosphatase) hydrolase(2) protein(1) hydrolase(1) detected in genetic screen for thiamin metabolic genes(1)" haloacid dehalogenase(1) hydrolase(1)

52 8 142 5.633802817 5 0.004626 >Cluster 2759 4-diphosphocytidyl-2C-methyl-D-erythritol kinase(30) 4- diphosphocytidyl-2-C-methyl-D-erythritol kinase(27) 4-(cytidineprotein(3) Phosphatase(3) HMP-PP hydrolase (pyridoxal Cof(3) cof-like hydrolase(2) HAD superfamilyCof-like hydrolase(2) cof-like hydrolase family haloacid dehalogenase-like family "HMP-PP hydrolase (pyridoxal phosphatase) Cof,5'-diphospho)-2-C-methyl-D-erythritol kinase(13) hypotheticalprotein(3) hypotheticalprotein(3)

53 12 142 8.450704225 5 2301 permease(32) putative permease(9) transporter/malic acid transport family protein(8) transporter/malic acid transport protein(6) protein(5) exfoliative toxin A/B(4) Transporter(4) exfoliative toxin A(4) conserved hypothetical protein(3)

hypotheticalconservedMW0450(1)0.006384 >Cluster C4-dicarboxylate C4-dicarboxylate putative membraneprotein(2) C4-dicarboxylate transporter/malic acidtransporter(2) "exfoliative toxin, putative(1)" resistance/dicarboxylate transporter, putative(1)" exfoliative toxin(1) Exfoliative toxin A(1) tellurite/dicarboxylate transporter(1)

54 11 142 7.746478873 3 2000 ATP:guanido phosphotransferase(26) phosphotransferase(11) Putative ATP:guanido phosphotransferase YacI(8) putative phosphotransferase(6) Putative ATP:guanido phosphotransferase(5) "ATP:guanido phosphotransferase, C-terminal catalytic domain protein(4)" D-isomer specific 2-hydroxyacid dehydrogenase family protein(4) guanido phosphotransferase domain protein(3) protein arginine kinase(2) Arginine kinase(2)"guanido phosphotransferase, C-terminal catalytic domain protein(2)" "guanido phosphotransferase, catalytic domain protein(1)" "ATP:guanido phosphotransferase, putative(1)" putative ATP:guanido phosphotransferase(1)hypothetical protein(1) possible phosphotransferase(1) probable creatine kinase(1) MW0479(1)

55 4 142 2.816901408 2 0.008247 >Cluster 2019 secretion protein(31) hypothetical protein(18) cysteine- rich secretory family protein(9) conserved hypothetical protein(5) SCP-like extracellular protein(4) SCP-like extracellular family protein(2) Cysteine-rich secretory protein family protein(2) Hypotheticalprotein(1) Putativeexportedprotein(1) "conserved hypothetical protein, putative(1)" SCP family extracellularprotein(1)protein(1)

56 4 2151 phage head morphogenesis protein(5) Phage minor capsid protein(4) minor head protein(3) phage minor head protein(2)phagecapsidprotein(2) hypotheticalprotein(1) conserved hypotheticalprotein(1) "phageheadmorphogenesis,SPP1gp7family domain protein(1)" protein_id=AKC04651.1(1) putative phage minor head protein(1) putative minor head protein(1) SPP1 family phage head morphogenesis protein(1) "phage putative head morphogenesis protein, SPP1 gp7 family(1)"putative head morphogenesis protein(1) protein_id=AKC04718.1(1)

57 4 142 2.816901408 2 0.008247 >Cluster 3303 two-component response regulator(23) two-component system response regulator(9) Autolysis response regulater LytR(8)autolysin response regulator protein(7) "two component transcriptional regulator, LytR(4)" sensory transduction proteinFIG01108071: hypothetical protein(1) SCP-like SCP-like extracellular protein domain protein(1)142 2.816901408 2 0.008247 >Cluster"tellurite-probableTDT family0.007931 >Cluster ATP--guanidoLytR(4) Sensory transduction protein LytR(4) response regulator(3) Sensory transduction protein lytR(3) DNA-binding response regulator(3) sensory transduction protein lytR(3) Autolysin response regulator LytR(1) sensory transduction protein LytT(1) autolysin two-component response regulator(1) "two- component response regulator LytR, putative(1)" sensory transduction protein lytT(1) response regulator receiver(1) two-componentsensorhistidinekinase(1) "two-componentresponse regulator,putative(1)" responseregulatorLytR(1)

58 4 142 2.816901408 2 0.008247 >Cluster 4473 general stress protein(27) ThiJ/PfpI family protein(21)conserved hypothetical protein(4) intracellular protease(4) protein yhbO(3) "intracellular protease, PfpI family protein(3)" hypotheticalprotein(3) protease(3) PfpIfamilyintracellular protease(2) "intracellularprotease,PfpIfamily(2)" peptidase C56 family protein(2) general stress protein 18(1)Uncharacterized protein(1)(Intracellular protease I)(1)general stress protein GSP18(1)amidotransferase (GATase1)-like protein(1)

59 10 142 7.042253521 3 1639 tellurite resistance protein TelA(30) resistance protein(9) TelA-like protein(9) protein(6) toxic anion resistance protein TelA(5) conserved intracellular protease 1C56 family endopeptidase PfpI(1)ProteaseI(1)type 1 glutaminehypotheticalprotein(4) hypothetical protein(3) protein, putative(2)" probabletoxicionresistanceprotein(1) MW1294(1) toxicanion resistance family protein(1) toxic anion resistance protein (TelA) family protein(1) toxic ion resistance protein(1)telluriteresistanceprotein(3) TelA-protein(2) "tellurite resistance "toxic anion resistance protein, TelA(1)"

60 7 142 4.929577465 2 0.01114 >Cluster 701 phageportalprotein(9) "phageportalprotein,SPP1 family(7)" Phageportalprotein(3) "phageportalprotein,SPP1 family; phi 11 orf31(1)" hypothetical phage-related protein(1) hypothetical protein(1)

61 7 142 4.929577465 2 0.01114 >Cluster 1116 Zn-dependent hydrolase(25) metallo-beta-lactamase superfamily protein(7) metallo-beta-lactamase family protein(5) conserved hypothetical protein(3) Zn-dependent hydroxyacylglutathione hydrolase / Polysulfide binding protein(2)beta-lactamase domain protein(1) Metal-dependent hydrolase with rhodanese-homology domain (RHOD)(1) probable hydroxyacylglutathione hydrolase(1) hypothetical protein(1)0.01031 >Cluster Tellurite telA-like

62 7 142 4.929577465 4 0.01114 >Cluster 1269 aminoacyltransferase(30) Lipid II:glycine glycyltransferase(6) tRNA-dependent lipid II--glycine ligase (FmhB)(6) aminoacyltransferase femX(6) putative peptidoglycan pentaglycine interpeptide biosynthesis protein(5) FmhB protein(4) peptidoglycan pentaglycine interpeptide biosynthetic protein FmhB(4) glycine glycyltransferase(3)aminoacyltransferase FemX(2) putative peptidoglycan pentaglycine interpeptidebiosynthesis protein(2) methicillin resistance factor peptidoglycan pentaglycine interpeptide biosynthetic protein(1) "FmhB protein, putative(1)"Aminoacyltransferase femX(1) acetyltransferase domain protein(1) peptidoglycan pentaglycine interpeptide biosynthesis proteinFmhB(1) UDP-N-acetylmuramoylpentapeptide-lysine N(6)- glycyltransferase(1) peptidoglycan pentaglycine interpeptide biosynthesisprotein(1) femABfamilyprotein(1) lipidII--glycine glycyltransferase(1) aminoacyltransferase FemX (Factor essential forexpression of methicillin resistance X)(1) Methicillin resistance protein(1) femX protein(1) FmhB protein of FemAB family involved in peptidoglycan interpeptide biosynthesis(1)

63 5 142 3.521126761 4 0.01124 >Cluster 656 CoA-transferase(27) coenzyme A transferase family protein(9) acetyl-CoA/acetoacetyl-CoA transferase(8) hypotheticalprotein(7) putativeacetyl-CoAtransferase(6) putative acetyl-CoA/acetoacetyl-CoA transferase(4) propionate CoA-transferase(3) acyl CoA:acetate/3-ketoacid CoA transferase(3) conserved hypothetical protein(3) putative propionate CoA- transferase(3) 3-oxoacid CoA-transferase(1) Hypothetical protein(1) Coenzyme A transferase(1)Acetyl-CoA:acetoacetyl-CoA transferase alpha subunit(1) coenzyme A transferase(1)FIG01108629: hypothetical protein(1)

64 5 142 3.521126761 2922 dihydropteroate synthase(54)CoA transferase(1)2 0.01124 >Cluster Dihydropteroate synthase(16)dihydropteroate synthase chain A synthetase(6)dihydropteroate synthase chain A(1) "dihydropteroate synthase, subunit A(1)"

65 5 142 3.521126761 2 0.01124 >Cluster 4290 hypothetical protein(67) conserved hypothetical protein(7) Hypotheticalprotein(5) HypotheticalproteinSAV2322(1)

66 5 142 3.521126761 3 0.01124 >Cluster 4465 hypothetical protein(47) conserved hypothetical protein(5) FIG01108090: hypothetical protein(2) Hypothetical protein(1) protein of unknown function DUF1643(1)

67 22 142 15.49295775 7 0.0113 >Cluster57 DNA-directed RNA polymerase subunit beta'(40) DNA-directed RNA polymerase beta' chain protein(12) "DNA-directed RNA polymerase, beta' subunit(11)" DNA-directed RNA polymerase beta' subunit(10) RNA polymerase beta-prime chain(2) DNA- directed RNA polymerase subunit beta(2) "DNA directed RNA polymerase beta-prime chain, putative(2)" DNA-directed RNA polymerase beta' chain(1) DNA-directed RNA polymerase beta subunit(1) "DNA- directed RNA polymerase, beta" subunit(1)"

68 6 142 4.225352113 2 0.01173 >Cluster 669 ribonuclease Y(21) ribonuclease(18) Hydrolase (HAD superfamily)(9) phosphodiesterase(8) conserved hypothetical protein(7) putative exported protein(6) "2',3'-cyclic- nucleotide 2'-phosphodiesterase(3)" Ribonuclease Y(3) FIG002344: Hydrolase (HAD superfamily)(1) "2', 3'-cyclic-nucleotide 2'-phosphodiesterase, putative(1)" uncharacterized domain HDIG domainprotein(1) hypotheticalprotein(1) YmdA/YtgFfamily protein(1) metal dependent phophohydrolase(1) possible hydrolase(1)

69 6 142 4.225352113 2 0.01173 >Cluster 4673 antibiotic biosynthesis monooxygenase(44) hypothetical protein(16) antibiotic biosynthesis monooxygenase family protein(7) conserved hypothetical protein(4) Signal transduction protein TRAP(3) Antibiotic biosynthesis monooxygenase(2) FIG01108277: hypothetical protein(1) Hypothetical protein(1) putative antibiotic biosynthesis monooxygenase(1)

70 9 142 6.338028169 2 0.01434 >Cluster 2457 1-phosphofructokinase(40)phosphofructokinase(17) fructose 1- phosphate kinase(13) putative phosphofructokinase(7) "fructose 1-phosphate kinase, putative(2)" "hexose kinase, 1- phosphofructokinase family protein(1)" hypothetical protein(1)

71 10 142 7.042253521 4 0.01552 >Cluster 231 AraC family transcriptional regulator(39) Transcriptional regulator of biofilm formation (AraC/XylS family)(7) AraC familyregulatory protein(5) "bacterial regulatory helix-turn-helix s, AraCfamilyprotein(4)" AraCfamilytranscriptionregulator(4) helix-turn helix domain protein(3) conserved hypothetical protein(3) transcriptional regulator(3) "transcriptional regulator, AraC family(2)" putative transcriptional regulator(2) "Transcriptional regulator, AraC family protein(1)"hypotheticalprotein(1) "bacterialregulatoryhelix-turn- helix proteins, AraC family protein(1)" "bacterial regulatory helix- turn-helix s, AraC(1)" helix-turn-helix- domain containing proteinAraC type(1)putative(1)"

72 12 135 ATP-dependent helicase(30) DinG family ATP-dependent helicaseYoaA(8)hypotheticalprotein(8) putativeATP-dependent helicase DinG like protein(4) "DNA polymerase III, epsilon subunit/ATP-dependent helicase DinG(4)" "DnaQ family exonuclease/ DinG family helicase, putative(3)" "exonuclease, DNA polymerase III, epsilon subunit family domain protein(3)" ATP-dependent DNA helicase DinG(2) probable ATP-dependent DNA helicase(2) ATP- dependent helicase dinG(2) conserved hypothetical protein(2) ATP-dependent helicase DinG-like protein(2) DnaQ family exonuclease(1) dnaQ family exonuclease/DinG family helicase(1) putative DnaQ family exonuclease/DinG family helicase(1) exonuclease family protein(1) probable ATP-dependent helicase(1) DNA-directed DNA polymerase III epsilon subunit(1) "DNA polymerase III, epsilon subunit(1)" "ATP-dependent helicase, DinG family"AraC/XylS family transcriptional regulator,MW0629(1)142 8.450704225 4 0.01838 >Clusterprotein(1)"protein(1)"

73 12 1984 O-sialoglycoprotein endopeptidase(22) tRNA threonylcarbamoyl adenosine modification protein TsaD(8) putative glycoprotease(7) putative DNA-binding/iron metalloprotein/AP endonuclease(7) tRNA threonylcarbamoyl adenosine modification protein YgjD(6) putative tRNA threonylcarbamoyladenosine biosynthesis protein Gcp(6) "TsaD/Kae1/Qri7protein,requiredfor threonylcarbamoyladenosine t(6)A37 formation in tRNA(5)" tRNA N6- adenosine(37)-threonylcarbamoyltransferase complex transferase subunit TsaD(3) "O-sialoglycoprotein endopeptidase, putative(2)" tRNA threonylcarbamoyladenosine modification protein TsaD(2) hypotheticalprotein(2) "putativemetalloendopeptidase,glycoprotease family(1)" Probable O-sialoglycoprotein endopeptidase(1) putative glycoprotease GCP(1) O-sialoglycoprotein endopeptidase (Glycoprotease)(1) tRNA threonylcarbamoyladenosine biosynthesis protein TsaB(1) "metalloendopeptidase, glycoprotease familyprotein(1)"MW1973(1)

74 11tRNA N6-adenosine threonylcarbamoyltransferase(1)M22 family O-sialoglycoprotein endopeptidase(1)142 7.746478873 4 0.01935 >Cluster"putative ATP-dependent helicase, DinG familyprobable ATP-dependent DNA helicase dinG(1)142 8.450704225 3 0.01838 >Cluster679 gluconokinase(39) gluconate kinase(17) Gluconokinase(12) putative gluconokinase(11) "FGGY family of carbohydrate kinase, N-terminal domain protein(2)" unnamed protein product(1) "carbohydrate kinase, FGGY family(1)"

75 9 142 6.338028169 3 0.02005 >Cluster 1400 pyrimidine nucleoside transporter NupC(31)putative nucleoside permease(7) na+ dependent nucleoside transporter family protein(7) putative pyrimidine nucleoside transport protein associated with pseudouridine catabolism(5) Na+ dependent nucleosidetransporterfamilyprotein(4) nucleosidepermease(4) Pyrimidine nucleoside transport protein(3)"nucleoside permease NupC, putative(2)" conserved hypothetical protein(2) Na dependent nucleoside transporter domain-containing protein(2) probable pyrimidine nucleoside transport protein(2)pyrimidine nucleoside transporter(2) nucleoside permease nupC (Nucleoside-transport system protein nupC)(1) Nucleoside permease nupC(1) putative nucleoside transporterpermease(1) probablenucleosidetransporterpermease(1)

Probable pyrimidine nucleoside transport protein associated with pseudouridine catabolism(1) Na dependent nucleoside transporter domain protein(1) Probable pyrimidine nucleoside transport protein(1) pyrimidine nucleoside transport protein(1)"nucleoside transporter, NupC family(1)" possible CNT family concentrative nucleoside transporter(1)putative pyrimidine nucleoside transport protein(1)

76 9 142 6.338028169 2 0.02005 >Cluster 4766 hypothetical protein(58) putative exported protein(7) conserved hypothetical protein(7) exported protein(3) Hypotheticalprotein(2) probableexportedprotein(1) FIG01108521: hypothetical protein(1)

77 13 142 9.154929577 2 0.02114 >Cluster 2292 histidine kinase(30) sensor kinase protein(9) "histidine kinase-, DNA gyrase B-, and HSP90-like ATPase family protein(8)" Two-componentsystemhistidinekinase(7) integralmembranesensor signal transduction histidine kinase(5) putative sensor histidine kinase graS(2) sensor protein(2) two-component sensor histidine kinase(2) "sensor histidine kinase, putative(2)" sensor histidine kinase(2) sensor kinase(2) ATP-binding region ATPase domain protein(1) "two-component sensor histidine kinase,ATPase/histidine kinase/DNA gyrase B/HSP90 domain probable two-component sensor histidine kinase(1) hypotheticalprotein(1) Two-componentsensorputative(1)"protein(1)MW2544(1)histidinehistidinekinase(1)kinase(1) nisin susceptibility-associated sensor kinase(1) Putative two-component sensor histidine

78 20 142 14.08450704 4 0.02306 >Cluster 1885 histidine kinase(27) histidine protein kinase saeS(8) histidine protein kinase SaeS(6) Sensor histidine protein kinase SaeS (exoprotein expression protein S)(6) Histidine protein kinase SaeS(5) histidine protein kinase(5) histidine kinase protein(4)integral membrane sensor signal transduction histidine kinase(4) two-componentsensorhistidinekinase(3) sensor histidine kinase SaeS(3) "sensor histidine kinase SaeS, putative(2)"Sensor histidine protein kinase SaeS(2) histidine kinase protein SaeS(2) ATP-binding region ATPase domain protein(1) Sensory transduction protein kinase SaeS(1) two-component system sensor histidine kinase(1)

79 20 142 14.08450704 4 0.02306 >Cluster 4506 hypothetical protein(21) putative exported protein(7) domain-containing protein(5) antigen SsaA-like protein(3) antigen ssaA(3) putative secretory antigen(3) secretory antigen SsaA-like protein(2) Secretory antigen SsaA(2)"secretory antigen precursor SsaA, putative(2)" N-acetylmuramoyl-L-alanine amidase Sle1(1) staphylococcal secretory antigen ssaA(1) putative secretory antigen SsaA(1) secretory antigen precursorSsaA(1) "staphyloxanthin biosynthesis protein, putative(1)" ssaA(1) CHAP domain containing protein(1) secretory antigen ssaA2(1)Secretory antigen precursor SsaA(1) exported secretory antigen precursor(1) Secretory antigen SsaA -like protein(1) Secretory antigen SsaA homolog(1)

80 18 142 12.67605634 6 0.02556 >Cluster 97type VII secretion protein EsaA(40) Putative secretionaccessory protein EsaA/YueB(9) protein EsaA(4) Protein EsaA(3) phage infection protein(2) protein(2) esaA(2) EsaA(1) virulence protein EsaA(1)EsaA domain protein(1) MW0259(1) Putative membrane spanning protein(1) protein esaA(1) Protein esaA(1)secretory antigen precursor(7) secretory antigen SsaA(5) CHAP CHAP domain protein(4) Secretory Secretory antigen ssaA(3) secretoryputative membrane protein(8) conserved hypothetical protein(3) hypothetical protein(2) EsaA probable membrane protein(1)

81 13 142 9.154929577 6 0.02559 >Cluster 351 AraC family transcriptional regulator(31) Transcription regulator(8) putative transcriptional regulator(4) "bacterial regulatory helix-turn-helix s, AraC familyprotein(4)" "Transcriptional regulator, AraC family protein(4)" helix-turn-helix domain protein(3) AraC familyegulatory protein(3) AraC family HTH-type transcriptional regulator(3) transcriptional regulator(3) "transcriptional regulator, AraC family(2)" transcription regulator(2) helix-turn-helix- domain containing protein(2) "helix-turn-helix-domaincontainingprotein,AraCtype(2)" probabletranscriptionalregulator(1) "bacterial regulatory helix-turn-helix proteins, AraC family protein(1)" conserved hypothetical protein(1) hypotheticalprotein(1) MW2216(1) helix-turn-helix-domain containing protein AraC type(1) transcriptional regulator AraC family protein(1) "transcriptional regulator, putative(1)"

82 13 142 9.154929577 3 0.02559 >Cluster 648 hypothetical protein(30) membrane-flanked domain-containing protein(10) membrane protein(9) putative membrane protein(8)bacterial PH domain protein(6) conserved hypothetical protein(4) putative transmembrane protein(3) bacterial membrane flanked domain protein(2)"Hypothetical protein, ydbT(1)" probable membrane protein(1) Putative membrane spanning protein(1) Hypotheticalprotein(1) putativebacterialmembraneflankeddomain protein(1) ydbT-like protein(1) "Hypothetical protein, ydbT homolog(1)" bacterial membrane flanked domain family(1) hypothetical membrane protein(1)

83 13 142 9.154929577 3 0.02559 >Cluster 976 membrane protein(18) hypothetical protein(14) Teicoplanin resistance associated membrane protein TcaA(9) membrane-associated protein TcaA(7) teicoplanin resistance associated membrane protein(7)teicoplanin resistance associated membrane protein TcaA protein(5) Membrane-associated protein TcaA(4) TcaA protein(4) tcaA protein(2) membrane-associated protein tcaA(2)truncated teicoplanin resistance associated membrane protein TcaA protein(2) Teicoplanin-associated protein TcaA(1) bicyclomycin and teicoplanin resistance protein(1) teicoplanin resistance protein TcaA(1) "teicoplanin resistance-associated membrane protein, putative(1)" Membrane-associated protein tcaA(1)conserved hypothetical protein(1) membrane-associated TcaAdomain protein(1)A(1)

84 6 142 ATPase(26)teicoplanin resistance associated protein4.225352113 3 0.02608 >Cluster 83 hypothetical protein(14) Superfamily I DNA/ conservedhypotheticalprotein(4)ATPaseRNAhelicaseprotein(6) associated with various cellular activities family protein(3) Helicase domain-containing protein(3) RecBCD enzyme subunit RecD(1) viral (Super1) RNA helicase family protein(1) "helicase, RecD/TraA family protein(1)" MW0068(1)

85 6 142 4.225352113 4 0.02608 >Cluster 776 monovalent cation/H+ antiporter subunit D(19) cation:proton antiporter(14) "NADH-Ubiquinone/plastoquinone (complex I), various chains family protein(8)" Na(+) H(+) antiportersubunitD(8) putativeNADH-Ubiquinone/plastoquinone (complex I) oxidoreductase protein(5) Putative antiporter subunit mnhD2(5) putative monovalent cation/H+ antiporter subunit D(2)MrpD protein(2) "monovalent cation antiporter-3, MnhD_1(2)" "monovalent cation antiporter-3, MnhD(1)" Na+ H+ antiportersubunitD(1) putativeantiportersubunit mnhD2(1) "monovalent cation/H antiporter, subunit D, putative(1)" "CPA3 family monovalent cation (K+ or Na+):proton (H +) antiporter-3, MnhD subunit(1)" MrpD(1) MnhD homologue(1) "monovalent cation antiporter-3, MnhD1(1)"Putative NADH Ubiquinone plastoquinone complex I oxidoreductase protein(1) NADH dehydrogenase (quinone)(1) Sodium/proton antiporter protein shaD(1) conserved hypothetical protein(1) probable Na+/H+ antiporter protein(1) MW0588(1) "Na+/H+ antiporter, MnhD component(1)"

86 6 142 4.225352113 3 0.02608 >Cluster 846 aminoacidpermease(39) aminoacidpermeasefamily protein(19) Amino acid transporter(8)APC family amino acid- polyamine-organocation transporter(5) Amino acid permease(4) hypotheticalprotein(1) conservedhypotheticalprotein(1)cationic amino acid transporter(1)MW2522(1) amino acid permease- associated region(1)

87 3 142 2.112676056 2 0.02717 >Cluster 1250 maltose ABC transporter substrate-binding protein(30) bacterial extracellular solute-binding family protein(11) "Maltose/ maltodextrin ABC transporter, substrate binding periplasmic protein MalE(9)" putative extracellular sugar-binding lipoprotein(7) "maltose/maltodextrin ABC superfamily ATP binding cassette transporter, binding protein(5)" Maltose/ maltodextrin transport system substrate-binding protein(2) "maltose ABC transporter, maltose-binding protein(2)" hypothetical protein(2) "extracellular solute-binding protein, family 1(2)" probable maltose/maltodextrin transport system protein(1) "maltose ABC transporter, substrate binding protein(1)" "ABC transporter, solute-binding protein(1)" extracellular solute-binding protein family 1(1) putative extracellular sugar-bindinglipoprotein(1) "bacterial extracellular solute-binding protein, putative(1)" MW0190(1) Maltose/ maltodextrin-binding protein(1) conserved hypothetical protein(1) "maltose ABC transporter, maltose-binding protein, putative(1)"

88 3 142 2.112676056 2 0.02717 >Cluster 1451 diaminopimelate decarboxylase(36) pyridoxal-dependent decarboxylase decarboxylase(11) Siderophore staphylobactin biosynthesis protein SbnH(7) pyridoxal-dependent decarboxylase(4) "pyridoxal-dependent decarboxylase, C-terminal sheet domain protein(4)" "pyridoxal-dependent decarboxylase, pyridoxal binding domain protein(3)" Siderophore biosynthesis protein SbnH(3) hypothetical protein(2) alanine racemase(2) conserved hypothetical protein(1) Diaminopimelate decarboxylase(1) probablediaminopimelatedecarboxylaseprotein(1) putativediaminopimelate decarboxylase protein(1) putative diaminopimelate decarboxylase(1) Orn/DAP/Arg decarboxylase 2(1) "alanine racemase, N-terminal domain protein(1)" "Pyridoxal-dependent decarboxylase, C-terminal sheet domain protein(1)"

89 3 142 2.112676056 3 0.02717 >Cluster 2106 CAAX protease(24) hypothetical protein(14) abi-likefamily protein(6) Abi family protein(6) infection bacteriophage resistance protein(5) hypotheticalprotein(3) Hypotheticalprotein(2)infection protein(1)MW0344(1)

90 3 142 2279 2-hydroxyacid dehydrogenase(22) hydroxyacid dehydrogenase(13) putative D-isomer specific 2-hydroxyacid dehydrogenase(7) D-isomer specific 2-hydroxyacid dehydrogenase family protein(5) Glyoxylate reductase / Glyoxylate reductase / Hydroxypyruvate reductase(5) "D-isomer specific 2-hydroxyacid dehydrogenase, NAD binding domain protein(4)" Putative 2- hydroxyacid dehydrogenase(4) glycerate dehydrogenase(4) "D- isomer specific 2-hydroxyacid dehydrogenase, catalytic domain protein(2)" glyoxylate reductase (Glycolate reductase)(2) Glyoxylatereductase(2) D-isomerspecific2-hydroxyaciddehydrogenase NAD-binding(1) 4-phosphoerythronate dehydrogenase(1) glyoxylate reductase(1) (R)-2-hydroxyacid dehydrogenase(1) Glyoxylate reductase(NADP+)(1) hypotheticalprotein(1) NADbindingdomain of 6-phosphogluconate dehydrogenase family protein(1) "glycerateAbi-family protein(1)FIG01108853: hypothetical protein(1)2.112676056 2 0.02717 >Clusterdehydrogenase, putative(1)" MW2224(1) dehydrogenase(1)

91 3 142 2.112676056 2 3852 riboflavin synthase subunit alpha(40) synthase, alpha subunit(16)" riboflavin synthase alpha chain(10)Riboflavin synthase eubacterial/eukaryotic(6) Riboflavin synthase alpha chain(5) riboflavin synthase(2) riboflavin synthase alpha subunit(1)

92 3 142 2.112676056 2 0.02717 >Cluster 3938 hypothetical protein(53) conserved hypothetical protein(9) putative phosphate uptake regulator(4) pit accessory protein(3) Phosphate transport regulator(3) Putative pit accessory protein(2) conserved hypothetical protein TIGR00153(1) protein YkaA(1) Phosphate transport regulator -like protein(1) "phosphate transportregulator,putative(1)"Hypotheticalprotein(1) proteinof unknown function DUF47(1)FIG01107977: hypothetical protein(1)putative abortive conserved putativeabortive Abi-like protein(1)probable0.02717 >Cluster "riboflavin

93 3 142 2.112676056 2 0.02717 >Cluster 4256 DNA-binding protein(31) hypothetical protein(23) conserved hypothetical protein(11) protein in cluster with ribosomal protein L32p(3) "protein in cluster with ribosomal protein L32p, Firmicutes subfamily(2)" "Hypothetical protein in cluster with ribosomal protein L32p, Firmicutes subfamily(1)" putative cytosolic protein(1) "putative ACR, COG1399(1)" protein of unknown function DUF177(1) ribosomal protein L32p(1)YlbN-like protein(1) Putative cytosolic protein(1) conserverd hypotheticalprotein(1) "COG1399proteininclusterwithribosomal protein L32p, Firmicutes subfamily(1)"

94 8 142 5.633802817 2 0.02729 >Cluster 712 histidine ammonia-lyase(57) Histidine ammonia-lyase(16)putative histidine ammonia-lyase(6) histidine ammonia- lyasedomainprotein(2) histidineammonialyase(1)

95 18 142 12.67605634 5 0.02903 >Cluster 5763 30S ribosomal protein S10(60) ribosomal protein S10(11)SSU ribosomal protein S10p (S20e)(7) SSU ribosomal protein S10P(1)

96 5 142 3.521126761 3 0.0294 >Cluster 575 arginine--tRNA ligase(36)arginyl-tRNA synthetase(18) Arginyl-tRNA synthetase(10) putative arginyl-tRNA synthetase(10)Arginine--tRNA ligase(6)

97 5 142 3.521126761 3 0.0294 >Cluster 1060 histidine kinase(27) Two component system histidine kinase ArlS(9) signal transduction histidine-protein kinase ArlS(8)Signal transduction histidine-protein kinase ArlS(6) two-componentsensorhistidinekinase(5) sensorkinaseprotein(5) signal transduction histidine kinase(4) HAMP domain protein(4) signal transduction histidine-protein kinase arlS(2) histidine kinase ArlS(2) conserved hypothetical protein(1) Two-component sensor kinase arlS(1) putative protein histidine kinase ArlS(1) putative signal transduction histidine-protein kinase ArlS(1) sensor histidine kinase(1) putative protein histidine kinase(1)sensor histidine kinase ArlS(1) ATP-binding region ATPase domain protein(1) "two-component sensor histidine kinase ArlS, putative(1)"

98 5 142 3.521126761 2 0.0294 >Cluster1350 imidazolonepropionase(54) Imidazolonepropionase(16) putative imidazolonepropionase(10)

99 5 142 3.521126761 2 0.0294 >Cluster 1606 acetyl-CoA acetyltransferase(31) acetyl-CoA C- acetyltransferase family protein(7) putative ketoacyl-CoA thiolase(6) acetyl-CoA C-acetyltransferase VraB(5) Putative acetyl-CoA C-acetyltransferase VraB(4) acetyl-CoA C-acetyltransferase(4) 3-ketoacyl-CoA thiolase(4) acetyl-CoA c-acetyltransferase(3) "acetyl-CoA acetyltransferase, putative(2)"putative acetyl-CoA C-acetyltransferase(2)acetyl-CoA C- acetyltransferase vraB(2)Acetyl-CoA acetyltransferase(2) 3-ketoacyl- CoA thiolase @ Acetyl-CoA

acetyltransferase(1) 3-ketoacyl-CoA thiolase / Acetyl-CoA acetyltransferase(1)3-ketoacyl-CoA thiolase; Acetyl-CoA acetyltransferase(1) "3-ketoacyl-CoA thiolase, Acetyl-CoA acetyltransferase(1)" acetyl-CoA acetyltransferases family protein(1) Putative acetyl-CoA C-acetyltransferase vraB(1)

100 5 142 3.521126761 2 0.0294 >Cluster 1621 nitric oxide dioxygenase(35) flavohemoprotein(21) Flavohemoprotein(5) Flavohemoprotein (Hemoglobin-like protein) (Flavohemoglobin) (Nitric oxide dioxygenase)(5) globin(3) putative flavohemoprotein(2) "flavohemoprotein, putative(2)" hypotheticalprotein(1) MW0216(1) flavohemo(Hemoglobin-like protein) (Flavohemoglobin) (Nitricoxide dioxygenase) (NO oxygenase) (NOD)(1)"oxidoreductase,FAD-bindingprotein(1)" possiblenitricoxide dioxygenase(1)oxide dioxygenase)(1)

101 4 142 4892 murein hydrolase transporter LrgA(30) Antiholin-like protein LrgA(14) holin-like protein(8) lrgA family protein(8) murein hydrolase regulator(5) murein hydrolase regulator LrgA(5)LrgA family protein(2) antiholin-protein lrgA(2)holin-like murein hydrolase regulator LrgA(1) holin-like protein A(1) conserved hypothetical protein(1) holin-like protein LrgA(1) Murein hydrolase exporter LrgA(1) antiholin-like protein LrgA(1)

102 10 142 7.042253521 4 0.03364 >Cluster 837 inosine-5'-monophosphate dehydrogenase(20)inosine-5- monophosphate dehydrogenase(17) Inosine-5'-monophosphate dehydrogenase(14) IMP dehydrogenase(10) putative inosine-5'-monophosphate dehydrogenase(9) inositol-monophosphate dehydrogenase(5) Inosine-5'-monophosphate dehydrogenase / CBS domain(3) nitronate monooxygenase familyFlavohemoprotein (Flavohemoglobin) (NitricGlobin domain protein(1)2.816901408 2 0.03117 >Clusterprotein(2) "inosine-5'-monophosphate dehydrogenase, GuaB(1)" Inosine-5-monophosphate dehydrogenase(1)

103 10 142 7.042253521 2 0.03364 >Cluster 3442 serine protease(32) serine protease SplA(10) Serine proteaseSplA(4)serineproteasesplA(3) SerineproteasesplA(3) peptidase S1 and S6 chymotrypsin/Hap(1) S1B family serine protease SplA(1) "peptidase S1 and S6, chymotrypsin/Hap(1)"

104 12 142 8.450704225 5 0.03498 >Cluster 5105 hypothetical protein(26) Phage protein(8) conserved hypothetical protein(4) putative phage protein(3)SLT orf 129-like protein(3)protein(1)hypothetical phage-related"Hypothetical protein, phi-ETA orf42(1)"142 5.633802817 2 0.03853 >Cluster

105 8 1477 acetyl-CoA acetyltransferase(33) 3-ketoacyl-CoA thiolase(12)acetyl-CoA C-acetyltransferase family protein(7) putative thiolase(7) putative acyl-CoA acetyltransferase FadA(6) acetyl-CoA C-acetyltransferase(4) thiolase(2) "3-ketoacyl-CoAacetyltransferase FadA, putative(1)" transferase(1) hypothetical protein(1) acetyltransferases family protein(1) homologue(1) acetyl-CoA acetyltransferase homolog(1) MW0207(1) "3-ketoacyl-CoA thiolase A, peroxisomal (Beta-ketothiolase A)(Acetyl-CoA acyltransferase A) (Peroxisomal 3-oxoacyl-CoA thiolas>(1)"staphylocoagulase precursor(1) "acetyl-CoA acetyltransferase, putative(1)"

106 8 142 5.633802817 2 0.03853 >Cluster 2442 ferrochelatase(63) "Ferrochelatase, protoheme ferro-lyase(8)" Ferrochelatase(8)hemH(1)

107 8 142 5.633802817 2866 pseudouridine synthase(21) family protein(13) RNA pseudouridine synthase(12) RNApseudouridylate synthase(10) pseudouridine synthase D(5) pseudouridine synthase D(4) pseudouridinesynthaseD-likeprotein(2) "RluA,Pseudouridylate synthases, 23S RNA-specific(2)" "RluA, Pseudouridylate synthase, 23S RNA-specific(2)" conserved hypothetical protein(2) probable RNA pseudouridylate synthase(1) "ribosomal large subunit3-ketoacyl-CoAacetyl-CoAacetyl-CoA acetyltransferase3 0.03853 >Cluster RNA pseudouridylate synthase ribosomal large subunit Ribosomal large subunit ribosomal large subunit pseudouridine synthase, RluD subfamily, putative(1)" pseudouridine synthase D(1) "ribosomal large subunit pseudouridine synthase D, YhcT type -like protein(1)" putative ribosomal large subunit pseudouridine synthase D(1) "Similar to ribosomal large subunit pseudouridine synthase D, Bacillus subtilis YhcT type(1)" "ribosomal large subunit pseudouridine synthase, RluD subfamily(1)" pseudouridylate synthase(1) "pseudouridine synthase, RluA family(1)"

108 8 142 5.633802817 2 0.03853 >Cluster 3304 carboxylesterase(50) Carboxylesterase(13) putative carboxylesterase(7) carboxyesterase precursor-like protein(3) carboxyesterase -like protein(2) "carboxylesterase precursor, putative(2)" esterase/lipase-protein(2) probable carboxylesterase precursor(1) conserved hypothetical protein(1) carboxyesterase precursor homologue(1) MW0741(1)

109 8 142 5.633802817 2 0.03853 >Cluster 4801 monovalent cation/H+ antiporter subunit G(18) cation:proton antiporter(14) "monovalent cation/proton antiporter, MnhG/PhaG subunit(9)" Na(+) H(+) antiporter subunit G(8) Putative antiporter subunit mnhG2(4) putative antiporter subunit mnhG2(3) conserved hypothetical protein(3) Na+/H+ antiporter subunit(2) putative monovalent cation/H+ antiporter subunit G(2) Na /H antiporter(2) putative membrane protein(2) "monovalent cation antiporter-3, MnhG_1(2)" MrpG(1) "CPA3 family monovalent cation (K+ or Na+):proton (H+) antiporter-3, MnhG subunit(1)" "monovalent cation antiporter-3, MnhG1(1)" probable membrane protein(1) "monovalent cation antiporter-3, MnhG(1)" Na+ H+ antiporter subunit G(1) "Na+/H+ antiporter, MnhG component(1)" hypothetical protein(1) Sodium/ proton antiporter protein shaG(1) "monovalent cation/H antiporter, subunit G, putative(1)"

110 8 142 5.633802817 2 0.03853 >Cluster 5333 glyoxalase(30) glyoxalase/bleomycin resistance protein/ dioxygenase superfamily protein(12) Glyoxalase/bleomycin resistance protein/dioxygenase superfamily protein(9) glyoxalase/ Bleomycin resistance /Dioxygenase superfamily protein(7) glyoxalase family protein(6) glyoxalase/bleomycin resistance protein/ dioxygenase(4) conserved hypothetical protein(4) lactoylglutathione lyase(3) "glyoxalase/bleomycin resistance protein, putative(1)" lactoylglutathione lysase(1) Glyoxalase/bleomycin resistance protein/dioxygenase(1) glyoxalase/Bleomycin resistance protein/ Dioxygenase superfamily protein(1) Lactoylglutathione lyase(1)

111 8 142 5.633802817 2 0.03853 >Cluster 6049 hypothetical protein(62) conserved hypothetical protein(11) Hypotheticalprotein(4) Putativecytosolicprotein(1) Hypothetical protein SAV2173(1) UPF0457 protein SA1975.1(1)

112 43 142 30.28169014 9 0.03906 >Cluster 14 methicillin resistance protein FmtB(22) FmtB protein(10) sasC/Mrp/FmtB intercellular aggregation

domain protein(6) methicillin resistance determinant FmtB protein(4) LPXTG surface-anchored protein(3) FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis(3) LPXTG cell wall anchor domain protein(3) YSIRK family Gram-positive signal peptide(2) Extracellular matrix binding protein(2) LPXTG family cell wall anchor protein(2) fmtB protiein(2) truncated FmtB protein(2) sasB protein(2) hypothetical protein(2) LPXTG surface protein(1) "Gram-positive signal peptide protein, YSIRK family(1)" truncated FmtB(1) LPXTG-motif cell wall anchor domain protein(1) fmtB protein(1) truncated methicillin resistance-related surface protein(1) LPXTG-motif cell wall anchor domain(1) motif)(1)

113 10 169 RecD/TraA family helicase(33) RecD-like DNA helicase YrrC(7) hypothetical protein(6) exodeoxyribonuclease V alpha subunit(4) Exodeoxyribonuclease V alpha chain(4) viral (Super1) RNA helicase family protein(4) conserved hypothetical protein(4) "helicase, RecD/TraA family(3)" exodeoxyribonuclease V alpha chain(3) "helicase, RecD/TraA family protein(3)" exodeoxyribonuclease V subunit alpha(2) ATP-dependent DNA helicase(2)"RecD-TraA family helicase, putative(1)" possible exodeoxyribonuclease V alpha subunit(1) probable deoxyribonuclease(1) MW1569(1) helicase RecD/TraA family protein(1)

114 10 142 7.042253521 2 0.04274 >Cluster 1538 UDP-N-acetylglucosamine 2-epimerase(44) UDP-N-acetyl glucosamine 2-epimerase(10) capsular polysaccharide synthesis enzyme(7) capsular polysaccharide biosynthesis protein Cap5P(5) Capsular polysaccharide synthesis enzyme Cap8P(5) capsular polysaccharidesynthesisenzymeCap5P(3) UDP-N-acetylglucosamine2- epimerase (UDP-GlcNAc-2-epimerase). domain protein(2) UDP-N- acetylglucosamine 2-epimerase Cap5P(1) "capsular polysaccharide synthesis UDP-N-acetylglucosamine 2-epimerase CapP, putative(1)" capsular polysaccharide biosynthesis protein CapP(1) capsular polysaccharide synthesis enzyme CapP(1) capsular polysaccharide synthesis enzyme Cap8P(1)

115 10 142 7.042253521 3 0.04274 >Cluster putative cell-wall-anchored protein SasC (LPXTG conserved hypothetical protein(1) 142 7.042253521 2 0.04274 >Cluster 4469 acetyltransferase(35) putative acetyltransferase(10) Acetyltransferase (GNAT) family(8) acetyltransferase family protein(6) conserved hypothetical protein(4) "acetyltransferase, GNATfamilyprotein(3)" GNATfamily acetyltransferase(2) "acetyltransferase, gnat family(2)" GCN5 N-acetyltransferase(2) hypothetical protein(1) "acetyltransferase, GNAT family, putative(1)" "acetyltransferase, GNAT family(1)" Acetyltransferase(1) Acetyltransferase GNAT family(1) possible acetyltransferase(1) acetyltransferase (GNAT) family protein(1) GCN5-related N-acetyltransferase(1)

116 28 142 861 amidase(34) SH3 domain protein(9) amidase(8)" bacteriophage amidase(4) N-acetylmuramoyl-L-alanine amidase family protein(4)putative phage amidase(3)SH3 domain protein(3) "phage lysin, N-acetylmuramoyl-L-alanine amidase(3)" 19.71830986 7 0.04691 >Cluster peptidoglycan hydrolase(21) bacterial "Phage lysin, N-acetylmuramoyl-L-alanine N-acetylmuramoyl-L-alanine amidase(3) truncated amidase(2) phage amidase(2) "peptidoglycan hydrolase, putative(1)" truncated phage amidase(1) amidase (fragment) (1) hypotheticalprotein(1) SH3domain-containingprotein(1) lysin(1)"Truncatedamidase,phageassociated(1)" "petidoglycan hydrolase, putative(1)" CHAP domain protein(1) putative amidase(1)

117 13 142 9.154929577 4 0.04699 >Cluster 3618 methionine ABC transporter ATP-binding protein(30) ABC transporter ATP-binding protein(14) YbbL ABC transporter ATP- binding protein(9) ABC transporter family protein(6) "ABC superfamily ATP binding cassette transporter, ABC protein(5)" ABC transporter(4) hypothetical protein(3) "ABC transporter, ATP- binding protein(3)" "ABC transporter, ATP-binding protein, putative(2)" MW2378(1) ABC transporter related(1) ATP-binding ABC transporter(1) Methionine ABC transporter ATP- binding protein(1)

118 9 142 6.338028169 3 0.04884 >Cluster 1392 thiamine biosynthesis protein ThiI(37) putative thiamine biosynthesis protein(7) tRNA sulfurtransferase ThiI(6) thiamine biosynthesis/tRNA modification protein ThiI(5) tRNA S(4)U 4- thiouridine synthase (former ThiI)(5) putative tRNA sulfurtransferase(4) tRNA 4-thiouridine(8) synthase ThiI(3) thiamine biosynthesis protein thiI(2) Thiamine biosynthesis protein thiI(2) tRNA sulfurtransferase(2)tRNA S(4)U 4-thiouridine synthase(2) thiamine biosynthesis protein(1) Probable thiamine biosynthesis protein thiI(1) probable thiamine biosynthesis protein(1) MW1658(1) hypothetical protein(1)

119 9 142 6.338028169 2 0.04884 >Cluster 2116 ribonuclease HIII(60) Ribonuclease HIII(10) putative ribonuclease(5) probable ribonuclease HIII(1) hypothetical protein(1) MW1023(1) ribonuclease hiii(1)

120 9 142 6.338028169 5 0.04884 >Cluster 3707 deoxyribose-phosphate aldolase(91) 2-deoxyribose-5- phosphate aldolase(20) Deoxyribose-phosphate aldolase(20) deoxyribose-phosphate aldolase 1(9) putative deoxyribose- phosphate aldolase(8) Deoxyribose-phosphate aldolase 2(6) Deoxyribose-phosphate aldolase 1(5) "deoxyribose-phosphate aldolase, putative(1)"

121 9 142 6.338028169 2 4281 DNA polymerase III subunit epsilon(33) protein(8) DNA polymerase III polC-type(8) exonuclease(8) exonuclease(7) "Exonuclease, RNase T and DNA polymerase III(6)" "DNA polymerase III, alpha chain PolC- type(2)" DNA polymerase III alpha subunit(1) hypothetical protein(1) conserved hypothetical protein(1) DNA-directed DNA polymerase III(1) MW1835(1) "3'-5' exonuclease, putative(1)" Exonuclease RNase T and DNA polymerase III(1) probable DNA polymerase III alpha chain(1)

122 11 142 7.746478873 3 0.04963 >Cluster 939 sodium:proton antiporter(29) putative Na+/H+ antiporter(11) Na+/H+ antiporter NhaC(21) na+/H+ antiporter NhaC(7) Na /H antiporter NhaC(2) Na putative Na(+)/H(+) antiporter nhaC(3) +/H+antiporternhaC(2) NhaCfamilysodium:proton(Na+:H) antiporter(1) MW2219(1) "NA+/H+ antiporter NhaC, putative(1)" truncated probable Na+/H+ antiporter(1) hypothetical protein(1)

123 11 142 7.746478873 4 0.04963 >Cluster 1266 zinc protease(31) peptidase M16 inactive domain protein(10) hypothetical protein(10) Zinc protease(7) conserved hypotheticalprotein(5) "processingproteinase-likeprotein,pqqL(4)" putative metalloprotease(4) peptidase M16(2) peptidase M16 domain protein(1) MW1161(1) "zinc-dependent peptidase, putative(1)" FIG001621: Zinc protease(1) "Non-proteolytic protein, peptidase family M16(1)" possible M16C subfamily peptidase(1) peptidase M16 inactive domain family(1) "M16C subfamily peptidase, putative(1)"

124 7 142 4.929577465 2 0.05195 >Cluster 0.04884 >Cluster exonuclease family putative 2380 ABC transporter(30) hypothetical protein(14) ABC-2 transporter family protein(10) putative membrane protein(9) conserved hypothetical protein(5) putative ABC-2 type transport system permeaseprotein(3) Hypotheticalprotein(2) ABC-2family transporter protein(2) probable membrane protein(2) ABC-2 type transport system permease(2) FIG01107872: hypothetical protein(1)

125 7 142 4.929577465 2 0.05195 >Cluster 5614 anti-sigma-B factor antagonist(26) anti-anti-sigma factor(15) anti-sigma B factor antagonist(8) Anti-sigma-B factor antagonist(6) "anti-sigma-B factor, antagonist(4)" anti-sigmaB factor antagonist(4) Anti-sigma F factor antagonist (spoIIAA-2)(3) anti-sigma-B factor(2) Anti-sigma F factor antagonist (spoIIAA-2); Anti-sigma B factor antagonist RsbV(2) anti-sigma- B factor antagonist (Anti-anti-sigma-B factor)(2) anti-sigma F factor antagonist (spoIIAA-2); anti sigma b factor antagonist RsbV(2) "STAS domain, putative(1)" anti-anti-sigma factor RsbV(1) Anti-sigma F factor antagonist (spoIIAA-2) / Anti-sigma B factor antagonist RsbV(1) Anti-sigma B factor antagonist(1) STAS domain protein(1) anti-sigma B factor antagonist RsbV(1) Anti-sigma B factor antagonist RsbV(1)

126 13 142 9.154929577 3 0.05373 >Cluster 1135 potassium transporter KtrB(32) putative sodium transport protein(10) ktrsystempotassiumuptakeproteinB(7) "Trkfamily potassium (K+) transporter, ABC protein(5)" Na+-transporting ATP synthase(5) "Potassium uptake protein, integral membrane component, KtrB(4)" trk system potassium uptake protein TrkH(3) Trk-type K+ transport system membrane component(2) cation transport family protein(2) H+-transporting two-sector ATPase(2) "potassium uptake protein, TrkH family(1)""TrkH family potassium uptake protein, putative(1)" Potassium uptake protein ktrB(1) H(+)-transporting two-sector ATPase(1) "cation transport protein, putative(1)" MW1946(1) sodium transport family protein(1) conserved hypothetical protein(1) hypotheticalprotein(1) probablesodiumtransportprotein(1)

127 9 142 6.338028169 3 0.05816 >Cluster 1216 succinyl-diaminopimelate desuccinylase(41)putative succinyl- diaminopimelate desuccinylase(13) Acetylornithine deacetylase(8) putative peptidase(6) conserved hypothetical protein(2) Succinyl- diaminopimelate desuccinylase(1) MW1943(1) probable succinyl- diaminopimelate desuccinylase (sdap)(1) probable succinyldiaminopimelatedesuccinylase(1) "succinyl-diaminopimelate desuccinylase, putative(1)" M20A family peptidase(1) acetylornithine deacetylase or succinyl-diaminopimelate desuccinylase(1) Probable succinyl-diaminopimelate desuccinylase(1) "peptidase, ArgE/DapE family protein(1)"hypothetical protein(1)

128 9 142 6.338028169 3 0.05816 >Cluster 2579 Vitamin B12 ABC transporter substrate-binding protein(30) "Vitamin B12 ABC transporter, B12-binding component BtuF(8)" periplasmic binding family protein(8) putative transport system lipoprotein(7) iron complex transport system substrate- binding protein(6) "iron (Fe3+) ABC superfamily ATP binding cassette transporter, binding protein(5)" periplasmic binding protein(4) iron compound ABC transporter periplasmic component(2) "iron compound ABC transporter, substrate-binding protein, putative(2)" probable iron-binding transport protein(1) MW0573(1) protein(1)" protein(1) protein, putative(1)" "iron compound ABC transporter, iron compound-binding protein(1)" Cobalamin-binding protein(1) "Iron compound ABC transporter, iron compound-binding iron-bindingprotein(1) hypothetical "iron compound ABC transporter, iron compound-binding

129 9 142 6.338028169 4 0.05816 >Cluster 5004 monovalent cation/H+ antiporter subunit B(20) cation:proton antiporter(14) Na(+) H(+) antiporter subunit B(7) putative membrane protein(6) na(+)/H(+) antiporter subunit B(6) Putative antiporter subunit mnhB2(5) "monovalent cation antiporter-3, MnhB_1(2)" putative monovalent cation/H+ antiportersubunitB(2) hypotheticalprotein(2) multicomponent Na :H antiporter subunit B(2) Na(+)/H(+) antiporter subunit B(1) domain related to MnhB subunit of Na+/H+ antiporter family protein(1) putative(1)" MnhB1(1)" MnhB component(1)" Na+ antiporter MnhB subunit-related protein(1) conserved hypothetical protein(1) probable Na+/H+ antiporter protein(1) "CPA3 family monovalent cation (K+ or Na +):proton (H+) antiporter-3, MnhB subunit(1)" Na(+)/H(+) antiporter subunit B (Multiple resistance and pHhomeostasis protein B) (Mrp complex subunit B)(1) "monovalent cation antiporter-3, MnhB(1)" Sodium/proton antiporter protein shaB(1) "monovalent cation/H antiporter, subunit B, MW0586(1) "monovalent cation antiporter-3, Na+ H+ antiporter subunit B(1) "Na+/H+ antiporter,

130 5 142 3.521126761 3 adhesin(8) collagen adhesin(8) precursor(6) Collagen binding protein Cna(3) adhesin(2) "Collagen Adhesin, precursor(1)" wall anchor domain protein(1) 0.05885 >Cluster 47 collagen adhesin Collagen LPXTG-motif cell 0.05885 >Cluster putative DNA polymerase(3) bacterophage DNA-directed

131 5 142 3.521126761 3 345 DNA polymerase(19) DNA polymerase A family protein(4) DNApolymerase(3) putativephage-encodedDNApolymerase(3) DNA polymerase I(2) "DNA polymerase I, phage-associated(1)" phage DNA polymerase(1) phage encoded DNA polymerase I(1) putative DNA- directed DNA polymerase(1) DNA-directed DNA polymerase(1)

132 5 142 3.521126761 2 0.05885 >Cluster 465 multidrug ABC transporter permease(33) ABC transporter ATP-bindingprotein(14) TransportATP-bindingproteinCydD(8) ABC transporter family protein(6) "ABC superfamily ATP binding cassette transporter, ABC/membrane protein(4)" ABC transporter ATP-binding/permease protein(3) ABC transporter transmembrane region family protein(2) ABC transporter ATP-binding and permease protein(1) MW2352(1) "ABC transporter, ATP-binding protein(1)" truncatedATP-bindingABCtransporter(1) "ABC-type multidrug transport system, ATPase and permease components, putative(1)" ABC transporter related(1) "ABC transporter, ATP-binding/permease protein(1)" conserved hypothetical protein(1) hypothetical protein(1)

133 5 142 3.521126761 2 0.05885 >Cluster 512 antibiotic ABC transporter ATP-binding protein(29) ABC transporter ATP-binding protein(8) Lipid A export ATP-binding/ permease protein MsbA(8) ATP-binding cassette transporter A(6) Multidrug resistance ABC transporter ATP-binding/permease protein BmrA(6) "ABC superfamily ATP binding cassette transporter, ABC protein(5)" multidrug resistance ABC transporter ATP-binding/ permease protein BmrA(4) ABC transporter family protein(3) ABC transporter(2) ABC transporter transmembrane region family protein(1) conserved hypothetical protein(1) "ABC transporter, ATP-binding/permease protein(1)" ABC transporter related(1) "multidrug ABC transporter ATP-binding protein, putative(1)" Multidrug resistance ABC transporter ATP-binding and permease protein(1) "ABC transporter, ATP-binding protein(1)" multidrug resistance ABC transporter ATP-binding and permease protein(1)

134 5 142 3.521126761 2 0.05885 >Cluster 1243 serine protease(31) putative protease(8) 2-alkenal reductase(6) "Serine protease, DegP/HtrA, do-like(5)" trypsin family protein(4) Trypsin-like serine endoprotease(3) serine protease Do-like protein HtrB(3) PDZ domain protein(3) "serine protease HtrA, putative(2)" "Serine protease,DegP/HtrA,do-likeprotein(2)" conservedhypothetical protein(2) Serine protease(1) S1 family peptidase(1) trypsin-like serine endoprotease(1) probable serine protease(1) trypsin domain protein(1)Endopeptidase degP(1) hypotheticalprotein(1) trypsin(1) MW1670(1) putative serine protease do-like htrA(1) trypsin-like serine protease(1)

135 5 142 3.521126761 2 0.05885 >Cluster 1301 glutaryl-CoA dehydrogenase(32) Long-chain-acyl-CoA dehydrogenase(8) putative acyl-CoA dehydrogenase(7) "acyl-CoA dehydrogenase, C-terminal domain protein(6)" NRAMP family Mn2+/ Fe2+ transporter(4) Acyl-CoA dehydrogenase(3)acyl-CoA dehydrogenase(3) Glutaryl-CoA dehydrogenase(2) Mn2+ and Fe2+ transporter of the NRAMP family-like protein(2) acyl-CoA dehydrogenase family protein(2) acyl-CoA dehydrogenase FadD homolog(1) "acyl-CoA dehydrogenase, N-terminal domain protein(1)" "acyl-CoA dehydrogenase, domain protein(1)" putative fatty oxidation complex protein(1) "acyl-CoA dehydrogenase, putative(1)" MW0209(1) hypothetical protein(1) conserved hypothetical protein(1) "acyl-CoA dehydrogenase,middledomainprotein(1)" acyl-CoAdehydrogenase domain protein(1)

136 5 142 3.521126761 2 0.05885 >Cluster 3144 capsular biosynthesis protein(30) putative capsule synthesis protein(10) Manganese-dependent protein-tyrosine phosphatase(7) PHP domain protein(6) capsular polysaccharide biosynthesis protein Cap1C(5) capsular polysaccharide biosynthesis(4) tyrosine- protein phosphatase CapC(3) protein-tyrosine- phosphatase(2) "capsular polysaccharide biosynthesis, CapC(2)" putative tyrosine-protein phosphatase CapC(2) polysaccharide biosynthesis protein, Cap5C(1)" polysaccharide biosynthesis protein, CapC(1)" protein phosphatase (capsular polysaccharide biosynthesis)(1) putative tyrosine-protein phosphatase capC(1) Protein-tyrosine- phosphatase(1) capsular polysaccharide biosynthesis protein(1) tyrosine-protein phosphatase YwqE(1) capsular polysaccharide biosynthesis capC(1) capsular polysaccharide biosynthesis protein CapC(1)

137 52 142 36.61971831 matrix-binding protein(15) protein Ebh(8) hypothetical protein(7) protein(6) Putative surface anchored protein(6) extracellular matrix-binding protein ebh(4) truncated cell surface fibronectin-binding protein(4) Putative Staphylococcal surface anchored protein(3) Extracellular matrix-binding protein ebh(3) cellwallassociatedfibronectin-bindingproteinEbh(2) very large surface anchored protein(2) conserved hypothetical protein(2) Putative Staphylococcal surface anchored protein; adhesin emb(2) extracellular matrix-binding protein ebhA(2) EbhA protein(1) very large surface anchored protein (pseudogene) (1) "putative methyl-coenzyme M reductase, alpha subunit(1)" large surface anchored protein(1) "surface protein, ECM binding protein-like protein(1)" Extracellular matrix-binding protein ebhB(1) protein of unknown function DUF1542(1) "surface protein, "capsular "capsular Phosphotyrosine-16 0.05953 >Cluster 0 hyperosmolarity resistance extracellular matrix bindingECM binding protein-like protein B(1)" Extracellular matrix binding protein(1)

138 4 142 2.816901408 2 0.07104 >Cluster 1302 aminopeptidase(19) aminopeptidase T(16) putative aminopeptidase(9) thermophilic metalloprotease family protein(6) "Aminopeptidase S (Leu, Val, Phe, Tyr preference)(6)" aminopeptidase PepS(5) "peptidase M29, aminopeptidase II(4)" peptidase M29(3) aminopeptidase AmpS(2) aminopeptidase ampS(2) aminopeptidase pepS(2) thermophilic metalloprotease (M29) (1) "aminopeptidase PepS, putative(1)" Aminopeptidase PepS(1) M29 family aminopeptidase(1) Aminopeptidase(1) peptidase M29 aminopeptidase II(1)

139 4 142 2.816901408 2 0.07104 >Cluster 1455 GTP cyclohydrolase(27) riboflavin biosynthesis protein(13) "3,4-dihydroxy-2-butanone 4-phosphate synthase / GTP cyclohydrolase II(7)" GTP cyclohydrolase II(5) "bifunctional 3,4- dihydroxy-2-butanone-4-phosphate synthase/GTP cyclohydrolase II(5)" RiboflavinbiosynthesisproteinRibBA(4) "bifunctional3,4- dihydroxy-2-butanone 4-phosphate synthase/GTP cyclohydrolase II(3)" riboflavinbiosynthesisproteinRibBA(3) "3,4-dihydroxy-2-butanone4- phosphate synthase(3)" cyclohydrolase-2(2) cyclohydrolase II(1) riboflavinbiosynthesisproteinribBA(1) "riboflavinbiosynthesis protein, putative(1)" "3,4-dihydroxy-2-butanone-4-phosphate synthase(1)" "3,4-dihydroxy-2-butanone-4-phosphate synthase-GTP cyclohydrolase II(1)"Riboflavin biosynthesis protein ribBA(2) GTP riboflavin biosynthesis protein GTP riboflavin biosynthesis protein ribAB(1)

140 4 142 2.816901408 3 0.07104 >Cluster 6050 hypothetical protein(13) membrane protein(6) putative membrane protein(2) "conserved hypothetical protein, transposon- related protein(2)" conserved hypothetical protein(2) hypothetical protein transposon-related(1)Hypothetical protein(1) hypothetical membrane protein(1)

141 7 142 4.929577465 3 0.07243 >Cluster 449 choline dehydrogenase(54)Choline dehydrogenase(16)putative choline dehydrogenase(8) Oxygen-dependent choline dehydrogenase(1) choline dehydrogenase CDH(1)

142 12 142 8.450704225 3 0.0731 >Cluster 926 magnesium transporter(28)magnesium transporter MgtE(28) putative divalent cation transport protein(6) magnesium (Mg2+)/cobalt (Co2+) transporter-E(6) transporter MgtE / CBS domain(5) MW0890(1) protein(1) Magnesium transporter(1) "magnesium transporter, MgtE family Mg/Co/Ni hypothetical putative(1)" probable magnesium transporter(1) Mg2+ transporter mgtE(1) Mg/Co/Ni transporter MgtE / CBS domain protein(1)

143 6 142 terminase(5) measure protein(3) phage-like protein(2) hypotheticalprotein(1) phage tail protein(1)

144 6 142 1797 threonine aldolase(32) aromatic amino acid beta-eliminating lyase/threonine aldolase(10) Low-specificity L-threonine aldolase(9) hypotheticalprotein(7) beta-eliminatinglyase family protein(6) conserved hypothetical protein(5) aminotransferase class I and II family protein(3) aromatic amino acid beta-eliminating lyase(2)probable low specificity threonine aldolase(1) "Aromatic amino acid beta-eliminating lyase, putative(1)" "Aspartate aminotransferase (AAT) superfamily protein, putative(1)" Beta-eliminating lyase(1)

145 6 142 4.225352113 2 0.07413 >Cluster 2092 phage head morphogenesis protein(8) "phage head morphogenesis, SPP1 gp7 family domain protein(3)" Phage protein(3) putative phage head morphogenesis protein(2) "phage putative head morphogenesis protein, SPP1 gp7 family Mu50B(1)" hypotheticalprotein(1) phageminorheadprotein(1) "phage putative head morphogenesis protein, SPP1 gp7 family domain protein(1)"

hypothetical phage-related protein(1) "phage putative head morphogenesis protein, SPP1 gp7 family(1)"

146 6 142 4.225352113 2 0.07413 >Cluster 2857 pyrroline-5-carboxylate reductase(57) Pyrroline-5- carboxylate reductase(12)putative pyrroline-5-carboxylate reductase(6) hypotheticalprotein(1) "pyrroline-5-carboxylatereductase, putative(1)" MW1457(1) probable pyrrolidine-5-carboxylate reductase(1)

147 15 142 10.56338028 3 0.07777 >Cluster 2220 peptidase M23(30) peptidoglycan hydrolase(21) glycyl-glycine endopeptidase lytM(8) Glycyl-glycine endopeptidase LytM precursor(7) glycyl-glycine endopeptidase LytM(4) M23 family lysostaphin(2) "peptidoglycan hydrolase, putative(2)" Peptidoglycan hydrolase(1) Glycyl-glycine endopeptidase lytM 4.225352113 2 0.07413 >Cluster 85 putative tape measure protein(4) phage tape Phage tail length tape-measure protein(3) hypothetical protein(1) conserved putativemembraneprotein(1) putative tape measure protein(1) 4.225352113 3 0.07413 >Cluster precursor(1) glycyl-glycine endopeptidase LytM domain protein(1) Lysostaphin(1) glycyl-glycine endopeptidase(1) Glycyl- glycine endopeptidase lytM(1) peptidase M23 family protein(1)

148 8 142 5.633802817 4 0.08245 >Cluster 53 nitrate reductase(28) "nitrate reductase, alpha subunit(12)" Respiratory nitrate reductase alpha chain(12) nitrate reductase alpha chain(8) respiratory nitrate reductase alpha chain(5) nitrate reductase subunit alpha(4) Nitrate reductase alpha chain(3) "respiratory nitrate reductase, alpha subunit(3)" respiratory nitrate reductase subunit alpha(2) molybdopterin oxidoreductase family protein(2) nitrate reductase alpha subunit(1) molybdopterin dinucleotide binding domain protein(1)

149 8 142 5.633802817 2 0.08245 >Cluster 186 monovalent cation/H+ antiporter subunit A(19) cation:proton antiporter(13) Na(+) H(+) antiporter subunit A(5) putative NADH-Ubiquinone/plastoquinone (complex I) oxidoreductase protein(5)"NADH-Ubiquinone/plastoquinone (complex I), various chains family protein(4)" Putative antiporter subunit mnhA2(4) hypotheticalprotein(4) putativeantiportersubunitmnhA2 domain protein(2) putative monovalent cation/H+ antiporter subunit A(2) "monovalent cation/H+ antiporter subunit A, MnhA_1(2)" Na(+) H(+) antiporter subunit A / Na(+) H(+) antiportersubunitB(2) na(+)/H(+)antiportersubunitA(2) Na /H antiporter(2) Na(+)/H(+) antiporter subunit A (Multiple resistance and pHhomeostasis protein A) (Mrp complex subunit A)(1) Na+ H+ antiporter subunit A / Na+ H+ antiporter subunit B(1) "monovalent cation/H+antiportersubunitA,MnhA(1)" "Na+/H+antiporter,MnhA component(1)" probable NADH dehydrogenase(1) conserved hypotheticalprotein(1) Na(+)/H(+)antiportersubunit A(1) "CPA3 family monovalent cation (K+ or Na+):proton (H+) antiporter-3, MnhA subunit(1)" Sodium/proton antiporter protein shaA(1) "monovalent cation/H antiporter, subunit A, putative(1)" Putative NADH Ubiquinone plastoquinone complex I oxidoreductase protein(1) NADH dehydrogenase (quinone)(1) "monovalent cation/ H+ antiporter subunit A, MnhA1(1)" MW0585(1) Na+ H+ antiportersubunitA(1) antiportersubunitmnhA2(1)

150 8 142 5.633802817 2 0.08245 >Cluster 589 "alpha-D-1,4-glucosidase(26)" "oligo-1,6-glucosidase(19)"glucohydrolase(10) Alpha-glucosidase(7) alpha amylase(4) alpha-glucosidase(4) "alpha amylase, catalytic region(2)" "alpha-glucosidase, putative(1)" alpha amylase catalytic region(1) "alpha amylase, catalytic domain protein(1)" "Exo-alpha-1,4-glucosidase(1)" "oligo-1,6-glucosidase (Oligosaccharide alpha-1,6-glucosidase) (Sucrase-isomaltase) (Isomaltase) (Dextrin 6-alpha-D-glucanohydr>(1)"

151 8 142 5.633802817 3 0.08245 >Cluster 902 dihydrolipoamide dehydrogenase(43) dihydrolipoyl dehydrogenase(14) putative dihydrolipoamide dehydrogenase(8) Dihydrolipoamide dehydrogenase of branched-chain alpha-keto acid dehydrogenase(8) pyridine nucleotide-disulfide oxidoreductase family protein(4) E3 component of branched-chain alpha-keto acid dehydrogenase complex(3) "2-oxoisovalerate dehydrogenase, E3 component, lipoamide dehydrogenase(1)" Dihydrolipoamide dehydrogenase(1)

152 8 142 5.633802817 3 0.08245 >Cluster 945 sodium:dicarboxylate symporter(31) L-cystine uptake protein TcyP(10) putative sodium:dicarboxylate symporter protein(8) L- cystine uptake protein tcyP(7) sodium/dicarboxylare symporter(4) sodium:dicarboxylate symporter family protein(3) sodium:dicarboxylate symporter protein(2) L-cystine transporter tcyP(2) Sodium:dicarboxylate symporter(2) hypothetical symporter YdjN(1) MW0359(1) "sodium:dicarboxylate symporter, putative(1)" Proton/sodium-glutamate symport protein(1)possible DAACS family dicarboxylate/amino acid:sodium (Na+) or proton (H+) symporter(1) proton/sodium-glutamate symport protein(1) "transporter, dicarboxylate/amino acid:cation Na+/H+ symporter family protein(1)" Sodium:dicarboxylate symporter family protein yhcL(1) conserved hypothetical protein(1) hypotheticalprotein(1) uncharacterizedsymporterydjN(1)

153 8 142 5.633802817 2 0.08245 >Cluster 1170 hypothetical protein(35) conserved hypothetical protein(3) orf1260(1) Hypothetical protein(1)

154 8 142 5.633802817 3 0.08245 >Cluster 1287 aminoacyltransferase(30) aminoacyltransferase FemB(7) glycine interpeptide bridge formation protein FemB(6) aminoacyltransferase femB(6) Aminoacyltransferase FemB(4) putative methicillin resistance expression factor(4) Aminoacyltransferase femB(3) tRNA-dependent lipid II-GlyGlyGly-- glycine ligase(3) FemB protein(2) "FemB, factor involved in methicillin resistance / Glycine interpeptide bridge formation(2)" femB protein(1) methicillin resistance protein FemB(1) femAB family protein(1) "methicillin resistance factor, putative(1)" aminoacyltransferase FemB (Factor essential forexpression of methicillin resistance B)(1) Methicillin resistance protein(1) methicillin resistance factor FemB(1) methicillin resistance factor protein(1) UDP-N-acetylmuramoylpentapeptide-triglycine glycyltransferase(1) "tRNA-dependent lipid II-GlyGlyGlyGly-- glycine ligase/FemB, factor involved in methicillin resistance(1)" putative methicillin resistance expressionfactor(1) "tRNA- dependent lipid II-GlyGlyGly--glycine ligase @ tRNA-dependent lipid II-GlyGlyGlyGly-- glycine ligase @ FemB, factor involv... at 99.60%(1)" "tRNA-dependent lipid II-GlyGlyGly--glycine ligase, tRNA-dependent lipid II-GlyGlyGlyGly--glycine ligase, FemB, factor involved>(1)" methicillin resistance expression factor FemB(1)

155 8 142 5.633802817 4 2291 hypothetical protein(74) lipoprotein(17) hypothetical protein(13) putative lipoprotein(9) class I (E and Q), catalytic domain protein(3)" functionDUF1672(2) Hypotheticalprotein(2) protein(2) FIG01107889: hypothetical protein(2) putative lipoprotein (pseudogene)(1) Similar to LMP group of surface- lacated membrane protein(1) possible lipoprotein(1) "lipoprotein, putative(1)"

156 8 142 5.633802817 2 0.08245 >Cluster 2323 phosphate ABC transporter ATP-binding protein(29) "phosphate ABC transporter, ATP-binding protein(12)" Phosphate transport ATP- bindingproteinPstB(7) ABCtransporterATP-bindingprotein(7) Phosphate import ATP-binding protein

PstB(6) "phosphate ABC superfamily ATP binding cassette transporter, ABC protein(5)" phosphate transporter ATP-binding protein(2) phosphate import ATP-binding protein pstB(2) phosphate import ATP-binding protein(2) Phosphate transport ATP-binding protein PstB (TC 3.A. 1.7.1)(1) "phosphate ABC transporter, ATPase subunit(1)" phosphate ABC transporter(1) Phosphate transport ATP-binding protein pstB(1) "phosphate ABC transporter ATP-binding protein, putative(1)" rad17 cell cycle checkpoint family protein(1) ABC transporter family protein(1)

157 8 142 5.633802817 3 0.08245 >Cluster 2882 SAM-dependent methyltransferase(34) methyltransferase domain protein(10) SAM dependent methyltransferase(6) conserved hypothetical protein(6) Ubiquinone/menaquinone biosynthesis methyltransferase UbiE(4)hypothetical protein(4) putative methyltransferase(3) Methyltransferase(2) methyltransferase(1) paralog)(1) Methyltransferase type 11(1) SAM-dependent methyltransferase (UbiE paralog)(1) methyltransferase domain family(1) probable methyltransferase(1) SAM-dependent methyltransferase HI0095 UbiE(1) methyltransferase type 11(2) "methyltransferase, putative(1)" SAM-dependent methyltransferase HI0095 (UbiE

158 8 142 5.633802817 2 0.08245 >Cluster 3230 pyruvate formate lyase-activating protein(29) pyruvate formate-lyase 1-activating enzyme(13) Pyruvate formate-lyase activating enzyme(11) putative pyruvate formate-lyase activating0.08245 >Cluster conserved "tRNA synthetases protein of unknown Putativecytosolicenzyme(7) pyruvate formate-lyase activating enzyme(5) formate acetyltransferase activating enzyme(5) Pyruvate formate- lyase-activating enzyme(5) pyruvate formate-lyase-activating enzyme(3) "pyruvate formate-lyase 1 activating enzyme, putative(1)" pyruvate formate lyase-activating enzyme(1) conserved hypothetical protein(1) -1

159 8 142 5.633802817 2 0.08245 >Cluster 5023 peroxiredoxin(30) Organic hydroperoxide resistance protein(10) OsmC-like protein(8) osmotically inducible protein OsmC(5) Organic hydroperoxide resistance protein-like protein(4) organic hydroperoxide resistance protein ohrB(4) OsmC/Ohr family protein(3) organic hydroperoxide resistance protein(3) "peroxiredoxin, Ohr subfamily protein(3)" organic hydroperoxideresistance-likeprotein(2) conservedhypothetical protein(2) MW0781(1) Organic hydroperoxide resistance protein-like(1) OsmC family protein(1) hypotheticalprotein(1) "peroxiredoxin, Ohr subfamily(1)" organic hydroperoxide reductase(1)

160 3 142 2.112676056 3 0.08393 >Cluster 686 1-pyrroline-5-carboxylate dehydrogenase(48) Delta-1- pyrroline-5-carboxylate dehydrogenase(10) aldehyde dehydrogenase family protein(8) putative delta-1-pyrroline-5-carboxylate dehydrogenase(8) "delta-1-pyrroline-5-carboxylate dehydrogenase, putative(3)" L-glutamate gamma-semialdehyde dehydrogenase(2) 1- pyrroline-5-carboxylate dehydrogenase 1(1)delta-1-pyrroline-5- carboxylate dehydrogenase(1)

161 3 142 2.112676056 2 0.08393 >Cluster 733 teichoic acid ABC transporter ATP-binding protein(30) putative membrane protein(7) Teichoic acid export ATP-binding protein TagH(7) ABC-type polysaccharide/polyol phosphate transport system ATPase component-like protein(4) "aBC superfamily ATP binding cassette transporter, ABC protein(3)" ABC transporter ATP-binding protein(3) hypotheticalprotein(3) teichoicacidtransport system ATP-binding protein(3) putative sugar ABC transporter ATPase(3) ABC-type polysaccharide/polyol phosphate transport system ATPase component-protein(2) conserved hypothetical protein(2) "ABC transporter, ATP-binding protein(2)" sugar ABC transporter ATPase(2) teichoic acid translocation ATP-binding protein(1) "ABC transporter, ATP-binding protein, putative(1)"sugar ABC transporter ATPase domain protein(1) teichoic acid translocation ATP-binding protein TagH(1) "teichoic acid translocation ATP-binding protein, putative(1)" MW1811(1) probable membrane protein(1) "Putative ABC transporter, ATP- binding protein(1)" teichoic acid translocation ATP-binding protein tagH(1) "teichoic acid ABC superfamily ATP binding cassettetransporter, ABC protein(1)"

162 3 142 2.112676056 2 0.08393 >Cluster 924 gamma-aminobutyrate permease(29) D-serine/D-alanine/glycine transporter(17) putative amino acid permease(11) amino acid permease(8) amino acid permease family protein(6) amino acid permease-associated region(1) "amino acid ABC transporter-like protein, putative(1)" amino acid ABC transporter homolog(1) APC family amino acid-polyamine-organocation transporter(1) D-alanine/ D-serine/glycine permease(1) amino acid ABCtransporter(1) "amino acid permease, putative(1)" MW2362(1)

163 3 142 2.112676056 2 0.08393 >Cluster 966 quinolone resistance protein(29) major Facilitator Superfamily protein(9) putative transport protein(9) hypotheticalprotein(8) transmembraneeffluxpumpprotein(5) MFS family major facilitator transporter(3) major facilitator transporter(2) "drug transporter, putative(2)" probable membrane transport protein(1) Quinolone resistance protein norB(1) Multidrug resistance protein B(1) MW0250(1) multidrug resistanceproteinB(1) majorfacilitatorsuperfamilyMFS_1(1)major facilitator superfamily transporter protein(1) "transporter, major facilitator family protein(1)" Hypotheticalprotein(1) "transmembraneeffluxpumpprotein, putative(1)" FIG01108109: hypothetical protein(1) conserved hypothetical protein(1)

164 3 142 2.112676056 2 0.08393 >Cluster 1107 exodeoxyribonuclease VII large subunit(35) "exodeoxyribonuclease VII, large subunit(12)" putative exodeoxyribonuclease VII large subunit(10) Exodeoxyribonuclease VII large subunit(10)Exodeoxyribonuclease 7 large subunit(7) exodeoxyribonuclease 7 large subunit protein(2) probable exodeoxyribonuclease VII large subunit(1) MW1476(1) hypotheticalprotein(1) "exodeoxyribonucleaseVII,largesubunit, putative(1)"

165 3 142 2.112676056 3 0.08393 >Cluster 1122 glucose-6-phosphate isomerase(54) Glucose-6-phosphate isomerase(18) putative glucose-6-phosphate isomerase(6) glucose-6- phosphate isomerase A(3) glucose-6-phosphate isomerase A (GPI A) (Phosphoglucoseisomerase A) (PGI-A) (Phosphohexose isomerase A) (PHI- A)(1)

166 3 142 2.112676056 2 0.08393 >Cluster 1472 methicillin resistance protein FmtA(31) autolysis and methicillinresistant-relatedprotein(7) Autolysisandmethicillinresistant-related protein(6) FmtA protein involved in methicillin resistance(6) protein FmtA(5) Protein FmtA(4) protein fmtA(4) beta-lactamase(3) fmtA protein(2) autolysis and methicillin resistant-related protein FmtA(2) FmtA protein involved in methicillin resistance; affects cell wall cross-linking and amidation(1)Protein fmtA(1) "methicillin resistance related protein, FmtA(1)" beta-lactamase family protein(1) "FmtA, autolysis and methicillin resistant-related protein(1)" autolysis and methicillin resistance-related protein FmtA(1) fmt protein(1) lactamase family protein(1) S12 familycarboxypeptidase(1) "fmt protein, putative(1)"

167 3 142 2.112676056 2 0.08393 2134 hypothetical protein(31) membrane protein(16) membrane protein(9) putative membrane protein(7)

hypotheticalprotein(6) fusaricacidresistanceprotein-likefamily protein(3) Fusaric acid resistance protein familyprotein(2) "Fusaric acid resistance protein-like membrane protein, putative(1)" probable membrane protein(1) UPF0421 protein SA1705(1) protein in map 5'region(1) hypothetical membrane protein(1)

168 3 142 2.112676056 2 0.08393 >Cluster 2907 teichoic acids export protein ATP-binding subunit(17) teichoic acid export protein ATP-binding subunit(14) Teichoic acid export ATP-binding protein TagH(9) teichoic acids export ATP- bindingproteinTagH(7) teichoicacidABCtransporterATP-binding protein(7) Teichoic acids export ATP-binding protein TagH(6) teichoic acid translocation ATP-binding protein(4) "Teichoic acid export ATP-binding protein, TagH(4)" teichoic acids export protein ATP-binding protein H(2) teichoic acids export ATP-binding protein tagH(2) ABC transporter related(1) O-antigen export system ATP-binding protein rfbB(1) "teichoic acid translocation ATP-binding protein, putative(1)" teichoic acids export ATP-binding protein TagH (Teichoicacid-transporting ATPase)(1) "teichoic acid ABC transporter ATP-binding protein, putative(1)" Teichoic acid translocation ATP-binding protein tagH(1) "ABC transporter, ATP- binding protein(1)" "ABC superfamily ATP binding cassette transporter, ABC protein(1)"

169 3 142 2.112676056 2 0.08393 >Cluster 3855 thymidylate kinase(44) Thymidylate kinase(16) putative thymidylate kinase(10) dTMP kinase(6) tmk(1) thymidylate kinase homologue(1)

170 3 142 2.112676056 3 0.08393 >Cluster 4337 hypothetical protein(68) conserved hypothetical protein(7) DUF697 domain-containing protein(2) Hypothetical protein(2)Beta->ClusterIntegralconservedFIG01108691: hypothetical protein(1)

171 3 142 2.112676056 2 0.08393 >Cluster 6897 hypothetical protein(14) phage protein(4) conserved hypothetical phage protein(2) FIG01108591: hypothetical protein(1) putative SaPI1 derepressor(1) protein(1)172 23 142 16.1971831 183 cation:proton antiporter(28) subunit(11) Na(+) H(+) antiporter subunit A(8) Na(+)/H(+) antiporter subunit A1(7) na(+)/H(+) antiporter subunit A1(6) "monovalent cation antiporter-3 subunit A, MnhA(4)" monovalent cation/H+ antiporter subunit A(4) Na /H antiporter subunit A1 protein(2) hypothetical protein(2) Na+/H+ antiporter subunit A(2) Na(+)/H(+) antiporter subunit A (Multiple resistance and pHhomeostasis protein A) (Mrp complex subunit A)(1) CPA3 family monovalent cation:proton (H+) antiporter-3 subunit A(1) NA+/H+ antiportersubunitA(1) "monovalentcation/H+antiporter,subunit 1(1)" Na+ H+ antiporter subunit A(1) monovalent cation:proton antiporter family protein(1) "Na+/H+ antiporter subunit, putative(1)" Sodium/proton antiporter protein shaA(1)

173 2 142 1.408450704 2 0.08994 >Cluster 703 hypothetical protein(56) putative exported protein(15) conserved hypothetical protein(7) exported protein(3) Hypotheticalprotein(2) FIG01108182:hypotheticalprotein(1) probable exported protein(1)

174 2 142 1.408450704 2 0.08994 >Cluster 770 hypothetical protein(11) signal peptide protein(4)conserved hypotheticalprotein(3) exportedprotein(2) Hypothetical protein within Pathogenicity island SaPIn2(1) "Gram-positive signalpeptideprotein,YSIRKfamily(1)" putativeexportedprotein(1) Hypothetical protein(1)

175 2 142 1.408450704 2 0.08994 >Cluster 1059 multidrug transporter MatE(18) MATE family efflux transporter(13) MATE family multi-antimicrobial extrusion protein(7)multidrug export protein mepA(6) "Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps(6)" putative membrane protein(5) multidrug export protein MepA(5) Multidrug export protein mepA(3) MATE efflux familyconserved hypothetical5 0.0893 >Cluster Na+/H+ antiporterprotein(3) conserved hypothetical protein(3) Multidrug export protein MepA(3) probable membrane protein(1) multidrug efflux MATE transporter MepA(1) hypothetical protein(1) Multi antimicrobial extrusion protein(1) MATE efflux family family protein(1) Na+ driven multidrug efflux pump(1) MDR efflux family protein(1)mepA: Multidrug export protein mepA(1)

176 2 142 1.408450704 2 1567 succinyl-CoA synthetase subunit beta(27) (11) putative CoA synthetase protein(7) ligase subunit beta(6) succinate--CoA ligase (ADP-forming) beta subunit(5) succinyl-CoAligase(4) "succinate-CoAligase,beta subunit(4)" "succinyl-CoA synthetase, beta subunit(3)"Succinyl- CoA ligase (ADP-forming) beta chain(3) succinyl-CoA synthetase beta chain (SCS-alpha)(Vegetative protein 63) (VEG63)(2) succinyl- CoA ligase subunit beta protein(2) succinyl-CoA synthetase(2)Succinyl-CoA ligase(1) succinyl-CoA synthetase beta chain(1) Succinyl-CoA ligase beta chain(1) "succinyl-CoA synthetase, beta subunit, putative(1)" Succinyl-CoA synthetase beta chain(1)

177 2 142 1.408450704 2 0.08994 >Cluster 1921 3-isopropylmalate dehydrogenase(81)

178 2 142 1.408450704 2 0.08994 >Cluster 2115 catabolite control protein A(59) Catabolite control protein A(16) "global transcriptional regulator, catabolite control protein A(4)" "catabolite control protein A, putative(1)"

179 2 142 1.408450704 2 0.08994 >Cluster 3001 metallophosphoesterase(25) hypothetical protein(14) conserved hypothetical protein(8) Phosphoesterase(7) "ABC superfamily ATP binding cassette transporter, binding protein(5)" calcineurin-like phosphoesterase family protein(5) phosphoesterase(5)ymdB-like family protein(3) putative metallophosphoesterase(2)putative phosphoesterase(2) metallophosphatase domain protein(1) FIG006542: Phosphoesterase(1) Phosphoesterase family protein(1) Hypothetical protein(1)

180 2 142 1.408450704 2 0.08994 >Cluster 3628 serine dehydratase(28) "L-serine dehydratase, iron-sulfur- dependent, beta subunit(17)" "L-serine dehydratase, beta subunit(10)" "putative L-serine dehydratase, beta chain(7)" "L-0.08994 >Cluster Succinyl-CoA ligase succinate--CoAserine dehydratase, iron-sulfur-dependent subunit beta(7)" "L-serinedehydratase, beta chain(3)" dehydratase subunit beta(2) subunit(1) MW2452(1) subunit(1) L-serine dehydratase beta subunit(1) hypothetical protein(1)L-serine ammonia-lyase(2)L-serine L-serine ammonia-lyase beta probable L-serine dehydratase beta

181 2 142 1.408450704 2 0.08994 >Cluster 3783 DtxR family transcriptional regulator(31) iron dependent repressor(9) Mn-dependent

transcriptional regulator MntR(8) putative metalloregulator(7) iron-dependentrepressor(6) "iron dependent repressor, N-terminal DNA binding domain protein(4)" "DtxR family transcriptional regulator, Mn- dependent transcriptional regulator(3)" feoA domain protein(3) metalloregulator(2) hypotheticalprotein(1) conserved hypotheticalprotein(1) "irondependentrepressor,DNAbindingdomain protein(1)" Iron-dependent repressor(1) iron dependent repressor DNA binding domain protein(1) possible metal-dependent transcriptional regulator(1) MW0596(1)

182 2 142 1.408450704 2 0.08994 >Cluster 3925 sortase A(40) sortase(12) sortase family protein(8) LPXTG specific sortase A(8) sortase A transpeptidase(4) class A sortase SrtA(3) "Sortase A, LPXTG specific(3)" "sortase A, peptide LPXTG peptidoglycan transferase(1)" "sortase, putative(1)" Sortase A(1)

183 2 142 1.408450704 2 0.08994 >Cluster 4259 hypothetical protein(24) Phage protein(3) conserved phage- associated protein(2) phage protein(2) hypothetical phage protein(2) conserved hypothetical phage protein(1) putative phage protein(1) hypothetical bacteriophage protein(1) phage-

related protein(1)
184 2 142 4380 dUTPase(23) protein(6) putative phage 2-deoxyuridine 5-triphosphate nucleotidohydrolase(3) conserved hypothetical protein(3) phage dUTPase(2) truncated phage dUTPase(1) hypothetical bacteriophage protein(1) Putative cytosolic protein(1) putative dUTP diphosphatase(1) conserved hypothetical phage protein(1)

185 2 142 1.408450704 2 0.08994 >Clusterconserved hypothetical protein(1)1.408450704 2 0.08994 >Cluster Dimeric dUTPase(10) dUTPase family4542 hypothetical protein(46) conserved hypothetical protein(12) ywpF-like family protein(7) ywpF protein(5)Hypothetical protein(4) YwpF protein(3) YwpF-like protein(2) Hypothetical protein SAV2097(1)

186 2 142 1.408450704 2 0.08994 >Cluster 4607 thiamine biosynthesis protein ThiW(18) energy coupling factor transporter S component ThiW(12) hypothetical protein(8) putative membrane protein(6) Substrate-specific component ThiW of predicted thiazole ECF transporter(6) thiamine transporter ThiW(4)putative thiamine-precursor transporter protein (ThiW)(4) thiamine- transporter family protein(4) conserved hypothetical protein(4) putative thiamine-precursor transporter protein(3) ThiW protein(3) probable membrane protein(1) Substrate-specific component ThiW of putative thiazole ECF transporter(1) "thiamine- precursor transporter protein ThiW, putative(1)" protein ThiW(1) "Thiamine-precursor transporter protein, putative(1)" thiW protein(1) thiamine-transporter protein (ThiW) family protein(1)Hydroxyethylthiazole permease(1)

187 2 142 1.408450704 2 0.08994 >Cluster 5693 hypothetical protein(46) conserved hypothetical protein(4) Hypotheticalprotein(1) FIG01108475:hypotheticalprotein(1)

188 10 142 7.042253521 3 0.09273 >Cluster 2844 phosphohydrolase(32) hypothetical protein(11) phosphoesterase family protein(7) putative phosphohydrolase(6) metallophosphoesterase(5)conserved hypothetical protein(4) calcineurin-like phosphoesterase(4) Metallophosphoesterase(3)"putative phosphoesterase, putative(1)" Predicted phosphohydrolase(1) Predicted phosphohydrolases(1) calcineurin-like phosphoesterase family protein(1) Phosphoesterase(1)putative phosphoesterase(1)

189 10 142 7.042253521 3 0.09273 >Cluster 4040 superoxide dismutase(45) superoxide dismutase (8) Manganese superoxidedismutase(6) superoxidedismutaseMn/Fefamilyprotein(5)Superoxide dismutase (5) superoxide dismutase SodA(3) Manganese superoxide dismutase; Superoxide dismutase (2) "superoxide dismutase,Mn,putative(2)" Superoxidedismutase(2) Manganese superoxide dismutase; Superoxide dismutase (Fe)(1) superoxide dismutase Mn/Fe(1)

190 6 142 4.225352113 4 0.09748 >Cluster 362 DNA gyrase subunit B(50) "DNA gyrase, B subunit(13)" DNAtopoisomerase IV subunit B(10) DNA topoisomerase (ATP-hydrolyzing) subunit B(2) DNA topoisomerase subunit B(2) GYRB STAAR DNA gyrase subunit B(1) "DNA topoisomerase (ATP-hydrolyzing) subunit B,GyrB(1)" DNAgyraseBsubunit(1) "DNAgyraseBsubunit, carboxyl terminus family protein(1)"

191 6 142 4.225352113 5 0.09748 >Cluster 1951 transcriptional regulator(30) Perfringolysin O regulator protein PfoR(10) putative membrane protein(7) "phosphotransferase system, EIIC family protein(6)" PfoR family transcriptional regulator(5) hypothetical protein(4) perfringolysin O regulator protein(3) Perfringolysin O regulatory protein(3) regulatory protein PfoR(3) conserved hypothetical membrane protein(2)conserved hypotheticalprotein(2) probableperfringolysinOregulatory protein(1) MW0287(1) phosphotransferase system IIC component-like protein(1)Transcriptional regulator pfoR(1) Regulatory protein(1)

192 6 142 4.225352113 2 0.09748 >Cluster 2010 LacI family transcriptional regulator(29) maltose operon transcriptional repressor(22) "Maltose operon transcriptional repressor MalR, LacI family(8)" maltose operon transcription repressor(4) HTH-type transcriptional regulator malR(3) hypotheticalprotein(2) transcriptionalregulator(2) Alanine racemase(1) maltose operon repressor(1) "Transcriptional regulator, LacI family protein(1)" HTH-type transcriptional regulator MalR(1) HTH-type transcriptional regulator MalR (Maltose operontranscriptional repressor)(1) maltose operon transcriptional repressor-like protein(1) "Maltose operon transcriptional repressor MalR,LacI family(1)" "transcriptional regulator,putative(1)" HTH-typetranscriptionalregulatormalR (Maltose operontranscriptional repressor)(1)

193 6 142 4.225352113 2 0.09748 >Cluster 3289 transcriptional regulator(18) two-component system response regulator(9) transcriptional regulatory protein srrA(7) transcriptional regulatory protein SrrA(6)response regulator protein(5) Staphylococcal respiratory response protein SrrA(4) winged helix family two component transcriptional regulator(4) DNA-binding response regulator SrrA(4) Transcriptional regulatory protein SrrA(3) DNA-binding response regulator(3) respiratory response protein SrrA(3) "two component transcriptional regulator, winged helix family(2)" transcriptional regulator srrA(2) staphylococcal respiratory response protein SrrA(1) Transcriptional regulatory protein resD(1)response regulator SrrA(1) transcriptional regulatory protein resD(1)"DNA-binding response regulator, putative(1)" response regulator receiver(1) DNA-

binding response regulator ResD(1) transcriptional regulatoryprotein ResD(1) response regulator receiver domain protein(1) staphylococcal respiratory response protein(1)

194 6 142 4.225352113 2 0.09748 >Cluster 3529 oxidoreductase(36) putative short chain dehydrogenase(13) hypotheticalprotein(7) Short-chain dehydrogenase/reductase SDR(4) clavaldehyde dehydrogenase(2) putative oxidoreductase(2) short chaindehydrogenase(2) "Oxidoreductase, short-chain dehydrogenase/ reductase family(2)" oxidoreductase family protein(1) MW2403(1)Short-chain alcohol dehydrogenase(1) "short chain dehydrogenase, putative(1)" dehydrogenase(1) Short chain dehydrogenase(1) NAD-dependent epimerase/dehydratase(1) conserved hypotheticalprotein(1) "oxidoreductase,shortchaindehydrogenase/ reductase family protein(1)" short chain dehydrogenase/reductase family oxidoreductase(1) short-chain dehydrogenase/reductase SDR(1)

195 6 142 4.225352113 2 0.09748 >Cluster 4030 FMN-dependent NADH-azoreductase(30) FMN-dependent NADPH-azoreductase(17) conserved hypothetical protein(7) putative flavoprotein(6) putative FMN-dependent NADPH-azoreductase(6) hypotheticalprotein(4) NADPH-dependentFMNreductasefamily protein(4) Predicted flavoprotein(2)Acyl carrier protein phosphodiesterase(1) "NADPH-dependent FMN reductase, putative(1)"fmn-dependent NADPH-azoreductase (nadph-dependent flavo- azoreductase) (nadph-flavin azoreductase)(1) NADPH-dependent FMN reductase(1)

196 13 142 9.154929577 5 0.09849 >Cluster 2716 hypothetical protein(62) lipoprotein(41) Tandem lipoprotein within Pathogenicity island(13) putative lipoprotein(11) tandem lipoprotein(11) staphylococcal tandem lipoprotein(11) conserved hypotheticalprotein(5) staphylococcustandemlipoproteinsfamily protein(3) Membranelipoprotein(3) satandemlipoprotein(2) putative membrane protein(2) hypothetical lipoprotein(1) staphylococcus tandem lipoprotein(1)

197 8 142 5.633802817 4 0.09909 >Cluster 471 histidine kinase(28) sensor protein srrB(9) integral membrane sensor signal transduction histidine kinase(6) sensor protein SrrB(5) Sensor protein SrrB(4) sensor kinase protein(4) Osmosensitive K+ channel histidine kinase KdpD(4) sensor histidine kinase SrrB(3) sensor kinase SrrB(3) two-component sensor histidine kinase(3) staphylococcal respiratory response protein SrrB(2) sensor protein SrrB (Staphylococcal respiratory responseprotein B)(1) Staphylococcal respiratory response protein SrrB(1) staphylococcal respiratory response protein(1) respiratoryresponse protein(1) staphylococcal respiratory response protein B(1) Osmosensitive K+ channel histidine kinase SrrB(1) sensor histidine kinase(1) "staphylococcal respiratory response protein SrrB, putative(1)" Histidine kinase(1) sensor protein srrB (Staphylococcal respiratory responseprotein B)(1)

198 8 142 5.633802817 2 0.09909 >Cluster 1828 UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase(24) UDP-diphospho-muramoylpentapeptide beta-N- acetylglucosaminyltransferase(16) undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase(10) UDP-N-acetylglucosamine--N-acetylmuramyl- (pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase(9) undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc GlcNAc transferase(4) "N-acetylglucosaminyltransferase,MurG(4)" N-acetylglucosamine transferase(3) putative UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide)pyr ophosphoryl-undecaprenol N-acetylglucosamine transferase(3) UDP-diphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase(2) undecaprenyldiphospho- muramoylpentapeptide beta-N- acetylglucosaminyltransferase(1) UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl- undecaprenol N-acetylglucosamine transferase(1) putative UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide)pyr ophosphoryl- undecaprenol N-acetylglucosamine transfera... at 86.83%(1) UDP-N-acetylglucosamin--N-acetylmuramyl-pyrophosphoryl-undecaprenol N- acetylglucosamine transferase(1) UDP-N-acetylglucosamine--N-acetylmuramyl-pentapeptide pyrophosphoryl-undecaprenol N- acetylglucosamine transferase(1) UDP-N-acetylglucosamine:LPS N-acetylglucosamine transferase(1)

199 8 142 5.633802817 3 0.09909 >Cluster 3599 heme uptake protein IsdC(20) heme transporter IsdC(18) NPQTN cell wall anchored protein IsdC(10) Iron-regulated surface determinant protein C(5) iron (Fe2+)-regulated surface determinant protein IsdC(5) iron-regulated surface determinant protein C(4) putative surface anchored protein(4) iron-regulated cell surface protein(4) conserved hypothetical protein(3) putative surface protein(2) NEAr transporter(1) Iron-regulated surface determinant protein C precursor(1) Heme uptake cell surface protein IsdC(1) heme-iron transport protein IsdC(1) iron- regulated surface determinant protein C (Staphylococcaliron-regulated protein D)(1) "sortase B signal domain, NPQTN class domain protein(1)"

200 8 142 5.633802817 4 0.09909 >Cluster 4621 hypothetical protein(68) conserved hypothetical protein(8) Hypotheticalprotein(2) FIG01108416:hypotheticalprotein(1) uncharacterized protein-like protein(1)

201 11 142 7.746478873 3 0.1018 >Cluster 259 DNA topoisomerase III(46)putative DNA topoisomerase(10) DNA topoisomerase 3(9) DNA topoisomerase III family protein(8) DNA topoisomerase III topB(2)"DNA topoisomerase III, putative(1)" DNA topoisomerase TopB(1) DNA topoisomerase(1) DNA topoisomerase 3 (DNA topoisomerase III)(1)

202 11 142 7.746478873 3 0.1018 >Cluster 444 hypothetical protein(22) phiETA ORF57-like protein(8) phage protein(3) phiETA ORF57-like protein-like protein(2) putative phageprotein(1)Hypotheticalprotein(1) conservedhypothetical protein(1)

203 11 142 7.746478873 2 0.1018 >Cluster 980 aldehyde dehydrogenase(43) putative aldehyde dehydrogenase(11) Aldehyde dehydrogenase(10) aldehyde dehydrogenase family protein(5) aldehyde dehydrogenase (NAD+)(3) acyl-CoA reductase family protein(2) "aldehyde dehydrogenase,putative(2)" aldehyde dehydrogenase (NAD(+))(1) dehydrogenase (NAD) family protein(1) "fatty aldehyde dehydrogenase (Aldehyde dehydrogenase,microsomal) (Aldehyde dehydrogenase family 3 member A2) (Aldehydedehydrog>(1)" aldehyde dehydrogenase ywdH(1) hypothetical protein(1) dehydrogenase (NAD(P)+)(1)aldehydeputativeAldehyde

204 11 142 7.746478873 2 0.1018 3050 hydrolase(32) putative carbon-nitrogen hydrolase(8) Aliphatic amidase AmiE(5)carbon-nitrogen hydrolase family protein(5) "hydrolase, carbon-nitrogen family(4)" carbon- nitrogenfamilyhydrolase(4) hypotheticalprotein(3) Aliphatic amidase

amiE(3) Nitrilase/cyanide hydratase and apolipoprotein N- acyltransferase(3) carbon-nitrogen hydrolase(2) conserved hypotheticalprotein(2) nitrilase/cyanidehydrataseand apolipoprotein N-acyltransferase(2) putative hydrolase in agr operon(2) Hydrolase in agr operon(1) "carbon-nitrogen hydrolase, putative(1)" Beta-ureidopropionase(1) amidohydrolase(1)hydrolase in agr operon (ORF 5)(1)

205 14 142 9.85915493 5 0.1058 >Cluster 588 hypothetical protein(39) Dihydroxyacetone kinase family protein(10) DAK2 domain fusion YloV family protein(6) dihydroxyacetone kinase family protein(6) conserved hypothetical protein(5) DAK2 domain fusion protein YloV(3) dihydroxyacetone kinase(2) Dak phosphatase(2) DAK2 domain>Clusterprotein(2) conserved hypotehtical protein(1) DAK2 domain-containing protein(1) "Dak phosphatase,putative(1)"putative(1)""dihydroxyacetone kinase domain protein YloV,glycerone kinase(1)142 11.97183099 5 0.1086 >Cluster 95

206 17 oxidoreductase(28) "formate dehydrogenase, alphasubunit(9)" Formatedehydrogenaserelatedprotein(8) putative bifunctional protein(6) molybdopterin oxidoreductase(4) Putative formate dehydrogenase(4) formate dehydrogenase subunit alpha(4) "formate dehydrogenase, alpha subunit, putative(3)" Formate dehydrogenase(3) formate dehydrogenase homolog(2) formate dehydrogenase alpha subunit(2) molybdopterin oxidoreductase family protein(2) formate dehydrogenase(2) MW2229(1) 4Fe-4S binding domain protein(1)Formate dehydrogenase alpha chain(1)

207 11 142 7.746478873 2 0.1123 >Cluster 258 translation initiation factor IF-2(64) Translation initiation factor 2(7) Translation initiation factor IF-2(5) Bacterial Protein Translation Initiation Factor 2 (IF-2)(1) "translation initiation factor IF-2, region family protein(1)"

208 11 142 7.746478873 3 0.1123 >Cluster 1081 glucarate transporter(33)putative transporter protein(8) Glucarate transporter(7) sugar (and other) transporter family protein(6) "MFS family major facilitator transporter, glucarate:cation symporter(5)" major Facilitator Superfamily protein(3) major facilitator superfamily transporter protein(3)putative MFS-type transporter(3) major facilitator superfamily MFS_1(2) major facilitator superfamily transporter MFS_1(2)hypotheticalprotein(2) conservedhypotheticalprotein(2) probable transport protein(1) "transporter,putative(1)"MW2431(1)putative(1)"

209 11 4393 peptide methionine sulfoxide reductase(32)peptide methionine sulfoxide reductase II(13) Peptide methionine sulfoxide reductase MsrA(8) peptide-methionine (S)-S-oxide reductase(7)Peptide methionine sulfoxide reductase MsrA 2(6) peptide methioninesulfoxidereductaseMsrA2(4) methioninesulfoxide reductase A(4) methionine-S-sulfoxide reductase(2) methionine sulfoxide reductase(1) Peptide methionine sulfoxide reductase msrA(1) peptide-methionine-(S)-S-oxide reductase(1) "methionine sulfoxide reductase A, putative(1)""transporter, major facilitator family protein(1)""major facilitator superfamily transporter,142 7.746478873 2 0.1123 >Cluster

210 14 142 9.85915493 4 0.1132 >Cluster 6827 hypothetical protein(55) phage protein(19) protein of unknown function DUF1270(5) conserved hypothetical protein(4) putative exported protein(3) phi PVL orf 38-like protein(3) conserved hypothetical phage protein(2) phi PVL orf 38-like protein- related protein(2) Phage protein(2) hypothetical phage protein(2) putative phage protein(1)Hypothetical protein(1) hypothetical bacteriophage protein(1) hypothetical protein within a prophage(1)

211 9 142 6.338028169 3 0.1138 >Cluster 211 single-stranded-DNA-specific exonuclease RecJ(27) single- stranded-DNA exonuclease(18) putative single-stranded-DNA- specific exonuclease(11) Single-stranded-DNA-specific exonuclease RecJ(8) single-stranded-DNA-specific exonuclease(3) Single- stranded-DNA-specific exonuclease recJ(2) ssDNA-specific exonuclease RecJ(2) exonuclease RecJ(2) "single-strand DNA-specific exonuclease, C terminal domain protein(1)"conserved hypotheticalprotein(1)protein(1)

212 9 303 PTS mannose transporter subunit IIABC(30) PTS system mannose- specific EIIBCA component(11) "putative PTS transport system, IIABC component(8)" "PTS system, mannose-specific IIAB component(3)" "PTS system, fructose-specific IIABC component(3)" "PTS system, mannose-specific IIB component / PTS system, mannose-specific IIC component / PTS system, mannose-specific IIA com>(2)" PTS system fructose subfamily transporter subunit IIC(2) PTSsystemmannose-specifictransportersubunit IIBCA(2) "PTS system, Fru family, IIB component domain protein(2)" PTSsystemcomponent(2) "PTSsystem,fructose-specificIIA component(1)" "PTS system, mannose-specific IIB component / PTS system, mannose-specific IIC component / PTS system, mannose-specific IIA com... at 99.80%(1)" MW2562(1) "PTS system, fructose-specific IIABC components(1)" "PTS system, fructose-specific IIA component / PTS system, fructose-specific IIB component / PTS system, fructose-specificIIC>...at97.70%(1)" fructose-specificIIArecombination protein RecJ(1) hypothetical single-strand DNA-specific exonuclease(1) MW1586(1)142 6.338028169 6 0.1138 >Clustercomponent(1) protein(1)" homolog(1) component(1) component(1)" component IIABC(1) "PTS system, fructose-specific IIABC component,putative(1)" "phosphotransferasesystem, EIIC(1)" "fructose-specific PTS system, IIABC component(1)" "PTS system, fructose subfamily, IIC subunit(1)" "PTS system,"PTS system, fructose subfamily, IIA component domainfructose phosphotransferase system enzyme fruAPTS system fructose-specific II"phosphotransferase system, fructose-specific iiabcpossible PTS family fructose/mannitol (fru) portermannose-specific IIB component / PTS system, mannose-specific IICcomponent / PTS system, mannose-specific IIA co... at 97.70%(1)"

213 9 142 6.338028169 3 0.1138 >Cluster 1363 tRNA CCA-pyrophosphorylase(31) CCA-adding enzyme(9) polyApolymeraseheaddomainprotein(8) tRNA nucleotidyltransferase(8)poly A polymerase family protein(7) polynucleotide adenylyltransferase(5) CCA tRNA nucleotidyltransferase(3)conserved hypothetical protein(2) cca-adding enzyme (trnanucleotidyltransferase) (trna adenylyl-/cytidylyl-transferase) (trnacca-pyrophosphorylase) (trna-nt)(1) hypotheticalprotein(1) MW1347(1) probablePolyApolymerase family protein(1) TRNA nucleotidyltransferase(1) tRNA nucleotidyltransferase/poly(A) polymerase family protein(1) Polynucleotide adenylyltransferase region(1)

214 9 142 6.338028169 3 0.1138 >Cluster 2077 iron ABC transporter permease(31) putative siderophore transport system permease(8) fecCD transport family protein(7) "Siderophore staphylobactin ABC transporter, permease protein SirC(6)" iron compound ABC transporter permeaseSirC(4) "iron compound ABC transporter, permease protein SirC(3)" Siderophore transport system permease protein SirC(3) Fe3+-siderophore ABC transporter permease(2) siderophore compound ABC transporter permease SirC(2) "Siderophore staphylobactin ABC transporter,permease protein SirC(2)" iron-regulated ABC transporter siderophore permease protein SirC(1) siderophore transport protein(1) iron-dicitrate transporter subunit FecD(1) "lipoprotein, SirC, putative(1)" "iron (Fe3+) ABC superfamily ATP binding cassette transporter, membrane

protein(1)" "ABC-type Fe3+-siderophore transport system, permease component(1)" siderophore compound ABC transporter permease protein SirC(1) transport system permease protein(1) Staphylobactin transport system permease protein SirC(1) "iron chelate uptake ABC transporter, FeCT family, permease protein(1)" putative siderophore transport system permease protein yfhA(1) lipoprotein(1)

215 9 142 6.338028169 3 0.1138 >Cluster 2162 NAD-dependent dehydratase(30) UDP-glucose 4-epimerase(15)NAD dependent epimerase/dehydratase family protein(12) NAD-dependent epimerase/dehydratase(6) NAD-dependent epimerase/ dehydratase family protein(5) polysaccharide biosynthesis family protein(4) 3-beta hydroxysteroid dehydrogenase/isomerase family protein(3) probable UDP-glucose 4-epimerase(1) "UDP- glucose 4-epimerase, putative(1)" conserved hypothetical protein(1) hypotheticalprotein(1) MW0102(1) putativeUDP-glucose4- epimerase (Galactowaldenase) (UDP-galactose 4-epimerase)(1)

216 9 142 6.338028169 3 0.1138 >Cluster 2249 2-hydroxyacid dehydrogenase(17) hydroxyacid dehydrogenase(12) D-isomer specific 2-hydroxyacid dehydrogenase family protein(9) D-3-phosphoglycerate dehydrogenase(7) hypotheticalprotein(6) "D-isomerspecific2-hydroxyacid dehydrogenase, NAD binding domain protein(6)" conserved hypotheticalprotein(4) "D-isomerspecific2-hydroxyacid dehydrogenase, catalytic domain protein(4)" Phosphoglycerate dehydrogenase and related dehydrogenase(3)"D-3-phosphoglycerate dehydrogenase, putative(2)" D-isomer specific 2-hydroxyacid dehydrogenase(2) Phosphoglycerate dehydrogenase(2) MW1802(1) Phosphoglycerate dehydrogenase and related dehydrogenases(1) Phosphoglycerate dehydrogenase-related dehydrogenase(1) Phosphoglycerate dehydrogenase-like dehydrogenase(1) 4-phosphoerythronate dehydrogenase(1) D-isomer specific 2- hydroxyacid dehydrogenase NAD-binding(1)

217 9 142 6.338028169 2 0.1138 >Cluster 3447 hypothetical protein(46) membrane protein(10) putative membrane protein(8) conserved hypothetical protein(5) ABC transporterpermease(3) ABCtransporterpermeaseprotein(2) hypothetical membrane protein(1) "hypothetical protein, DUF3169 superfamily(1)" probable membrane protein(1) "membrane protein, putative(1)" FIG01108133: hypothetical protein(1)Hypothetical protein(1)

218 9 142 6.338028169 5 0.1264 >Cluster50 cell division protein FtsK(29) FtsK/SpoIIIE familyprotein(9) protein(7) protein(5) protein(3) translocase stage III)(2)cell division protein SpoIIIE(2) cell division protein FtsK/SpoIIIE(1) SpoIIIE family cell division protein(1) conserved hypothetical protein(1) ftsk/spoiiie family protein(1) DNA translocase(1) DNA translocase stage III sporulation protein(1) DNA translocase stage III sporulation prot homolog(1) MW1684(1)Cell division protein FtsK(8) ftsK/SpoIIIE family FtsK/SpoIIIE (DNA translocase stage III) family putative DNA translocase SftA(3) ftsk gamma domain Cell division protein ftsK(2) FtsK/SpoIIIE (DNA

219 9 142 6.338028169 3 0.1264 >Cluster 428 hypothetical protein(38) Ferrous iron transport permease EfeU(8) iron permease FTR1 family protein(7) putative membrane protein(6) high-affinity iron transporter(5) conserved hypotheticalprotein(4) Putativehigh-affinityFe2+/Pb2+permease like protein(4)transporter(2)transporter(1)permease(1)protein(1)FTR1 family protein(2) High-affinity iron OFeT family oxidase-dependent iron (Fe2+) iron permease(1) high-affinity iron "membrane protein, putative(1)" probable membrane High-affinity iron permease(1)

220 9 142 6.338028169 3 0.1264 >Cluster 466 myosin-cross-reactive antigen(17) hypothetical protein(16) oleate hydratase(11) putative myosin-crossreactive antigen(7) Oleate hydratase(6) Myosin-crossreactive antigen(4) 67 kDa myosin-cross-reactive antigen like family protein(3) 67 kDa Myosin-crossreactive antigen(2) 67 kDa Myosin-crossreactive streptococcal antigen homologue(2) myosin-cross-reactive antigen family protein(2)67 kDa Myosin-crossreactive streptococcal antigen homolog(1) myosin-crossreactive streptococcal antigen- like protein(1) myosin-cross-reactive antigen like family protein(1)67 kDa myosin-cross-reactive antigen family protein(1) "antigen, 67 kDa(1)" MW0081(1) Hypotheticalprotein(1) putativemyosin-crossreactiveantigen(1) conserved hypothetical protein(1) 67 kDa Myosin-crossreactive streptococcal antigen(1)

221 9 142 6.338028169 4 0.1264 >Cluster 548 CDP-glycerol glycerophosphotransferase(29)hypothetical protein(15) Putative glycosyl/glycerophosphate transferase in teichoic acid biosynthesis(7) Poly(glycerophosphate) glycerophosphotransferase family protein(6) conserved hypotheticalprotein(5) CDP-Glycerol:Poly(glycerophosphate) glycerophosphotransferase family protein(4) putative glycerophosphotransferase(3) glycosyl glycerophosphate transferase involved in teichoic acid biosynthesis(2) glycosyl/ glycerophosphate transferasen(2) "glycerophosphotransferase, TarF(2)"TagF domain protein(2) tagF domain protein(1) Hypotheticalprotein(1) "TagF/TagB/EpsJ/RodC-liketeichoicacid biosynthesis glycosyl/glycerophosphate transferase, putative(1)"

222 12 142 8.450704225 4 0.1319 >Cluster98 nuclease SbcCD subunit C(33) Exonuclease SbcC(8)putativeexonuclease(7) exonucleaseSbcC(7) nucleasesbcCD subunit C(4) exonuclease subunit SbcC(4) Nuclease SbcCD subunit C(3) P-loop containing region of AAA domain protein(2) conserved hypothetical protein(2) nuclease SbcCD subunit C domainprotein(2) nuclease sbcCD subunit C protein(2) sbcCD subunit C(2) "exonuclease SbcCD, C subunit(2)" exodeoxyribonucleaseSbcC(1) hypotheticalprotein(1) exonuclease SbcC(1) MW1233(1) exonuclease(1) sbcC(1)

223 12 142 8.450704225 3 0.1319 5126 hypothetical protein(20) phi ETA orf 63-like protein(8) Phage protein(6) conserved hypothetical protein(4) phage protein(3) putative phage protein(1)NucleaseputativeExonuclease>Cluster

224 5 142 3.521126761 3 0.1377 >Cluster 410 N-acetylmuramoyl-L-alanine amidase(37) "N-acetylmuramoyl- L-alanine amidase, family 4(8)" N-acetylmuramoyl-L-alanine amidase domain-containing protein(6) putative exported protein(6) mannosyl-glycoendo-beta-N-acetylglucosaminidase family protein(6) N- acetylmuramoyl-L-alanine amidase domain protein(3) hypothetical protein(2) CHAP domain-containing protein(2) possible N- acetylmuramoyl-L-alanine amidase(1) conserved hypothetical protein(1) mannosyl-glycoprotein endo-beta-N- acetylglucosaminidase family protein(1) probable exported protein(1)N-acetylmuramidase(1) MW2565(1) containing protein(1)

225 5 142 3.521126761 2 664 peptide chain release factor 3(62) release factor 3(15) peptide chain release factor 3 protein(2) peptide chain

release factor RF3(1) Bacterial Peptide Chain Release Factor 3 (RF-3)(1)

226 7 142 4.929577465 3 0.138 >Cluster 238 transglycosylase(34) penicillin-binding protein 2(17) penicillin binding transpeptidase domain protein(6) Multimodular transpeptidase-transglycosylase / Penicillin-binding protein1A/1B(PBP1)(4) Multimodulartranspeptidase-transglycosylase(3) penicillin-binding protein 1A(3) PBP2(2) glycosyl transferase family protein(2) "glycosyl transferase, family 51(2)" penicillin binding protein 2(2) glycosyl transferase family 51(1) Penicillin-binding protein 1A/1B (PBP1)(1) penicillin-binding protein 1A/1B (PBP1)(1)Penicillin-binding protein 2(1) transglycosylase family protein(1)

227 7 142 4.929577465 2 0.138 >Cluster 3020 hypothetical protein(14) putative lipoprotein(4) sa tandem lipoprotein(1) Tandem lipoprotein within Pathogenicity island(1) Tandem lipoprotein within Pathogenicity island2(1) "lipoprotein, putative(1)" staphylococcus tandem lipoproteins(1) staphylococcus tandem lipoproteins family protein(1)tandem lipoprotein(1) staphylococcal tandem lipoprotein(1)

228 12 142 8.450704225 4 0.1455 >Cluster 152 DNA mismatch repair protein MutS(70) DNA mismatch repair protein(6) DNA mismatch repair protein mutS(3) mutS domain V family protein(1) mutS domain II family protein(1)CHAP domain0.1377 >Cluster Peptide chain

229 12 142 8.450704225 2 0.1455 >Cluster 2906 hypothetical protein(136)lipoprotein(22) putative lipoprotein(18) tandem lipoprotein(12) tandem lipo family protein(11) Tandem lipoprotein within Pathogenicity island(10) staphylococcal tandem lipoprotein(10) conserved hypothetical protein(8) Membranelipoprotein(6) satandemlipoprotein(5) staphylococcus tandem lipoproteins family protein(5) tandem lipos family protein(4) putative tandem lipoprotein(3) possible staphylococcal tandem lipoprotein(2) "putative lipoprotein, truncated(2)" staphylococcus tandem lipoproteins(1) Hypotheticalprotein(1) FIG01108564:hypotheticalprotein(1) Tandem lipoprotein(1)

230 10 142 7.042253521 4 0.1533 >Cluster 1396 transcription elongation factor NusA(32) transcription termination factor NusA(11) Transcription termination protein NusA(9) putative N utilization substance protein A(8) transcription termination-antitermination factor(6)transcription termination/antitermination protein NusA(3) hypotheticalprotein(3) TranscriptionelongationproteinNusA(3) NusA antitermination protein(2) transcription elongation protein nusA(1) N utilization substance protein A(1) N utilization substance A(1) "transcription termination-antitermination factor, putative(1)"

231 10 142 7.042253521 2 0.1533 >Cluster 5962 preprotein translocase subunit SecG(37) "preprotein translocase, SecG subunit(14)" putative protein-export membrane protein(8) Preprotein translocase subunit SecG(8) putative protein-export membrane protein SecG(4) Preprotein translocase subunit SecG (TC 3.A.5.1.1)(2) export membrane protein secG(2) probable protein-export membrane protein(2) Protein translocase subunit secG(1) Probable protein-export membrane protein secG(1) probable protein-export translocase membrane protein(1) possible Sec family Type I general secretory protein SecG(1)

232 17 142 11.97183099 6 0.1568 >Cluster64 transcription-repaircouplingfactor(56) Transcription-repair coupling factor(10) putative transcription-repair coupling factor(6) Transcription-repair-coupling factor(5) transcription-repair coupling factor helicase(1)

233 3 142 2.112676056 2 0.1631 >Cluster 233 competence protein ComEC(32) DNA internalization-related competence protein ComEC/Rec2(20) "Late competence protein ComEC, DNA transport(7)" hypothetical protein(7) putative membrane protein(5)comEC/Rec2-related domain protein(2) DNA internalization competence protein ComEC/Rec2(2) possible competence protein ComEC(1) "competence protein ComEC, putative(1)" MW1539(1) DNA internalization competence protein ComEC/Rec2-like protein(1) COME operon protein 3(1)

234 3 142 2.112676056 3 0.1631 >Cluster 253 hypothetical protein(18) RNA-binding transcriptional accessory protein(12) S1 RNA binding domain protein(8) Transcription accessory protein (S1 RNA-binding domain)(6) putative RNA binding protein(6) RNA binding protein(5) S1 domain RNA- binding protein(4) transcription accessory protein (S1 RNA binding)(4) conserved hypothetical protein(3) tex-like N-terminal domain protein(3) RNA binding S1 domain-containing protein(2)transcription accessory protein(1) S1 RNA-binding protein(1) RNA polymerase sigma factor SigB(1) S1 RNA- binding domain-containing protein(1) putative RNA-bindingprotein(1)protein(1)domain(1)accessory protein (S1 RNA binding domain)(1)protein YhgF(1) Tex-like protein N-terminal domain Transcription accessory protein S1 RNA-binding RNA binding S1 domain protein(1) Transcription

235 3 142 2.112676056 2 0.1631 >Cluster 985 long-chain fatty acid--CoA ligase(30) AMP-binding enzyme family protein(10) Long-chain-fatty-acid--CoA ligase(10) putative AMP-binding enzyme(6) "long chain fatty acid CoA ligase, VraA(4)" Putative long chain fatty acid-CoA ligase VraA(4) long chain fatty acid CoA ligase vraA(3) long chain fatty acid-CoA ligase VraA(2) long chain fatty acid-CoA ligase vraA(2) long-chain-fatty- acid--CoA ligase(1) "long-chain-fatty-acid--CoA ligase, putative(1)" AMP-dependent synthetase and ligase(1) AMP-binding enzyme(1) "substrate--CoA ligase, putative(1)" conserved hypothetical protein(1) hypothetical protein(1) vraA(1) probable long chain fatty acid CoA ligase(1) putative long chain fatty acid CoA ligase VraA (Acyl-CoAsynthetase)(1)

236 3 142 2.112676056 3 0.1631 >Cluster 1066 glutamine synthetase(37) "glutamine synthetase, type I(17)"Glutamine synthetase type I(9) Glutamine synthetase(8) glutamine-ammonia ligase(4) type I glutamate--ammonia ligase(3)glutamine synthetase FemC(1) glutamate--ammonia ligase(1)

237 3 142 2.112676056 2 0.1631 >Cluster 1759 histidine kinase(28) sensor histidinekinase(19) "histidine kinase-, DNA gyrase B-, and HSP90-like ATPase family protein(4)"sensor kinase protein(4) histidine kinase family protein(3) Two-component sensor histidine kinase(2) sensor kinase(2) "Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase domain protein(1)"hypothetical protein(1) "sensor histidine kinase, putative(1)" Sensory Transduction Histidine Kinase(1) MW1208(1) histidine kinase dimerisation and phosphoacceptor region(1)

238 3 142 2.112676056 2 0.1631 >Cluster 1987 iron ABC transporter(30) ferrichrome transport permease(13)fecCD transport family protein(7)

"ABC-type Fe3+-siderophore transportsystem,permeasecomponent(6)" "iron(Fe3+)ABCsuperfamily ATP binding cassette transporter, membrane protein(5)" Iron(3+)- hydroxamate import system permease protein fhuB(3) cell wall anchor domain-containing protein(3) Ferrichrome transport system permease protein fhuB(2) ferrichrome transport permease fhuB(1) LPXTG-motif cell wall anchor domain protein(1) Ferrichrome transport permease(1) ferrichrome transport system permease protein fhuB(1) "ferrichrome transport permease fhuB, putative(1)" iron- hydroxamate transport permease(1) LPXTG-motif cell wall anchor domain(1) ferrichrome transport system permease protein FhuB(1)"iron compound ABC transporter, permeaseprotein(1)"putative(1)"component(1)"

239 3 2187 Phage major capsid protein(4) major capsid protein(3) head protein(3) "phage major capsid protein, HK97 family(3)" phage head protein(2) Phage capsid family protein(2) phage- relatedheadprotein(2) hypotheticalprotein(2) phagemajorcapsid protein(1) "phage head protein, putative(1)" Phage head protein/ Phage major capsid protein(1)

240 3 142 2.112676056 3 0.1631 >Cluster 2309 "1,4-dihydroxy-2-naphthoate octaprenyltransferase(47)" "1,4-dihydroxy-2-naphthoate polyprenyltransferase(8)"UbiA prenyltransferase family protein(7) ubiAprenyltransferasefamilyprotein(7) DHNA- octaprenyltransferase(4) "1,4-dihydroxy-2-naphtoate prenyltransferase(2)" "1,4-dihydroxy-2-naphthoate octaprenyltransferaseMenA,putative(1)" MW0925(1) hypotheticalprotein(1) conservedhypotheticalprotein(1)"probable 1,4-dihydroxy-2-naphthoate octaprenyltransferase(1)"

241 3 142 2.112676056 2 0.1631 >Cluster "ferrichrome transport permease,"ABC-type Fe3+-siderophore transport system,permease142 2.112676056 3 0.1631 >Cluster2389 malonyl CoA-ACP transacylase(27) malonyl CoA-acyl carrier protein transacylase(25) Malonyl CoA-acyl carrier protein transacylase(13) putative malonyl CoA-acyl carrier protein transacylase(10) -2 malonyl-CoA-acyl carrier protein transacylase(2) Malonyl-CoA-(1)

242 3 142 2.112676056 2 0.1631 >Cluster 2547 replication protein DnaD(10) DNA replication protein DnaD(7) dnaD domain protein(5) Phage replication initiation protein(3) phage replication initiation protein(2) "primosome, DnaDsubunit(2)"hypotheticalprotein(2) putativephageregulatory protein(2) Replication initiation and membrane attachment(2) possibleDNAreplicationproteinDnaD(1) DNAreplicationDnaDdomain protein(1) Phage protein(1) conserved hypothetical protein(1) dnaD and phage-associated domain protein(1) hypothetical protein within prophage(1)

243 3 142 2.112676056 2 0.1631 >Cluster 2679 HAD family hydrolase(34) haloacid dehalogenase-like hydrolase(16) "HAD hydrolase, IIB family protein(7)" conserved hypotheticalprotein(4) haloaciddehalogenasesuperfamily hydrolase(3) Hydrolase (HAD superfamily)(3) hydrolase(2) HMP-PP hydrolase (pyridoxal phosphatase) Cof(2) Cof-like hydrolase(2) "conserved hypothetical protein, putative(1)" cof-like hydrolase family protein(1) Putative cytosolic protein(1) possible HAD superfamily hydrolase(1) haloacid dehalogenase(1) hypothetical protein(1) "HMP-PP hydrolase (pyridoxal phosphatase) Cof, detected in genetic screen for thiamin metabolic genes(1)"

244 3 142 2.112676056 2 0.1631 >Cluster 2794 16S rRNA methyltransferase(27) rRNA small subunitmethyltransferase I(9) family protein(9) family protein(8) tetrapyrrole (corrin/porphyrin) methylase(4) Ribosomal RNA small subunit methyltransferase I(3) 16S rRNA (cytidine(1402)-2'-O)- methyltransferase(3) tetrapyrrole methylase family protein(3) tetrapyrrole (corrin/porphyrin) methylase familyprotein(1) Tetrapyrrole (Corrin-Porphyrin) methylase family protein UPF0011(1) possible tetrapyrrole (corrin/porphyrin) methyltransferase(1) Uroporphyrin-III C/tetrapyrrole (Corrin/Porphyrin) methyltransferase(1) Tetrapyrrole methylase family protein(1) rRNA (cytidine-2'-O-)-methyltransferase(1)"S-adenosylmethionine- dependent methyltransferase, YraL family(1)"tetrapyrrole (corrin/porphyrin) methylasetetrapyrrole (Corrin/Porphyrin) Methylasesconserved hypothetical protein(5)

245 3 142 2.112676056 2 0.1631 >Cluster 2807 lipoate--protein ligase(30) lipoate-protein ligase A protein(14) Lipoate-protein ligase A(7) lipoate-protein ligase A family protein(6) protein N-octanoyltransferase(6) lipoate-protein ligase A(3) conserved hypothetical protein(3) Octanoyl-(3) "lipoate-protein ligase A, putative(2)" Protein:protein lipoyl transferase(2) lipoate-protein ligase A- protein(2) octanoyl-(1) lipoate--protein ligase A(1)

246 3 142 2.112676056 2 0.1631 >Cluster 3801 tail protein(11) phage major tail protein(7) phage tail protein(7) major tail protein(6) "phage major tail, phi13 family protein(5)" putative major tail protein(3) Phage major tail protein(3) "phage major tail protein, phi13 family(1)"

247 3 142 2.112676056 3 0.1631 >Cluster 4009 phosphoglycerate mutase(38) phosphoglycerate mutase family protein(23) histidine phosphatase super family protein(8)putative phosphoglycerate mutase family protein(4) truncated phosphoglycerate mutase family protein(1) "phosphoglycerate mutase Gpm3p, putative(1)" MW0351(1) Phosphoglycerate mutase(1)hypotheticalprotein(1) conservedhypotheticalprotein(1) Phosphoglycerate mutase family protein(1)

248 3 142 2.112676056 2 0.1631 >Cluster 4651 YbaK/EbsC protein(17) aminoacyl-tRNA deacylase(12) ybaK / prolyl-tRNA synthetases associated domain protein(7) hypothetical protein(7) Cys-tRNA(Pro) deacylase YbaK(7) putative prolyl-tRNA synthetase(4)conserved hypothetical protein(4) YbaK/ebsC protein(3) transcriptional regulation protein(3) putative transcription regulator(3) EbsC protein(2) probable transcriptional regulator protein(1) possible transcriptional regulator(1) Transcriptional regulator(1) Putative YbaK(1) ebsC protein(1) "amino acid trans-editing deacylase YbaK, putative(1)" protein ebsC(1) Cys-tRNAPro deacylase YbaK(1) ybaK/ebsC protein(1) MW0659(1)

249 7 142 4.929577465 3 0.1647 >Cluster 300 ferrous iron transporter B(29) ferrous iron transport protein B(19) Ferrous iron transport protein B(14) putative ferrous iron transport protein B(11) hypothetical protein(2) FeoB family ferrous iron (Fe2+) uptake protein(1) ferrous iron transport protein B (FeoB)(1) 50S ribosome-binding GTPase family protein(1) feoB(1) ferrous iron transport protein B homolog(1)

250 7 142 4.929577465 5 0.1647 >Cluster 372 adhesin(30) putative surface anchored protein(7) LPXTG cell wall surface anchor family protein(7) putative cell-wall- anchored protein SasF (LPXAG motif)(7) conserved hypothetical protein(4) LPXTG family cell wall surface anchor protein(3)

putative LPXAG surface protein(3) putative cell-wall-anchored protein SasF(3) Predicted cell-wall-anchored protein SasF (LPXAG motif)(2) hypotheticalprotein(2) LPXTG-motifcellwallanchordomain protein(2) Surface anchored protein(2) LPXTG family cell wall anchor protein(2) lpxtg cell wall surface anchor family protein(1) surface anchored protein(1) Hypothetical protein(1) LPXTG-motif cell wall anchor domain(1) probable surface anchored protein(1) putative surface anchored protein SasF(1)

251 7 142 4.929577465 3 0.1647 >Cluster 670 nitrate reductase(28) Respiratory nitrate reductase beta chain(13) "nitrate reductase, beta subunit(12)" nitrate reductase beta chain(10) nitrate reductase subunit beta(7) nitrate reductase beta subunit(3)"respiratory nitrate reductase, beta subunit(2)" respiratory nitrate reductase beta subunit protein(2)nitrate reductase beta chain NarH(2) "nitrate reductase, beta subunit domain protein(1)" Nitrate reductase beta chain(1) nitrate reductase beta chain narH(1)

252 7 142 4.929577465 4 0.1647 >Cluster 1076 carboxylesterase(35) Carboxylesterase type B(10) alpha/beta hydrolase fold family protein(8) putative carboxylesterase(7) para-nitrobenzyl esterase chain A(6) Carboxylesterase(4) para-nitrobenzyl esterase(3) carboxylesterase type B(2) conserved hypothetical protein(1) hypotheticalprotein(1) probablecarboxylesterase(1) carboxylesterase family protein(1) MW2375(1)nitrobenzyl esterase(1)

253 7 142 4.929577465 2 1210 seryl-tRNA synthetase(43) serine--tRNA synthetase(13) Serine--tRNA ligase(4)0.164 ligase(21)Para->ClusterSeryl-tRNA

254 7 142 4.929577465 2 1651 carbamoyl phosphate synthase small subunit(24) phosphate synthase small chain(14) carbamoyl-phosphate synthase small chain(10) carbamoyl-phosphate synthase small subunit(9) "putative carbamoyl-phosphate synthase, pyrimidine- specific, small chain(8)""carbamoyl-phosphate synthase, small0.1647>ClusterCarbamoyl-subunit(7)" "putative carbamoyl-phosphate synthase,pyrimidine-specific, small chain(2)"carbamoyl-phosphate synthase small subunit protein(2) "carbamoyl-phosphate synthase, small subunit PyrAA(1)" "carbamoyl-phosphate synthase, pyrimidine-specific, small chain(1)" carbamoyl-phosphate synthase pyrimidine-specific small chain (Carbamoyl-phosphate synthetase glutamine chain)(1) "carbamoyl-phosphate synthase (glutamine-hydrolyzing), small subunit(1)"

255 7 142 4.929577465 3 0.1647 >Cluster 1886 UDP-phosphate N-acetylglucosaminyl 1-phosphate transferase(27)putative glycosyl transferase(9) Undecaprenyl-phosphate N- acetylglucosaminyl 1-phosphate transferase(8) glycosyl transferase 4 family protein(7) UDP-N-acetylglucosamine:undecaprenyl-P N-acetylglucosaminyl 1-P transferase(3) undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1- phosphate transferase(3) glycosyl transferase(2) lipophilic regulator protein(2) glycosyl transferase familyprotein(2) "glycosyl transferase, family 4(2)" lipophilic protein affecting lysis rate and methicillin resistance level protein(1) "glycosyl transferase, group 4 family protein(1)" "Undecaprenyl-phosphate N-acetylglucosaminyl1- phosphate transferase, putative(1)" "glycosyltransferase, group 4 family(1)" lipophilic protein affecting bacterial lysis rate and methicillin resistance level protein(1) glycosyl transferase family 4(1) Undecaprenyl-phosphate alpha-N-acetylglucosaminephosphotransferase(1) conserved hypothetical protein(1) lipophilic protein affecting bacterial lysis rate and methicillin resistance level(1) putative undecaprenyl-phosphate N- acetylglucosaminyl 1-phosphatetransferase (UDP-GlcNAc:undecaprenyl-P GlcNAc 1-Ptransferase)(1)probable undecaprenyl-phosphate N- acetylglucosaminyl 1-phosphatetransferase (UDP-GlcNAc:undecaprenyl-P GlcNAc 1-Ptransferase)(1)undecaprenyl-phosphate N-acetylglucosaminyl 1-phosphate transferase(1) hypothetical membrane protein(1) putative glycosyltransferase(1) lipophilic protein affecting lysis rate and methicillin resistance level(1)

256 7 142 4.929577465 2530 S1 RNA-binding protein(16) conserved hypothetical protein(10) B(10) hypothetical protein(8) S1 RNA binding domain(7) virulence factor B(7) virulence factor B protein(2) S1 RNA binding domain protein(2) Virulence factor expression protein CvfB(1)putative virulence factor B(1) S1 RNA binding protein(1) poly(U) binding protein CvfB(1) Conserved virulence factor B(1)

257 7 142 4.929577465 3 0.1647 >Cluster 4481 acetyltransferase(39) "acetyltransferase, GNAT family(9)"protease synthase and sporulation negative regulatory protein2 0.1647 >Cluster RNA-binding protein(14) conserved virulence factorPAI 1(7)acetyltransferase (GNAT) family protein(6)putative N- acetyltransferase(3) "acetyltransferase, GNAT family protein(2)"Protease synthase and sporulation negative regulatory protein PAI 1(2) acetyltransferase domain protein(2) GCN5 N- acetyltransferase(2) conserved hypothetical protein(1) hypotheticalprotein(1) probabletranscriptionalregulator(1) GCN5-related N-acetyltransferase(1) "acetyltransferase, GNAT family hypothetical protein(1)" Acetyltransferase GNAT family protein(1) MW2290(1) "N-acyltransferase, putative(1)" putative protease synthase and sporulation negative regulatory protein PAI 1(1)

258 7 142 4.929577465 2 0.1647 >Cluster 4638 acetyltransferase(35) putative acetyltransferase(12) bacterial transferase hexapeptide family protein(7) Putative acetyltransferase(4) O-acetyltransferase(3) bacterial transferase hexapeptide domain protein(3) Putative acetyltransferase YvoF(2) conserved hypothetical protein(2) hypothetical protein(2) possible acetyltransferase(1) probable acetyltransferase(1) bacterial transferase hexapeptide repeat protein(1) MW0724(1)transferase hexapeptide repeat containing protein(1) maltose O-acetyltransferase (Maltose transacetylase). domain protein(1) "O-acetyltransferase, putative(1)"

259 10 142 7.042253521 6 0.1695 >Cluster 146 valine--tRNA ligase(38) valyl-tRNA synthetase(22)Valyl-tRNA synthetase(11) Valine--tRNA ligase(6) valine-tRNA ligase(2) valyl tRNA synthetase(1)

260 8 142 5.633802817 2 0.1766 >Cluster 180 ATPase P(29) copper-translocating P-type ATPase(15) Copper-exporting P-type ATPase A(7) putative copper importingATPase A(6) transporter(5) ATPase(4)" transporting ATPase(2) "Lead, cadmium, zinc and mercury transporting ATPase; Copper-translocating P-type ATPase(2)" Copper- translocating P-type ATPase(2) Copper-exporting ATPase(1) cation-transporting ATPase E1-E2 family protein(1) copper- translocating P-type ATPase CopA(1) copper-exporting ATPase(1)P-ATPase superfamily P-type ATPase copper (Cu2+) "Lead, cadmium, zinc and mercury transporting copper-transporting ATPase CopA(2) copper-copper-transporting ATPase copA(1) "cation- transporting ATPase, E1-E2 family, putative(1)"

261 8 142 5.633802817 2 0.1766 >Cluster 1855 NADH dehydrogenase(47) pyridine nucleotide-disulfide oxidoreductase(7) pyridine nucleotide-

disulfide oxidoreductasefamily protein(7) oxidoreductase(3) oxidoreductase(3) nucleotide-disulphide oxidoreductase(2) FAD-dependent pyridine nucleotide-disulfide oxidoreductase(2) hypothetical protein(1) putative NADH dehydrogenase(1) Pyridine oxidoreductase(1) pyridine nucleotide-disulphide oxidoreductase family protein(1) MW0820(1) probable NADH dehydrogenase(1) FAD-dependent pyridine nucleotide-disulphide oxidoreductase(1) "NADH dehydrogenase, FAD-containing subunit, putative(1)"

262 8 142 5.633802817 2 0.1766 >Cluster 1888 peptide ABC transporter permease(29) putative oligopeptide transport system permease(6) "ABC transporter, permease protein(4)" oligopeptide transporter membrane permease domain(4)binding-protein-dependent transport system inner membrane component family protein(4) "oligopeptide ABC superfamily ATP binding cassette transporter, membrane protein(4)" "ABC-type dipeptide/oligopeptide/nickel transport system, permease component(4)"binding--dependent transport system inner membrane component family protein(3) putative oligopeptide transport system permease protein oppB2(2)oligopeptide transport system permease oppB2(2) oligopeptide transporter permease(2) Putative oligopeptide transport system permease protein oppB2(2) Dipeptide transport system permease protein DppB(1) "oligopeptide ABC transporter membrane permease, putative(1)" oligopeptide ABC transporter membrane protein(1) probable oligopeptide membrane permease(1) oligopeptide transporter putative membrane permease domain(1) Oligopeptide transport system permease protein oppB(1) "peptide ABC transporter, permease protein(1)" binding- protein-dependent transport systems inner membrane component(1)

263 8 142 5.633802817 4 0.1766 >Cluster 2351 ribosomal protein L11 methyltransferase(51) Ribosomal protein L11 methyltransferase(16) putative methyltransferase(5) 50S ribosomal protein L11 methyltransferase(2)conserved hypothetical protein(2) probable methyltransferase(1) putative ribosomal protein L11 methyltransferase(1) ribosomal protein L11 methylase(1)Ribosomal Protein L11 methyltransferase(1)

264 8 142 5.633802817 4 0.1766 >Cluster 6325 hypothetical protein(16) phage protein(3) phage-like protein(3) virulence-related phage protein(2) Hypotheticalprotein(2) "Hypotheticalprotein,phi-ETAorf16(1)" hypothetical phage-related protein(1) conserved hypothetical phage-related protein(1) "Hypothetical protein, phi-ETA orf16 homolog (1)"putative pyridine nucleotide-disulfide putative pyridine nucleotide-disulphide conserved hypothetical protein(2) pyridine

265 5 142 3.521126761 4 0.1776 >Cluster 133 histidine kinase(30) Osmosensitive K+ channel histidine kinase KdpD(8) sensor kinase protein(7) sensor histidine kinase KdpD(6) osmosensitive K+ channel histidine kinase(4) "histidine kinase-, DNA gyrase B-, and HSP90-like ATPase family protein(4)" his Kinase A domain protein(3) sensor protein(3) Sensor protein KdpD(3) hypothetical protein(2) two component sensor protein(2) ATPase/histidine kinase/DNA gyrase B/HSP90 domain protein(1) his Kinase A (phosphoacceptor) domain protein(1) Sensor protein kdpD(1) "Osmosensitive K channel sensor histidine kinase KdpD, putative(1)"Osmosensitive K channel His kinase sensor(1)sensor protein KdpD(1) "sensor protein KdpD, putative(1)"

266 5 142 3.521126761 4 0.1776 >Cluster 532 formate--tetrahydrofolate ligase(50) Formate-- tetrahydrofolate ligase(16) formyltetrahydrofolate synthetase(8)formate-tetrahydrofolate ligase(4) formate-- tetrahydrofolate ligase (Formyltetrahydrofolatesynthetase) (FHS) (FTHFS)(1) "formate-tetrahydrofolate ligase, putative(1)"

267 5 142 3.521126761 2 0.1776 >Cluster 2332 peptide ABC transporter ATP-binding protein(29) Oligopeptide transport ATP-binding protein OppF(10) ABC transporter family protein(7) putative oligopeptide transport ATP- binding protein(6) "oligopeptide ABC superfamily ATP binding cassette transporter, ABC protein(4)" oligopeptide transport ATP- binding protein OppF(4) oppF(2) Oligopeptide transport ATP-binding protein oppF(2) "oligopeptide ABC transporter, ATP-binding protein(2)" oligopeptide transport system ATP-binding protein OppF(2) ABC transporter(2) "oligopeptide transport system ATP- binding protein OppF, putative(1)" "oligopeptide ABC superfamily ATP binding cassette transporter, ABC protein, Opp3F(1)"ABC transporter related(1) oligopeptide transport system ATP-binding protein(1) oligopeptide transport system ATP- binding protein OppF homologue(1) "oligopeptide ABC transporter, ATP-binding protein, putative(1)""ABC transporter, ATP-bindingtransport ATP-binding protein OppF (TCtransport system ATP-binding protein(1)

268 5 2641 cell wall amidase(31) N-acetylmuramoyl-L-alanine amidase(18) N-acetylmuramoyl-L-alanine amidase family protein(7)putative N-acetylmuramoyl-L-alanine amidase(5) putative cell wall amidase LytH(4)"N-acetylmuramoyl-L-alanine amidase,familyprotein(1)"3.A.1.5.1)(1)OligopeptideOligopeptide142 3.521126761 3 0.1776 >Cluster3(3)" bacterial SH3 domain protein(3) LytH protein involved in methicillin resistance / N-acetylmuramoyl-L-alanine amidase domain protein(2) cell wall amidase lytH(2)LytH protein involved in methicillin resistance(1)conserved hypothetical protein(1) cell wall hydrolase/autolysin(1) LytH protein involved in methicillin resistance / N-acetylmuramoyl-L-alanine amidase domain(1)

269 5 142 3.521126761 3881 heat shock protein GrpE(22)Heat shock protein GrpE(9)3 0.1776 >Cluster molecular chaperone GrpE(10) GrpE protein (Hsp-70cofactor)(5)protein(4)factor GrpE(3)protein(2)cofactor)(1)GrpE protein(1)GrpE(1)heat shock molecular chaperone protein(5) GrpE Protein GrpE(4) protein grpE(4) nucleotide exchange co-chaperone GrpE(3) protein GrpE(3) grpE chaperone GrpE(1) protein grpE (HSP-70 Heat shock protein grpE(1) Hsp-70 cofactor GrpE protein (HSP-70 cofactor)(1) heat -hock protein

270 11 142 7.746478873 4 0.1917 >Cluster 1444 aspartate kinase(40) Aspartokinase(11) aspartokinase II(10) aspartate kinase domain protein(7) aspartokinase(3) "aspartokinase, alpha and beta subunits(3)" aspartate kinase 2(3) aspartokinase 2 (Aspartokinase II) (Aspartate kinase 2)(2) aspartate kinase II(1) "aspartokinase II, putative(1)"

271 8 142 5.633802817 5 0.1968 >Cluster 150 hypothetical protein(26) membrane protein(22) putative membrane protein(8) bacterial membrane YfhO family protein(6) conserved hypothetical protein(6) putative membrane proteinYfhO(5) "membrane protein, putative(1)" FIG01108412: hypothetical protein(1) Hypotheticalprotein(1) Integralmembraneprotein(1)bacterial membrane protein YfhO(1) hypothetical membrane protein(1)

272 8 142 5.633802817 4 0.1968 >Cluster 352 ATPase(22) hypothetical protein(12) "ATPase involved in DNA repair, phage associated(5)" AAA domain protein(4) hypothetical phage protein(3) conserved hypothetical protein(3) RecF/RecN/SMC N terminal domain(1) RecF/RecN/SMC N terminal domainprotein(1) Hypotheticalprotein(1) phiPVLORF41-like protein(1)

273 8 142 5.633802817 2 0.1968 >Cluster 702 aminobenzoyl-glutamate transporter(32) Aminobenzoyl- glutamate transport protein(11) putative transport protein(8)aminobenzoyl-glutamate transport protein(4) Na+/H+ antiporter family protein(4) C4-dicarboxylate anaerobic carrier family protein(4) putative aminobenzoyl-glutamate transport protein(3)abgT transporter family protein(3) "transporter, putative(2)" p-aminobenzoyl-glutamate transporter(2) C4- dicarboxylate anaerobic carrier domain protein(1) MW2397(1) probable transport protein(1) AbgT family p-aminobenzoyl-glutamatetransporter(1)protein(1)family(1)hypothetical protein(1) conserved hypothetical AbgT putative transporter(1) AbgT transporter putative efflux pump component MtrF(1)142 5.633802817 3 0.1968 >Cluster

274 8 704 2-isopropylmalate synthase(80)

275 8 142 5.633802817 2 0.1968 >Cluster 4140 imidazole glycerol phosphate synthase subunit HisH(17) imidazole glycerol phosphate synthase(17) "imidazole glycerol phosphate synthase, glutamine amidotransferase subunit(13)"Imidazole glycerol phosphate synthase amidotransferase subunit(8) putative amidotransferase(7) Imidazole glycerol phosphate synthase subunit HisH(6) imidazole glycerol phosphate synthase subunit hisH(3) imidazole glycerol phosphate synthase subunit(2) amidotransferase hisH(2) "imidazole glycerol phosphate synthase, subunitH(1)" "imidazole glycerol phosphate synthase, subunit H (hisH), putative(1)" "Imidazole glycerol phosphate synthase, glutamine amidotransferase subunit(1)" imidazoleglycerol-phosphate synthase subunit HisH(1)

276 8 142 5.633802817 2 0.1968 >Cluster 5120 D-ribose pyranase(50) putative ribose transport protein(7)"Ribose ABC transport system, high affinity permease RbsD(6)""ribose ABC superfamily ATP binding cassette transporter, membrane protein(5)" ribose permease(2) "ribose permease ABC transport protein, putative(1)" high affinity ribose transport protein RbsD(1)RbsD or FucU transport(1)ribose ABC transporter protein(1) ribose permease transport protein(1) "Ribose ABC transport system, high affinity permease RbsD(TC3.A.1.2.1)(1)" RbsD/FucUtransportfamilyprotein(1) high affinity ribose transport protein rbsD(1) "ribose ABC transporter protein, putative(1)" RbsD / FucU transport protein family(1) D-ribose mutarotase(1)

277 6 142 4.225352113 3 0.1988 >Cluster46 hypothetical protein(13) NTPase(9) NACHT domain protein(5) conserved hypothetical protein(4) Hypotheticalprotein(1) -1

278 6 142 4.225352113 3 0.1988 >Cluster 263 transcriptional antiterminator(36) PRD domain protein(7) putative PTS multi-domain regulator(5) "putative galactitol operon regulator (Transcriptional antiterminator), BglG family / PTS system, mannitol/fructose-specific II>(3)" transcriptional antiterminator BglG family protein(3) hypotheticalprotein(2) mgahelix-turn-helixdomainprotein(2) "phosphoenolpyruvate-dependent sugar phosphotransferase system, EIIA 2 family protein(2)" putative PTS multidomain regulator(2) PRD domain-containing protein(2) BglG family transcriptional antiterminator(2) conserved hypotheticalprotein(1) "galactitoloperonregulator(Transcriptional antiterminator), BglG family(1)" "putative galactitol operon regulator (Transcriptional antiterminator), BglG family / PTS system, mannitol/fructose-specific II... at 98.47%(1)" "putative galactitol operon regulator (Transcriptional antiterminator), BglG family / PTS system, mannitol/fructose-specific II... at 98.90%(1)" "putative galactitol operon regulator (Transcriptional antiterminator), BglG family(1)" BglG family transcription antiterminator(1) putative PTS multi-domain regulatorcomponent(1) M trans-acting positive regulator (MGA) HTH domain protein(1) "Predicted galactitol operon regulator (Transcriptional antiterminator), BglG family / PTS system, mannitol/ fructose-specific I>(1)" "transcriptional antiterminator, BglG family(1)" "Transcription antiterminator, BglG family(1)" BglG family transcriptional antiterminator-like protein(1) MW0220(1)bglG family transcriptional antiterminator domain protein(1)possible BglG family transcriptional antiterminator(1) "Predicted galactitol operon regulator (Transcriptional antiterminator), BglG family / PTS system, mannitol/ fructose-specific I... at 98.81%(1)"

279 6 142 4.225352113 3 0.1988 >Cluster 299 excinuclease ABC subunit B(51) Excinuclease ABC subunit B(11) "excinuclease ABC, B subunit(5)" excision endonuclease subunit UvrB(5) UvrABC system protein B(4) exinuclease ABC subunit B(4) "excinuclease ABC subunit B, putative(1)"

280 6 142 4.225352113 3 0.1988 >Cluster 1523 D-alanine--D-alanine ligase(54) D-alanyl-alanine synthetase A(9) D-alanine--D-alanine ligase A(6) D-alanine-D-alanine ligase(6) D-alanine--D-alanine ligase family protein(3) D- alanine--D-alanine ligase (D-alanylalanine synthetase)(D-Ala-D-Ala ligase)(2) D-ala D-ala ligase N-terminal domain protein(1)

281 6 142 4.225352113 4 0.1988 >Cluster 1894 ribitol-5-phosphate dehydrogenase(33) Xylitol dehydrogenase(10) zinc-binding dehydrogenase family protein(7)putative zinc-binding dehydrogenase(6) alcohol dehydrogenase(3) putative ribitol-5-phosphate dehydrogenase(3) putative alcohol dehydrogenase(2) "alcohol dehydrogenase, zinc- containing(2)" truncated xylitol dehydrogenase(2) GroES-like protein(1) alcohol dehydrogenase GroES-like domain protein(1) putative ribitol-5-phosphate dehydrogenase (Ribulose-5-P reductase)(1)hypotheticalprotein(1) "alcoholdehydrogenase,zinc- containing, putative(1)" Alcohol dehydrogenase GroES domain protein(1)zinc-binding dehydrogenase(1) MW0228(1) dehydrogenase(1)zinc-containingalcoholdehydrogenase(1) phosphate 2-dehydrogenase(1)xylitolRibitol-5-

282 6 142 4.225352113 2 0.1988 2350 oxidoreductase(30) aldo/keto reductase family protein(10) Oxidoreductase ion channel(9) protein IolS(7) "oxidoreductase, aldo/keto reductase family(4)" aldo/keto reductase family oxidoreductase(3) aldo/keto reductase(3) NADP-dependent oxidoreductase(3) probable oxidoreductase(1) Putative aldo/keto reductase family protein(1) Oxidoreductase ion channelproteinIolS(1) proteiniolS(Vegetativeprotein147) (VEG147) (AKR11A)(1) MW0563(1) "aldo-keto reductase, putative(1)" dehydrogenase(1) "oxidoreductase, aldo/keto reductase family protein(1)" conserved hypothetical protein(1) hypothetical protein(1)

283 6 142 4.225352113 2 0.1988 >Cluster 3069 iron ABC transporter ATP-binding protein(30) ABC transporter ATP-binding protein(26) ABC transporter family protein(7) "ABC superfamily ATP binding cassette transporter, ABC protein(5)" "ABC transporter, ATP-binding protein(4)" ABC transporter(2) "ABC transporter, ATP-binding protein, putative(2)"Iron(III) dicitrate transport ATP-binding protein fecE(1)probable ATP-binding ATP transporter protein(1) protein(1) ABC transporter related(1)

284 6 142 4.225352113 3 3744 30S ribosomal protein S3(59) ribosomal protein S3(13) SSU ribosomal protein S3p (S3e)(7) SSU ribosomal

protein S3P(1)

285 6 142 4.225352113 2 0.1988 >Cluster 5975 hypothetical protein(21) Hypothetical protein(2)hypotheticalMW2079(1)0.1988 >Cluster>Cluster

286 4 142 2.816901408 4 0.206 >Cluster 129 isoleucine--tRNA ligase(40) isoleucyl-tRNA synthetase(24) Isoleucyl-tRNA synthetase(11) Isoleucine--tRNA ligase(3) Ile-tRNA synthetase(2) tRNA synthetases class I (C) catalytic domain protein(1) tRNA synthetases class I family protein(1)

287 4 142 2.816901408 3 697 GMP synthase(36) GMP synthetase(9) synthase/glutamine amidotransferase protein(7) putative GMP synthase(6) glutamine-hydrolyzing GMP synthase(5) GMP synthase (glutamine-hydrolyzing)(3) "GMP synthase (glutamine- hydrolyzing), amidotransferase subunit / GMP synthase (glutamine- hydrolyzing), ATP pyrophosphatase sub>(2)""GMP synthase, large subunit(1)" GMP synthase (glutamine-hydrolyzing) domain protein(1) "GMP synthase glutamine-hydrolyzing, amidotransferase subunit / GMP synthase glutamine-hydrolyzing, ATP pyrophosphatase subun... at 97.92%(1)" "GMP synthase, putative(1)" GMP synthase (glutamine-hydrolyzing) amidotransferasesubunit(1) "GMP synthase (glutamine-hydrolyzing), amidotransferase subunit(1)"

288 4 142 2.816901408 4 0.206 >Cluster 2076 LacI family transcriptional regulator(30) LacI family regulatory protein(9) Ribose operon repressor(7) "bacterial regulatory s, lacI family protein(4)" "sugar-binding transcriptional regulator, LacI(4)" ribose operonrepressor(3) "bacterial regulatory, lacI family protein(3)" LacI family HTH-type transcriptional repressor(3) transcriptional regulator(2) hypothetical protein(2) Transcriptional repressor(2)ribosetranscriptionalrepressorRbsR(2) "riboseoperon repressor, LacI family, putative(1)" regulatory protein LacI(1) "transcriptional regulator, LacI family(1)" probable ribose transcriptional repressor LacI family(1) MW0247(1) conserved hypothetical protein(1) "bacterial regulatory proteins, lacI family protein(1)"

289 4 142 2.816901408 2 0.206 >Cluster 3799 bacteriocin ABC transporter ATP-binding protein(28) ABC transporter ATP-binding protein(24) ABC transporter(5) hypotheticalprotein(4) putativebacteriocinABCtransporterfamily protein(3) conserved hypothetical protein(3) "ABC transporter, ATP-bindingprotein(2)" ABCtransporter(ATP-bindingprotein) (2) "ABC transporter, ATP-binding protein, putative(2)" "bacteriocin export ABC transporter, lactococcin 972 group family protein(1)" ABC transporter0.206 >Cluster bifunctional GMP GMP synthase (7)related(1) "putative bacteriocin export ABC transporter, lactococcin 972 group(1)"putative bacteriocin export ABC transporter(1) ABC transporter family protein(1) probable ATP- binding ABC transport protein(1) "bacteriocin export ABC transporter, lactococcin family protein(1)" bacteriocin export ABC transporter family protein(1)

290 4 142 2.816901408 4 0.206 >Cluster 4975 cell division protein FtsL(43) Cell division protein FtsL(14)cell division protein(11)putative cell division protein(7) "cell division and chromosome partitioning protein, FtsL(3)" conserved hypothetical protein(1) cell division septum formation protein FtsL(1)Cell division protein ftsL(1)

291 9 142 6.338028169 2 0.2123 >Cluster 2546 UDP pyrophosphate phosphatase(24) Undecaprenyl- diphosphatase(14) undecaprenyl-diphosphatase UppP(10) undecaprenyl-diphosphatase(10) putative undecaprenol kinase(7) undecaprenyl pyrophosphate phosphatase(6) "bacitracin resistance protein, undecaprenyl pyrophosphate phosphatase(2)" bacA(1) bacitracin resistance protein: Undecaprenyl-diphosphatase(1) probable undecaprenol kinase bacitracin resistanceprotein(1) "undecaprenyl pyrophosphate phosphatase, putative(1)" bacitracin resistance protein homologue(1)Undecaprenyl- diphosphatase (Bacitracin resistance protein)(1) "undecaprenolkinase, putative(1)"

292 12 142 hydrolase(4)undecaprenol kinase(1)8.450704225 6 0.213 >Cluster 39serine-aspartate repeat-containing protein D(3) adhesin of unknown specificity SdrD(2) "Ser-Asp richfibrinogen-binding, bone sialoprotein-binding protein(1)" hypotheticalprotein(1) AdhesinproteinSdrD(1) Adhesinofunknown specificity SdrE(1) Serine-aspartate repeat-containing protein E(1) Serine-aspartate repeat-containing protein D(1)

293 12 142 8.450704225 4 0.213 487 pyruvate kinase(64) Pyruvate kinase(17) kinase, barrel domain protein(1)"

294 12 142 8.450704225 4 0.213 495 transcriptional regulator(36) dihydrolipoamide dehydrogenase(30) hypotheticalprotein(9) transcriptional regulator family protein(8) pyridine nucleotide-disulfide>Cluster"pyruvate>Clusterreductase(6) putative DNA-binding protein(6) pyridine nucleotide-disulfide oxidoreductase family protein(5) pyridine nucleotide-disulphide oxidoreductase protein(5) conserved hypotheticalprotein(4) Pyridinenucleotide-disulfideoxidoreductase family protein(4) pyridine nucleotide-disulfide oxidoreductase protein(3) trkA-N domain protein(3) putative transcriptional regulator(3) putative pyridine nucleotide-disulfide oxidoreductase(3) pyridine nucleotide-disulfide oxidoreductase(3) mercuric reductase(2) AsnC family transcriptional regulator(2) "transcriptional regulator, BadM/ Rrf2 family(2)" Putative Dihydrolipoamide dehydrogenase(2)"Rrf2 family transcriptional regulator, group III(2)" probable DNA- binding protein(2) FAD-dependent pyridine nucleotide-disulphide oxidoreductase(1) MW0549(1) "Putative Dihydrolipoamide dehydrogenase; Mercuric ion reductase; PF00070 family, FAD-dependent NAD(P)-disulfide oxidoreducta... at 97.81%(1)" "Putative Dihydrolipoamide dehydrogenase; Mercuric ion reductase; PF00070 family, FAD-dependent NAD(P)-disulfide oxidoreductase(1)" "pyridine nucleotide-disulfide oxidoreductase, putative(1)" probable pyridine nucleotide-disulfide oxidoreductase YkgC(1) Hypothetical protein(1) protein of unknown function UPF0074(1)

295 2 142 1.408450704 2 0.2155 >Cluster 147 DNA polymerase I(69) DNA-directed DNA polymerase I(8) DNA polymeraseI(POLI)(2) "5'-3'exonuclease,C-terminalSAMfold family protein(2)" DNA-directed DNA polymerase(1)

296 2 142 1.408450704 2 0.2155 >Cluster 246 ribonucleotide-diphosphate reductase subunit alpha(34) "ribonucleoside-diphosphate reductase, alpha subunit(15)" "Ribonucleotide reductase of class Ib (aerobic), alpha subunit(7)" ribonucleotide-diphosphate reductase(4) ribonucleoside-diphosphate reductase alpha chain(3) ribonucleoside-diphosphate reductase subunit alpha(3) ribonucleoside-diphosphate reductase(2) ribonuceloside diphosphate reductase major subunit(2) "Ribonucleotide reductase of class Ib (aerobic),alpha subunit(2)" ribonucleotide-

diphosphate reductase alpha chain(1) "ribonucleotide-diphosphate reductase, alpha subunit(1)" ribonuceloside diphosphate reductase subunit alpha(1)Ribonucleoside-diphosphate reductase alpha chain(1) "Ribonucleoside-diphosphate reductase, large chain(1)" ribonucelotide diphosphate reductase alpha chain(1) "ribonucleotide-diphosphate reductase alpha chain, putative(1)"

297 2 142 1.408450704 2 0.2155 >Cluster 454 oligoendopeptidase F(28) oligopeptidase PepB(27) Oligoendopeptidase F(11) putative oligopeptidase(7) thimet oligopeptidase-like protein(3) "oligoendopeptidase F, putative(1)"thimet oligopeptidase homologue(1) M03 family oligopeptidase F(1) thimet oligopeptidase(1) MW0882(1)

298 2 142 1.408450704 2 0.2155 >Cluster 570 Na/Picotransporter(31) Sodium-dependentphosphate transporter(9) putative Na+/Pi-cotransporter protein(6) Na+/ phosphate symporter(4) conserved hypothetical protein(4) na+/Pi- cotransporter family protein(4) Na/Pi-cotransporter II-protein(4) phoU domain protein(3) putative phosphate:Na+ symporter(3) Na/ Pi cotransporter family protein(2) Na+ Pi-cotransporter protein(2) PnaS family phosphate:sodium (Na+) symporter(1) putative Na+/Pi - cotransporter protein(1)hypothetical protein(1) Na/ Pi cotransporter II-related protein(1) possible PnaS family phosphate:sodium (Na+) symporter(1) Na/Pi-cotransporter II- related protein(1) probable membrane transporter(1) Na+/Pi-cotransporter family protein(1) putative Na/Pi cotransporter family protein(1) Na/Pi-cotransporter II-like protein(1)

299 2 142 1.408450704 2 0.2155 >Cluster 645 ABC transporter ATP-binding protein(40) "Putative glutathione transporter, ATP-binding component(9)" nickel import ATP- bindingproteinNikE(7) oligopeptidetransportATP-bindingprotein(5)ABC transporter(2) Peptide/nickel transport system ATP- binding protein(2) ABC superfamily ATP binding cassette transporter protein(2) glutathione import ATP-binding protein gsiA(1) "peptide ABC transporter, ATP-binding protein(1)" putative phosphonate C-P lyase system protein PhnK(1) "oligopeptide ABC transporter, ATP-binding protein, putative(1)" glutathione import ATP-binding protein GsiA(1) "peptide ABC transporter, ATP binding protein,protein oppD(1)transporter, ABCrelated(1)protein(1)"putative(1)" Oligopeptide transport ATP-binding "oligopeptide ABC superfamily ATP binding cassette protein(1)" ABC transporter "oligopeptide ABC transporter, ATP-binding

300 2 142 1.408450704 2 0.2155 >Cluster 1089 sodium-dependent transporter(33) sodium:neurotransmitter symporter family protein(12) Sodium-dependent transporter(11) neurotransmitter symporter family protein(6) putative sodium- dependent transporter(3) SNF family Na+-dependenttransporter(2) "sodium-dependent transporter, putative(2)" Sodium:neurotransmitter symporter(2) Sodium/neurotransmitter symporter family protein(1) MW0413(1) sodium-dependent symporter protein(1) sodium:neurotransmitter symporter(1) probablesodium-dependenttransporter(1) Sodium:neurotransmitter symporter family protein(1) conserved hypothetical protein(1) hypothetical protein(1)

301 2 142 1.408450704 1168 enolase(35) Enolase(18) hydratase(17) putative enolase(6) dehydrogenase)(4) Phosphopyruvate hydratase(1) enolase 2- phosphoglycerate dehydratase(1)

302 2 142 1.408450704 2 0.2155 >Cluster 1381 nucleoside permease(30) nucleoside transport protein(10) putative Na+ dependent nucleoside transporter(7) "nucleoside permease NupC, putative(4)" purine nucleoside transport protein nupG(4) Na+ dependent nucleoside transporter domain-containing protein(4) na+ dependent nucleoside transporter family protein(3) "concentrative nucleoside transporter, CNT family(3)"Na+ dependent nucleoside transporter domain protein(2) conserved hypothetical protein(2) Na dependent nucleoside transporter domain-containing protein(2) Pyrimidine nucleoside transporter(1)na+ dependent nucleoside transporter C-terminus family protein(1) "nucleoside transporter, putative(1)" "nucleoside transporter, NupC family(1)" MW0607(1) Nucleoside permease nupC(1) hypothetical protein(1) CNT family concentrative nucleoside transporter(1) Na dependent nucleoside transporter domain protein(1)

303 2 142 1.408450704 2 0.2155 1552 alanine racemase(58) Alanine racemase(14) racemase 1(8)

304 2 142 1.408450704 2 0.2155 1679 UDP-N-acetylglucosamine 2-epimerase(46) UDP-N-acetyl glucosamine 2-epimerase(10) capsular polysaccharide synthesis enzyme(7) capsular polysaccharide biosynthesis protein Cap5G(4)capsular polysaccharide synthesis enzyme Cap5G(3) Capsular polysaccharidesynthesisenzymeCap8G(2) capsularpolysaccharide synthesis enzyme Cap8G(2)capsular polysaccharide synthesis enzyme CapG(2) Capsular polysaccharide synthesis enzyme Cap5G; UDP-N- acetylglucosamine 2-epimerase(1) UDP-N-acetylglucosamine 2-epimerase Cap5G(1) "UDP-2-acetamido-2,6-dideoxy-beta-L-talose 2-epimerase(1)"

305 2 142 1.408450704 2 0.2155 >Cluster 1854 dihydroneopterin aldolase(4) rhodanese-like domain- containing protein(3) rhodanese domain-containing protein(3) hypotheticalprotein(2) Rhodanesedomainprotein(1) "Hypothetical protein, sulfurtransferase domain protein(1)" conserved hypothetical protein(1) dsrE/DsrF-like2 0.2155 >Cluster phosphopyruvate enolase (2-phosphoglycerate>Clusteralanine>Clusterfamily protein(1) Disulfide bond regulator(1)

306 2 142 1.408450704 2 0.2155 >Cluster 2885 phosphotransferase(20) hypothetical protein(10) conserved hypothetical protein(10) phosphoenolpyruvate synthase regulatory protein(9) ATP/GTP-binding protein(6) "Putative pyruvate, phosphate dikinase regulatory protein(6)" putative phosphotransferase yqfL(4) kinase/pyrophosphorylase family protein(4)phosphoenolpyruvate synthetase regulatory protein(3) putative phosphotransferase(2) ATP/GTP-bindingprotein-likeprotein(1) "phosphotransferase, putative(1)" protein of unknown function DUF299(1) protein in Sigma70 operon (ORF30)(1)"ATP/GTP-binding protein, SA1392-like protein(1)" "ATP/GTP-binding protein, SA1392homolog(1)" "ADP-dependent kinase / inorganic phosphate- dependent pyrophosphorylase, putative(1)"

307 2 142 1.408450704 2 0.2155 >Cluster 2886 RecX family transcriptional regulator(26) regulatory protein RecX(15) recombination regulator RecX(10) putative regulatory protein(6) regulatory protein recX(6) Regulatory protein RecX(4) conserved hypothetical protein(3) Regulatory protein recX(2)Regulatory protein(2) recX family protein(1) regulatory protein(1) hypotheticalprotein(1) regulatoryproteinRecx(1)

308 2 142 1.408450704 2 0.2155 >Cluster 2888 short-chain dehydrogenase(18) short chain dehydrogenase(12) putative short chain dehydrogenase(9) "Oxidoreductase, short chain dehydrogenase/ reductase family(7)" short chain dehydrogenase family protein(5)KR

domain protein(3) short-chain dehydrogenase/reductase SDR(3) putativeoxidoreductase(3) conservedhypothetical protein(2) glucose 1-dehydrogenase(1) hypothetical protein(1) Glutamate dehydrogenase(1) putative short-chain dehydrogenase(1) (1) MW2396(1)

309 2 142 2990 hypothetical protein(45) tandem lipoprotein(10) putative lipoprotein(8) Tandem lipoprotein within Pathogenicity island(7) tandem lipo family protein(5) lipoprotein(4) staphylococcal tandem lipoprotein(4) Membrane lipoprotein(2) tandem lipos family protein(2) conserved hypothetical protein(2) staphylococcus tandem lipoproteins family protein(1) hypothetical lipoprotein(1) "tandem lipoprotein, Lpl8(1)" staphylococcus tandem lipoproteins(1) putative tandem lipoprotein(1)Glucose 1-dehydrogenase(1) 3-oxoacyl- "short chain dehydrogenase, putative(1)"1.408450704 2 0.2155 >Cluster

310 2 142 1.408450704 2 0.2155 >Cluster 3305 RNA methyltransferase(30)SpoU rRNA Methylase family protein(12) rRNA methylase(10) RNA 2'-O ribose methyltransferase substrate binding family protein(8) "RNA methyltransferase, TrmH family(4)" SpoU rRNA methylase family protein(3) rRNA methyltransferase(3)tRNA/rRNA methyltransferase(2) hypotheticalprotein(1) conserved hypotheticalprotein(1) FIG011178:rRNAmethylase(1) MW1020(1)tRNA/rRNA methyltransferase (SpoU)(1) TrmH family rRNA methyltransferase(1) 23S rRNA methyltransferase(1)

311 2 142 1.408450704 2 0.2155 >Cluster 4304 ADP-ribose pyrophosphatase(56) putative ADP-ribosepyrophosphatase(7) NUDIX hydrolase(3) domain protein(2) pyrophosphatase (adp-ribose diphosphatase)(adenosine diphosphoribose pyrophosphatase) (adpr-ppase) (adp-ribosephosp>(1) ADP-ribose diphosphatase(1) "nudix-type nucleoside diphosphatase, YffH/AdpP family(1)" "ADP-ribose pyrophosphatase, putative(1)" probable ADP-ribose pyrophosphatase(1)protein(1)protein(1)protein(1)putative(1)"protein //(1)putative helix-turn-helix protein YlxM/p13 familySigma70 region 4-domain containing"Sec family secretory protein signal,"helix-turn-helix protein, YlxM/p13 family(1)""ADP-ribose pyrophosphatase, NudF(4)" conserved hypothetical protein(2) NUDIX MutT/nudix family protein(1) adp-ribose

312 2 142 4935 crystallin(17) shock protein(10) protein(8)" hsp20/alphacrystallinfamilyprotein(7) heatshock protein Hsp20(6) Heat shock protein Hsp20(3) hsp20-like protein(2) "Hsp20 family small heat shock protein, putative(1)"1.408450704 2 0.2155 >Cluster hypothetical protein(16) putative small heat "Molecular chaperone, small heat shockSmall heat shock protein(1) acid shock protein(1) MW2307(1) Hsp20/alphacrystallinfamilyprotein(1) HSP20 family protein(1) small heat shock protein Hsp20(1) conserved hypothetical protein(1)

313 2 142 1.408450704 2 0.2155 >Cluster 5566 DNA-binding protein(32) putative DNA-binding protein(13) hypothetical protein(13) Signal recognition particle associated protein(10) conserved hypothetical protein(5) "helix-turn-helix protein, YlxM / p13 like family protein(1)" Hypothetical142 1.408450704 2 0.2155 >Cluster

314 2 5645 TrmB family transcriptional regulator(7) hypotheticalprotein(6) conserved hypothetical protein(3) Phage protein(3) putative phage protein(3)hypothetical phage protein(1) phage protein(1) conserved hypothetical phage protein(1)

315 2 142 1.408450704 2 0.2155 >Cluster 5824 hypothetical protein(59) conserved hypothetical protein(6) iron-sulfur cluster biosynthesis protein(5) HesB/YadR/YfhF- family protein(2) "HesB-like iron-sulfur cluster biosynthesis protein, putative(1)" iron-sulphur cluster biosynthesis family protein(1) FIG01108262: hypothetical protein(1) iron- sulphur cluster biosynthesis protein(1) Putative cytosolic protein(1) "conserved hypothetical protein, putative(1)" HesB-like protein(1) Hypothetical protein(1)

316 2 142 1.408450704 2 0.2155 >Cluster 6026 hypothetical protein(10) DNA-binding protein(9) putative DNA-binding protein(8) phage DNA-binding protein(8) putative phiPVL-likeprotein(4) helix-turn-helixdomainprotein(3)Phage protein(3) phi PVL ORF 37 analogue(2) PVL orf 37-like protein(2) "phiSLT ORF 87-like protein, putative DNA-binding protein(2)" conserved hypothetical protein(1) hypothetical protein within a prophage(1) homeo-like domain protein(1) phage protein(1) hypothetical bacteriophage protein(1)

317 6 142 4.225352113 3 0.226 >Cluster 278 sodium:proton antiporter(30) sodium/hydrogen exchanger family protein(22) Na+/H+ antiporter(10) CPA1 family monovalent cation:proton (H+) antiporter-1(3) sodium/hydrogen exchanger(3) putative Na(+)/H(+) antiporter(3) MW2363(1) conservedhypotheticalprotein(1)hypotheticalprotein(1) Na+/H+ antiporter nhaP(1) putative Na+/H+ antiporter(1) "Na+/H+ antiporter, putative(1)" sodium hydrogen exchanger family protein(1)Na+/H+ antiporter family protein(1) "NhaP-type NA+/H+ antiporter, putative(1)"

318 6 142 4.225352113 2 0.226 >Cluster 578 urocanate hydratase(64) Urocanate hydratase(16)

319 6 142 4.225352113 2 0.226 >Cluster 782 monovalent cation/H+ antiporter subunit D(18) cation:proton antiporter(11) Na(+) H(+) antiporter subunit D(9)Na+/H+ antiporter subunit(9) Na(+)/H(+) antiporter subunit D1(6) na(+)/H(+) antiporter subunit D(6) CPA3 familymonovalent cation:proton (H+) antiporter-3 subunit D(4) Na+/H+ antiporter subunit D(3) Na /H antiporter subunit D1 protein(2) Na +/H+-antiporter subunit(2) putative monovalent cation/H+ antiportersubunitD(2) "monovalentcationantiporter-3subunitD, MnhD(1)"Sodium/protonantiporterproteinshaD(1) multicomponentK+:H +antiporter family protein(1) "Na+/H+ antiporter, MnhD component, putative(1)" Na(+)/H(+) antiporter subunit D(1) NA+/H+ antiportersubunitD(1) "multicomponentK+/H+antiporter,subunit 4(1)" Na+ H+ antiporter subunit D(1) Na(+)/H(+) antiporter subunit D (Multiple resistance and pHhomeostasis protein D) (Mrp complex subunit D)(1)

320 6 142 4.225352113 2 0.226 >Cluster 1569 cysteine protease(30) Staphopain A precursor(10) staphopain A(9) staphopain protease(6) Staphopain A(6) cysteine protease precursor SspB(4) staphopain peptidase C47 family protein(3) staphopain thiol proteinase(2) "staphopain A, cysteine proteinase(2)" Staphopain(2) staphopain A (Staphylopain A) (Staphylococcalcysteine proteinase A)(1) C47 family staphopain protease(1) Staphopain Cysteine Proteinase(1) staphopain A domain protein(1) staphopain(1) cysteine proteinase staphopain A(1) staphopain cysteine proteinase(1)

321 6 142 4.225352113 2 0.226 >Cluster 2198 ribonucleotide-diphosphate reductase subunit beta(34) ribonucleoside-diphosphate reductase subunit

beta(10) ribonucleoside-diphosphate reductase beta chain(7) "Ribonucleotide reductase of class Ib (aerobic), beta subunit(7)" "ribonucleoside-diphosphate reductase, beta subunit(4)" "ribonucleotide reductase, small chain family protein(4)" ribonucleoside-diphosphate reductase class Ib beta subunit protein(2) class 1b ribonucleoside-diphosphate reductase subunit beta(2)ribonucleoside-diphosphate reductase minor subunit(2) "Ribonucleotide reductase of class Ib (aerobic),beta subunit(2)" Ribonucleoside-diphosphate reductase beta chain(1) ribonucelotide diphosphate reductase beta chain(1) Ribonucleoside- diphosphate reductase(1) ribonucleotide-diphosphate reductase beta chain(1) "ribonucleotide-diphosphate reductase, betasubunit(1)" "ribonucleotide-disphosphate reductase beta chain, putative(1)" ribonucleoside-diphosphate reductase subunit beta (Ribonucleotide reductase small subunit)(1)

322 6 142 4.225352113 2 0.226 >Cluster 3359 enterotoxin I(14) extracellular enterotoxin type Iprecursor(4) enterotoxin(2) protein(2) enterotoxin I(2) enterotoxin sei(1) enterotoxin K(1) "toxin, beta-grasp domain protein(1)" Enterotoxin(1) "staphylococcal/extracellular enterotoxin type I(2) enterotoxin type I(2) toxin beta-grasp domain Superantigen enterotoxin SEK(2) staphylococcalStreptococcal toxin, beta-grasp domain protein(1)"

323 9 142 6.338028169 5 0.227 >Cluster 907 aspartate kinase(45) Aspartokinase(10) putative aspartate kinase(9) aspartate kinase domain protein(7) aspartokinase(3) amino acid kinase family protein(2) MW1214(1)"aspartate kinase, putative(1)" probable aspartate kinase(1) putative aspartokinase(1)aspartate kinase homolog(1)

324 9 142 6.338028169 4 0.227 >Cluster 6678 hypothetical protein(33) phage protein(15) Hypotheticalprotein(3) conservedhypotheticalphageprotein(2) conserved hypothetical protein(2) putative phage protein(1)Phage protein(1) "Hypothetical protein, PV83 orf23(1)"hypothetical phage protein(1) PV83 orf 23-like protein-related protein(1) "Hypothetical protein, PV83 orf23 homolog (1)" "Hypothetical protein, PV83 orf23-like protein(1)" Hypothetical Protein(1)

325 17 142 11.97183099 10 0.2334 >Cluster 173 hyaluronate lyase(49) Hyaluronate lyase precursor(12) hyaluronate lyase precursor(9) "polysaccharide lyase family 8, super-sandwich domain protein(7)" hyaluronate lyase precursor 2(7) Hyaluronate lyase(4) hyaluronate lyase 2(4) "polysaccharide lyase family 8, N terminal alpha-helical domain protein(4)"hyaluronate lyase (Hyaluronidase) (HYase)(2) hyaluronate lyase precursor1(2) "polysaccharide lyase family 8, C-terminal beta-sandwich domain protein(2)" "hyaluronate lyase, putative(1)" polysaccharide lyase family 8(1)

326 13 142 9.154929577 4 0.2334 >Cluster 1280 ATP-dependent Clp protease ATP-binding subunit ClpX(36) ATP-dependent protease(17) "ATP-dependent Clp protease, ATP- binding subunit ClpX(16)"ATP-dependent protease ATP-binding subunit ClpX(5) ATP-dependent Clp protease(2) protease ClpX(1) ATP- dependent endopeptidase clp ATP-binding subunit clpX(1) S14 family endopeptidase ClpX(1) protease(1) ATP-dependent Clp protease ATP-binding subunit(1)

327 13 142 9.154929577 4 0.2459 >Cluster 154 membrane protein(19) hypothetical protein(18) MMPL family protein(9) Putative antibiotic transport-associated protein(9)putative transport protein(7) RND superfamily resistance- nodulation-cell division:proton (H+) antiporter(6) MmpL efflux pump(3)Membrane protein ydfJ(3) MMPL domain-containing protein(2) mmpl domain protein(1) "Transporter, MMPL family protein(1)" MMPL domain protein(1) conserved hypothetical protein(1) "MmpL efflux pump, putative(1)""RND superfamily drug exporter, putative(1)"probable membrane transport protein(1) MW2473(1)

328 18 142 12.67605634 6 0.2463 >Cluster 108 fibronectin-binding protein(12) Fibronectin binding protein FnbB(5) fibronectin-binding protein A(3) fibronectin-binding protein FnbB(2) LPXTG family cell wall anchor protein(2) fibronectin-binding protein B(2) hypothetical protein(2) fibronectin-binding protein precursor(1) Fibronectin-binding protein fnbB(1) fibronectin binding protein b(1) fnbB(1) fibronectin-binding protein homolog(1) LPXTG-motif cell wall anchor domain(1) LPXTG-motif cell wall anchor domain protein(1)

329 18 142 12.67605634 6 0.2463 >Cluster 614 acyl--CoA ligase(30) AMP-dependent synthetase and ligase(9) putative AMP-binding enzyme(6) acetate-CoA ligase(6) AMP-binding enzyme family protein(5) hypotheticalprotein(3) "acetyl-CoAsynthetase,putative(3)" Acyl-coenzyme A synthetase/AMP-(fatty) acid ligase(3) Acetyl- coenzyme A synthetase(2) putative acetyl-CoA synthetase(2) conserved hypothetical protein(1) AMP-binding enzyme(1) Acyl-coenzyme A synthetases/AMP-(fatty) acid ligase(1) "Acyl-coenzyme A synthetase/AMP-(fatty) acid ligase, YtcI-like protein(1)" ligase, AMP-dependent(1)"acetate--CoA ligase(1) synthetase/AMP-(fatty) acid ligase, YtcI(1)" protein(1) "Acyl-coenzyme A synthetases/AMP-(fatty) acid ligases, YtcI homolog(1)"acetyl-CoA synthetase(1)

330 4 142 2.816901408 2 0.2498 >Cluster 174 virulence-associatedEfamilyprotein(9) hypothetical protein(5) phage-like protein(4) virulence-associated protein E(3) "DNA primase, phage associated(3)" conserved hypothetical phage protein(3) truncated phage primase(2) hypothetical phage protein(2) Virulence-associated protein E(1) hypothetical bacteriophage protein(1) E family protein(1)

331 4 142 2.816901408 3 0.2498 >Cluster 1528 hypothetical protein(26) membrane protein(23) putative membrane protein(8) conserved hypothetical protein(8) Putative lipoprotein(4) fusaric acid resistance protein-like family protein(2) Fusaric acid resistance protein family protein(2) probable membrane protein(1) Integral membrane protein(1) protein of unknown function DUF939(1) hypothetical membrane"acetate-CoA"Acyl-coenzyme AAMP-bindingprotein(1) Putative lipoprotein SAV1865(1) fusaric acid resistance family protein(1) "lipoprotein, putative; Hypothetical protein(1)"

332 4 142 2.816901408 3 0.2498 >Cluster 1622 2-isopropylmalate synthase(36) hypothetical protein(12) HMGL-like family protein(8) isopropylmalate synthase-related protein(4) 2-isopropylmalate synthase-like protein(3)putative 2- isopropylmalate synthase(3) "2-isopropylmalate synthase, putative(2)" MW0859(1) FIG01108309: hypothetical protein(1)conserved hypothetical protein(1) HMGL-like protein(1) putative hMGL-like domain protein(1) Hypothetical protein(1)

333 4 142 2.816901408 4 0.2498 >Cluster 1682 hypothetical protein(40) membrane protein(19) conservedhypotheticalprotein(8) Hypothetical protein(2) membrane protein(1) Putative membrane associated protein(1)putativemembraneprotein(7) Hypothetical protein SAV1846(1) probable protein of unknown function DUF445(1)

334 4 142 2.816901408 2 0.2498 >Cluster 3435 3-dehydroquinate dehydratase(50) "3-dehydroquinate dehydratase,typeI(9)" 3-dehydroquinatedehydrataseI(8)putative type I 3-dehydroquinase(6) type I 3-dehydroquinate dehydratase(2) MW0782(1) 3-dehydroquinase(1) hypotheticalprotein(1) 3-dehydroquinatedehydrataseIAroCI(1)

335 4 142 2.816901408 2 0.2498 >Cluster 3440 geranylgeranylglyceryl phosphate synthase(21) geranylgeranylglyceryl/heptaprenylglyceryl phosphate synthase(9) PcrB family protein(8) geranylgeranylglyceryl phosphate synthase family protein(8) (S)-3-O-geranylgeranylglyceryl phosphate synthase(6) Heptaprenylglyceryl phosphate synthase(6) putative glycerol-1-phosphate prenyltransferase(5) geranylgeranylglyceryl phosphate synthase-like protein(3) conserved hypothetical protein(3) pcrB protein(2) Geranylgeranylglyceryl phosphate synthase-like protein YerE(2) geranylgeranylglyceryl phosphate synthase family family protein(1) protein PcrB(1) PcrB family replication protein(1) PcrB-like replication protein(1) geranylgeranylglyceryl phosphate synthase family protein PcrB(1) Putative glycerol-1-phosphate prenyltransferase(1) Geranylgeranylglyceryl diphosphate synthase(1)

336 4 142 2.816901408 2 0.2498 >Cluster 3795 hypothetical protein(52) putative lipoprotein(10) conserved hypothetical protein(4) lipoprotein(3) putative lipoproteinprecursor(2) "lipoprotein, putative(2)" uncharacterized lipoprotein SA2158(1) hypothetical lipoprotein(1) MW2289(1)putative TpgX-like protein(1) lipoprotein(1) Hypothetical protein(1) to TpgX(1)TpgX protein(1) Membrane hypothetical protein similar

337 7 142 4.929577465 283 protein map(31) Extracellular adherence protein of broad specificity Eap/Map(7) map-ND2C protein(4) hypothetical protein(4) map protein(3) truncated cell surface protein map- w(3) MHC class II-like protein(2) "major histocompatibility complexclassIIanalogprotein,Map(2)" map-likeprotein(2) membrane protein(2) MHC class II analog protein(2) "map protein,programmed(2)" truncatedmap-wprotein;extracellular adherence protein of broad specificity Eap/Map(1) cell surface protein(1) Outer membrane protein(1)cell surface protein MapW2(1) "major histocompatibility complex class II -like protein, Map(1)" truncated map-w protein(1) MHC class II analog(1) MHC class II antigen-like protein(1) 65 kDa membrane protein(1) Extracellular adherence protein(1) MAP domain protein(1) adherence protein Eap/Map(1) 338 7 142 4.929577465 4 0.2694 >Cluster 767 pyridine nucleotide-disulfide oxidoreductase(36) hypothetical protein(22) FAD-NAD(P)-binding family protein(6) conservedhypotheticalprotein(5)Hypotheticalprotein(3) Pyridine nucleotide-disulfide oxidoreductase(1) uncharacterized protein-like protein(1) Hypothetical protein SAV1772(1) pyridine nucleotide-disulphide oxidoreductase(1) "NAD(P) binding protein,pyridine nucleotide-disulfide oxidoreductase familyPutative exported protein(1)

339 7 1213 glutamate-1-semialdehyde aminotransferase(37) "glutamate-1-semialdehyde-2,1- aminomutase(15)" "glutamate-1-semialdehyde 2,1-aminomutase(11)" Glutamate-1-semialdehyde aminotransferase(8) "Glutamate-1- semialdehyde 2,1-aminomutase 1(6)" "glutamate-1-semialdehyde 2,1-aminomutase 1(2)" "Glutamate-1-semialdehyde 2,1-aminomutase(1)"

340 10 142 7.042253521 3 0.2729 >Cluster 2045 alcohol dehydrogenase(30)zinc-type alcohol dehydrogenase-like protein(10) putative zinc-binding dehydrogenase(6) Zinc-type alcohol dehydrogenase-like protein(6) conserved hypothetical protein(3) alcohol dehydrogenase zinc-binding type 1(3) zinc-type alcohol dehydrogenase(2) zinc-binding dehydrogenase(2)hypotheticalprotein(2) zinc-containingalcohol dehydrogenase(2) Bifunctional protein: zinc-containing alcoholputative(1)"protein(1)142 4.929577465 4 0.2694 >Cluster2 0.2694 >Clusterdehydrogenase(2) Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase); Similar to argi... at 98.00%(1) "alcohol dehydrogenase, zinc-binding domain protein(1)" MW2112(1) Bifunctional protein: zinc- containing alcohol dehydrogenase; quinone oxidoreductase(1)"alcohol dehydrogenase, zinc-containing(1)" Alcohol dehydrogenase. zinc- binding type 1(1) arginate lyase-family protein(1) zinc- binding alcohol dehydrogenase family protein(1) Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase)(1) zinc-binding alcohol dehydrogenase family family protein(1) zinc-containing alcohol dehydrogenase - quinone oxidoreductase(1)possible alcohol dehydrogenase(1) Alcohol dehydrogenase zinc-binding type 1(1)

341 11 142 7.746478873 4 0.2812 >Cluster 3540 phosphoribosylformylglycinamidine synthase(28) phosphoribosylformylglycinamidine synthase I(21) putative phosphoribosylformylglycinamidine synthaseI(10) "Phosphoribosylformylglycinamidine synthase, glutamine amidotransferase subunit(8)" Phosphoribosylformylglycinamidine synthase 1(6) phosphoribosylformylglycinamidine synthase 1(2) "Phosphoribosylformylglycinamidine synthase,glutamine amidotransferase subunit(2)" phosphoribosylformylglycinamidine synthase I PurQ(1) Phosphoribosylformylglycinamidine synthase(1)phosphoribosylformyl glycinamidine synthase 1 PurQ(1)phosphoribosylformylglycinamidine synthase subunit I(1)

342 28 142 19.71830986 12 0.2905 >Cluster24 peptidase(30) putative cell-wall-anchored protein SasC(LPXTG motif)(7) sasC/Mrp/FmtB intercellular aggregation domain protein(6) cell wall surface anchor family protein(5)cell-wall- anchored protein SasC(4) putative surface anchored protein(4) Surface protein C(3) LPXTG-motif cell wall anchor domain protein(3) Extracellular matrix binding protein(2) LPXTG surface protein(2) conserved hypothetical protein(2) FmtB- protein(2) Predicted cell-wall-anchored protein SasC (LPXTG motif)(2) Mrp protein(2) cell surface anchored protein(2) surface anchored protein(1) FmtB-like cell wall anchored protein(1) SasC/Mrp/FmtB intercellular aggregation domain-containing protein(1) LPXTG-motif cell wall anchor domain(1) lpxtg-motif cell wall anchor domain(1) MW1699(1) "Gram- positive signal peptide protein, YSIRK family(1)"

343 22 142 15.49295775 8 0.2986 >Cluster51 mannosyl-glycoprotein endo-beta-N-acetylglucosamidase(30)bifunctional autolysin(8)Bifunctional autolysin(7)bifunctional autolysin precursor(7) "bifunctional N-acetylmuramoyl-L-alanine amidase/endo-beta-N-acetylglucosaminidase, Atl(4)" "Phage lysin, N- acetylmuramoyl-L-alanine amidase(4)" mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase(3) autolysin(3) Bifunctional autolysin Atl / N-acetylmuramoyl-L-alanine amidase/ endo-beta-N- acetylglucosaminidase(2) "autolysin, N-acetylmuramyl-L-alanine amidase and endo-b-N-acetylglucosaminidas(2)" N-acetylmuramoyl-L-alanine amidase family protein(1)bifunctional N-acetylmuramoyl-L-alanine amidase/mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase(1) "bifunctional autolysin precursor, putative(1)" Peptidoglycan endo-beta-

N-acetylglucosaminidase(1) autolysin Atl(1) Bifunctional autolysin Atl / N-acetylmuramoyl-L- alanine amidase(1) Bifunctional autolysin Atl / N-acetylmuramoyl-L-alanine amidase / Endo-beta-N- acetylglucosaminidase(1) N-acetylmuramoyl-L-alanine amidase(1) partial autolysin(1)

344 16 142 11.26760563 8 0.3044 >Cluster18 peptide synthetase(18) non-ribosomal peptide synthetase(15) putative non-ribosomal peptide synthetase(10) thioesterreductase domain protein(8) aureusimine non-ribosomal peptide synthetase(4) gramicidin S synthetase 2 related protein(4) surfactin/siderophore synthetase(4) Malonyl CoA-acyl carrier protein transacylase(4) putative peptide synthetase(2) Long-chain- fatty-acid--CoA ligase(2)conserved hypothetical protein(2) amino acid adenylation domain protein(1) nonribosomal peptide synthetase ausA(1) hypothetical protein(1) thioester reductase domain(1) non-ribosomal peptide-like protein and polyketide synthesis(1)AMP-binding enzyme(1) MW0153(1) synthetase, putative(1)"

345 16 142 11.26760563 phage tape measure protein(7)"non-ribosomal peptide4 0.3044 >Cluster68hypothetical protein(5) putative membrane protein(3) membrane protein(2) Phage taillength tape-measure protein(2) phage tail length tape-measure protein(1) hypothetical phage-related protein(1) putative tape measure protein(1)

346 12 142 8.450704225 4 0.3052 >Cluster 1781 hypothetical protein(18) membrane protein(17) PIN domain protein(11) putative membrane protein(8) PIN and TRAM-domain containing protein(5) Integral membrane protein (PIN domain superfamily)(4) conserved hypothetical protein(3) Membrane-associated protein containing RNA-binding TRAM domain and ribonuclease PIN- domain(3) PilT protein domain-containing protein(2) Pili retraction protein pilT(2) PIN-YacL domain protein(1)PilT domain-containing protein(1) PIN/TRAM domain-containing protein(1) N-terminal deoxyribonuclease(1) Putative membrane associated protein(1) "Membrane-associated protein containing RNA- binding TRAM domain and ribonuclease PIN-domain, YacL(1)" PilT protein domain protein(1)probable membrane protein(1)

347 8 142 5.633802817 5 0.3077 >Cluster67 pyruvate carboxylase(57) putative pyruvate carboxylase(11)Pyruvatecarboxylase(8) Pyruvatecarboxyltransferase(4)

348 8 142 5.633802817 2 0.3077 >Cluster 1186 hypothetical protein(30) HD domain protein(10) "dNTP triphosphohydrolase, putative(8)" putative phosphohydrolase(7) conserved hypothetical protein(5) metal-dependent phosphohydrolase(4)HD domain-containing protein(3) Metal-dependentphosphohydrolase(3) phosphohydrolase(2) triphosphohydrolase(2) putative(1)" DGTP triphosphohydrolase(1) deoxyguanosinetriphosphate triphosphohydrolase(1) metal-dependent phosphohydrolase HD sub domain(1)phosphohydrolase(3) metal dependent Deoxyguanosinetriphosphate "metal-dependent HD-motif phosphohydrolase,

349 5 142 3.521126761 2 0.3194 >Cluster 445 glucosamine--fructose-6-phosphate aminotransferase(29) glutamine--fructose-6-phosphate aminotransferase(11) glutamine- fructose-6-phosphate transaminase(7) Glucosamine--fructose-6- phosphate aminotransferase(5) glucosamine-fructose-6-phosphate aminotransferase(5) Glucosamine--fructose-6-phosphate aminotransferase (5) Glucosamine--fructose-6-phosphate aminotransferase (isomerizing)(5) glucosamine--fructose-6-phosphate aminotransferase (4) glutamine-fructose-6-phosphate transaminase (isomerizing)(3) Glutamine--fructose-6-phosphate

aminotransferase(3) "glucosamine--fructose-6-phosphate aminotransferase, isomerizing(2)" "glucosamine--fructose-6-phosphate aminotransferase, putative(1)" glutamine--fructose-6-phosphate transaminase (isomerizing)(1) "glucosamine-fructose-6-phosphate aminotransferase, isomerizing(1)"

350 5 142 3.521126761 2 0.3194 >Cluster 629 phosphoenolpyruvate carboxykinase(36) phosphoenolpyruvate carboxykinase(11) hypotheticalprotein(8) Phosphoenolpyruvate carboxykinase(8) phosphoenolpyruvate carboxykinase (ATP)(7) Phosphoenolpyruvate carboxykinase (ATP)(6)Phosphoenolpyruvate carboxykinase (4) ATP-dependent phosphoenolpyruvate carboxykinase(1)

351 5 142 3.521126761 2 0.3194 >Cluster 2047 abi-like family protein(3) CAAX protease(3) abi-like protein(2) hypotheticalprotein(2) hypotheticalproteinin superantigen-encoding pathogenicity islands SaPI(1) Abi-likeprotein(1)

352 5 142 3.521126761 4 0.3194 >Cluster 2095 sphingomyelin phosphodiesterase(21) phospholipase(19)phosphodiesterase(18) Beta-hemolysin(17) truncated beta-hemolysin(10) phospholipase C(9) phospholipase C domain protein(7) beta-hemolysin(6) phospholipase C precursor(4) Phospholipase C(4) hypothetical protein(3) truncated beta-hemplysin(3) truncated betahemolysin(2) truncated(2)" (truncated)(1) hypothetical phage protein(1) Phospholipase C precursor (truncated)(1) truncated 6449-6450 beta-hemolysin(1)"Sphingomyelin phosphodiesterase, Beta-hemolysin, Phospholipase C precursor(1) phospholipase C phospholipase C precursor (pseudogene)(1) conserved

353 5 142 3.521126761 2 0.3194 >Cluster 2129 1-phosphatidylinositol phosphodiesterase(57) Phosphatidylinositol-specific phospholipase C(8) phosphatidylinositol-specific phospholipase C(4) 1- phosphatidylinositol phosphodiesterase domain protein(2) 1- phosphatidylinositol phosphodiesterase precurosr(2) conserved hypotheticalprotein(1) Phosphatidylinositolphosphodiesterase(1) "Phosphatidylinositol-specific phospholipase C,Xdomainprotein(1)" Phosphatidylinositoldiacylglycerol-lyase(1) phosphatidylinositol diacylglycerol-lyase(1) 1-phosphatidylinositol phosphodiesterase precursor(1) 1- phosphatidylinositol phosphodiesterase (Phosphatidylinositol diacylglycerol-lyase) (Phosphatidylinositol-specific phospholip>(1) "1- phosphatidylinositol phosphodiesterase precursor, putative(1)"

354 5 142 3.521126761 2 2168 2-oxoisovalerate dehydrogenase(30) dehydrogenase E1 component beta subunit(11) dehydrogenase E1 component, beta subunit(7)" dehydrogenase E1 component subunit beta(7)Pyruvate dehydrogenase E1 component subunit beta(6)pyruvate dehydrogenase E1 component beta subunit(5) "putative pyruvate dehydrogenase E1 component,beta subunit(2)" "pyruvate dehydrogenase E1 component subunit beta (Scomplex, 36 kDa subunit)(2)" putative pyruvate dehydrogenase E1 component subunit beta(2)pyruvate dehydrogenase E1 component subunit beta protein(2) "pyruvate dehydrogenase E1 component, beta subunit(1)" "pyruvate dehydrogenase complex, E1 component,

pyruvate dehydrogenase beta subunit, putative(1)" Transketolase domain protein(1) pyruvate dehydrogenase (acetyl-transferring) beta subunit(1) "pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase beta subunit PdhB(1)" "Transketolase, C- terminal domain protein(1)" "pyruvate dehydrogenase complex E1 component, beta subunit(1)"0.3194 >Cluster Pyruvate "putative pyruvate pyruvate

355 5 142 3.521126761 2 0.3194 >Cluster 2170 HrcA family transcriptional regulator(27) heat-inducible transcription repressor HrcA(20) Heat-inducible transcription repressor HrcA(13) heat-inducible transcription repressor(9) heat-inducible transcriptional repressor(2) heat-inducible transcription repressor hrcA(2) Heat-inducible transcriptional repressor(2) Heat-inducible transcription repressor(2) heat shock transcriptional repressor HrcA(1) Heat-inducible transcription repressor hrcA(1)

356 5 142 3.521126761 2 0.3194 >Cluster 2216 iron-dicitrate transporter subunit FecD(30) FecCD transport family protein(8) "Heme ABC type transporter HtsABC, permease protein HtsC(6)""iron (Fe3+) ABC superfamily ATP binding cassette transporter, membrane protein(6)"iron-dicitrate ABC transporterpermease(4) fecCDtransportfamilyprotein(3)ABC3 transport family protein(3) Iron(III) dicitrate transport system permease protein fecD(2) Fe(3+)-citrate import system permease protein yfmE(2) cobalamin Fe3+-siderophores ABC transporter permease(2) transport system permease(2) iron(III) dicitrate transport system permease protein FecD(1) MW2101(1) "FecCD iron compound ABC transporter,permeasefamilyprotein(1)" fe(3+)dicitratetransport system permease protein fecD(1) conserved hypothetical protein(1) hemetransportsystempermease(1)hypotheticalprotein(1) ferrichrome ABC transporter(1) iron(III) dicitrate transport system permease protein fecD(1) "iron chelate uptake ABC transporter, FeCT family, permease protein(1)" transport system permeaseprotein(1) "iron compound ABC transporter, permease protein(1)"

357 5 142 3.521126761 2 0.3194 >Cluster 2341 primosomal protein DnaI(46) putative primosomalprotein(12) protein(3) loader DnaI(2) like protein(1) Primosomal DnaI domain protein(1) primosomal DnaI family protein(1) conserved hypothetical protein(1) primosomal protein dnaI(1)HelicaseloaderDnaI(8) istB-likeATPbindingfamily primosomal protein(3) replicative DNA helicase Primosomal protein dnaI(2) primosomal DnaI-

358 5 142 3.521126761 2 0.3194 >Cluster 3121 hypothetical protein(26) membrane protein(16) putative membrane protein(7) sulfite exporter TauE/SafE-like protein(7) sulfite exporter TauE/SafE family protein(7) conserved hypotheticalprotein(5) PutativemembraneproteinYfcA(2)probable membrane protein(1) FIG011065: hypothetical

protein(1) "permease,putative(1)" proteinofunknownfunction DUF81(1) transporter(1) Hypothetical protein(1) Putative membrane spanning protein(1) Sulfite exporter TauE/SafE-like protein(1)

359 5 142 3.521126761 2 0.3194 3169 hypothetical protein(8) tandem lipoprotein(1) tandem lipoprotein(1) "lipoprotein, putative(1)">Clusterputative sa

360 5 142 3.521126761 5 0.3194 3709 potassium transporter Trk(30) Trk system potassium uptake protein TrkA(7) putative potassium transport protein(7) Ktr system potassium uptake protein C(5) NAD+ binding potassium transporter(4) conserved hypothetical protein(4) ktr system potassium uptake protein A(4) NADH(P)-binding family protein(3) TrkA potassium uptake family protein(2) Trk system potassium uptake protein trkA(2) K+ transport system NAD-binding component(2) TrkA-N domain-containing protein(2) Potassium uptake protein ktrA(1) "possible Trk family potassium (K+) transporter, NAD+ binding protein(1)" "TrkA family potassium uptake protein, putative(1)"pottasium uptake protein KtrA(1) TrkA N-terminal domain protein(1) TrkA-N domain protein(1) potassium uptake protein(1)potassium uptake protein TrkA(1) trkA-C domain protein(1)

361 5 142 3.521126761 2 0.3194 >Cluster 3895 guanylate kinase(57) Guanylate kinase(16) putative guanylatekinase(5) hypotheticalprotein(1) guanosine monophosphatekinase(1) guanylatekinasehomolog(1) gmk(1)

362 5 142 3.521126761 2 0.3194 >Cluster 4609 phosphinothricin acetyltransferase(30) Phosphinothricin N- acetyltransferase(9) acetyltransferase (GNAT) family protein(7) acetyltransferase family protein(3) putative phosphinothricin N- acetyltransferase(3) "Acetyltransferase, GNAT family protein(3)" "acetyltransferase, GNAT family(3)" GCN5 N- acetyltransferase(2) acetyltransferase domain protein(2) "acetyltransferase, GNAT family protein(2)" phosphinothricin N-acetyltransferase(2) putative antibiotic resistance(2) acetyltransferase(2) GNAT family acetyltransferase(2) GCN5-related N-acetyltransferase(1) AcetyltransferaseGNATfamilyprotein(1) "sortase-related acyltransferase, putative(1)" MW2449(1) probable phosphinothricin N-acetyltransferase(1) hypothetical protein(1) conserved hypothetical protein(1) hypothetical acetyltransferase YncA(1)

363 5 142 3.521126761 2 0.3194 4623 hypothetical protein(17) Hypothetical protein(1) low-complexity protein(1)putative exported protein(1) hypotheticalprotein(1) putativelow-complexityprotein(1)>Cluster>Clusterconservedconserved

364 5 142 3.521126761 2 0.3194 >Cluster 4970 50S ribosomal protein L11(58) ribosomal protein L11(14)LSU ribosomal protein L11p (L12e)(8) LSU ribosomal protein L11P(1)

365 5 142 3.521126761 2 0.3194 >Cluster 5014 formyl peptide receptor-like 1 inhibitory protein(35) FPRL1 inhibitory protein(13) hypothetical protein(11) conserved hypotheticalprotein(3) FPRL1familyinhibitoryprotein(2) FIG017917: hypothetical protein(1) fprl1 inhibitory protein (flipr)(1) formyl peptide receptor-like 1 inhibitory domain protein(1) FPRL1 inhibitory protein (FLIPr)(1)Hypothetical protein(1)

366 5 142 3.521126761 2 0.3194 >Cluster 5269 hypothetical protein(53) putative membrane protein(7) conserved hypothetical protein(7) Inner membrane protein YbaN(2) conserved hypothetical membrane protein(2)Putative membrane spanning protein(1) Hypothetical protein DUF454(1) putative heme utilization protein(1) hypothetical protein related to heme utilization(1) Hypothetical protein(1) FIG039061: hypothetical protein

related to heme utilization(1) membrane spanning protein(1)hypothetical membrane protein(1) protein of unknown function DUF454(1)

367 5 142 3.521126761 2 0.3194 >Cluster 5532 hypothetical protein(15) phage protein(13) Phage protein(4) conserved hypothetical protein(3) "Hypothetical protein, PVL orf39(1)" hypothetical phage-related protein(1) putative phage protein(1)protein of unknown function DUF1108(1)3.521126761 2 0.3194 >Cluster "holin, phage phi LC3 family(10)" Phage holin protein(2) bacteriophage holin(1) hypotheticalprotein(1) phage-associated

368 5 142 5786 holin(34) holin(9) phage holin(7) Holin(1)MW1887(1) holin(1) holin homolog(1)

369 8 142 374 hypothetical protein(10) Putative major teichoic acid biosynthesis protein C(8)minor structural protein(7) putative minor structural protein(6) phage protein(5) phi ETA orf 56-like protein(5) conserved hypothetical protein(3) putative phage5.633802817 3 0.3285 >Clusterprotein(1) putative minor tail protein(1) related protein(1)

370 8 142 5.633802817 4 1729 mannitol-1-phosphate 5-dehydrogenase(50) phosphate 5-dehydrogenase(16) putative mannitol-1-phosphate 5-dehydrogenase(10) mannitol dehydrogenase domain protein(1) "mannitol-1-phosphate 5-dehydrogenase, putative(1)"Mannitol dehydrogenase domain(1)

371 8 142 5.633802817 2 0.3285 >Cluster 4042 protein-disulfide isomerase(35) putative lipoprotein(10) "Protein-disulfide isomerase, related to DsbA(6)" DSBA-like thioredoxin domain protein(4) thioredoxin-like domain protein(3) conserved hypothetical protein(3) DSBA-like thioredoxin domain-containing protein(3) lipoprotein(2) "Protein- disulfide isomerase, DsbA-like protein(2)"conserved hypothetical secreted protein(2) Disulfide bond formation protein D precursor(2) "chain A, Crystal Structure Of Staphylococcus aureus subsp. aureus ECT-R 2 Dsba(1)" Protein-disulfide

isomerase(1) "disulfide bond protein A, putative(1)" Thiol:disulfide interchange protein dsbA(1) probablelipoprotein(1)lipoprotein(1)hypothetical protein(1) possible "lipoprotein, putative(1)"

372 13 142 9.154929577 6 0.335 >Cluster 202 recombination and DNA strand exchange inhibitor protein(35) Recombination inhibitory protein MutS2(9) MutS2 protein(8) mutS2 protein(7) MutS family DNA mismatch repair protein(6) endonuclease MutS2(3) MutS-like protein(2) DNA mismatch repair MutS2familyprotein(2) DNAmismatchrepairprotein(1)Endonuclease MutS2(1) mutS domain V family protein(1) MutS2 familyprotein(1) mutS2domainprotein(1) recombinationand DNA strand exchange inhibitorprotein(1) smr domain protein(1) recombination and DNA strand exchange inhibitor(1) DNA mismatch repair protein mutS(1)

373 9 142 6.338028169 4 0.3414 >Cluster 1212 zinc protease(29) peptidase M16 inactive domainprotein(10) protease(8) peptidase(4) hypotheticalprotein(2) M16Csubfamilypeptidase(1) putative peptidaseM16inactivedomainprotein(1) Zincprotease(1)probable protease (zinc) protein(1) "FIG009210: peptidase, M16 family(1)" MW1162(1) peptidase M16 family protein(1) conserved hypothetical protein(1) insulinase (Peptidase family M16)"peptidase, M16 family(8)" putative "zinc-dependent peptidase, M16 family(4)" M16 family putative zinc protease(3)peptidase M16(2)hypothetical phage-0.3285 >Cluster Mannitol-1-family protein(1) peptidase M16 domain protein(1) "zinc- dependent peptidase, putative(1)" "M16 family peptidase, putative(1)"

374 9 142 6.338028169 3 0.3414 >Cluster 1347 phage tail protein(7) tail protein(6) phage tail fiber(6)hypotheticalprotein(5) Phagetailfiber(4) phagetail fiber protein(3) putative tail fiber(2) Tail fiber protein(2) tail fiber(2) "phage tail fiber protein, putative(2)" collagentriple helix repeat family protein(1) phage protein(1) Tail fiber protein (1)

375 9 142 6.338028169 3083 DNA replication protein DnaC(10)DNA replication protein dnaC(4) protein(3) hypotheticalprotein(2) protein(2) DnaC protein(2) DNA replication protein(2) Transposase-associated ATP/GTP binding protein(2) istB-like ATP binding family protein(2)"DNA replication protein dnaC, contains dnaC domains, no evidence for helicase(2)" phage protein(2) dnaC(1) IstB-like ATP-binding protein(1) conserved DnaC-like protein(1) DnaC(1) phage like DNA replication protein dnaC(1)

376 9 142 6.338028169 4 0.3565 >Cluster 979 ATP-dependent DNA helicase RecQ(19) recombinase RecQ(13) "ATP-dependent DNA helicase, RecQ family protein(8)" DEAD/DEAHboxhelicasefamilyprotein(7) ATP-dependentDNA helicase(7) ATP-dependent DNA helicase RecS (RecQ family)(4) ATP-dependent DNA helicase RecQS-like protein(3) "ATP-dependent DNA helicase, RecQ family(3)"DEAD/DEAH box helicase(3)ATP-dependent DNA helicase recQ(3) probable ATP-dependent DNA helicaseRecQ(2) "ATP-dependent DNA helicase RecQ, putative(2)" ATP- dependent DNA helicase RecQ domain protein(1) ATP-dependent DNA helicase RecS(1) conserved hypothetical protein(1) ATP-dependent helicase RecQ(1) Probable ATP-dependent DNA helicase RecQ(1) probable ATP-dependent DNA helicase(1) dead/deah box helicase family protein(1)

377 9 142 6.338028169 4 0.3565 >Cluster 2446 LysR family transcriptional regulator(33) LysR family regulatory protein(22) "bacterial regulatory helix-turn-helix, lysR family protein(4)" lysR substrate binding domainprotein(4) "transcriptional regulator, LysR family(2)" transcriptional regulator(2) LysR family HTH-type transcriptional regulator(2) "bacterial regulatory helix-turn-helix protein, lysR family protein(1)" "LysR family transcriptional regulator, putative(1)" hypothetical protein(1) conserved hypothetical protein(1) truncated hypothetical protein(1) LysR-family regulatory protein(1) LysR substrate binding domain protein(1)tail fiber protein(1)2 0.3414 >Cluster phage DnaC-like protein(4) conserved hypothetical putativephageDnaC-likeLysR substrate-binding(1)MW2253(1) LysR family protein(1)"

378 5 142 3.521126761 DNA polymerase III PolC(19)"Transcriptional regulator,2 0.3578 >Cluster43"DNA polymerase III, alpha subunit, Gram-positive type(13)" DNA polymerase III PolC-type(10) DNApolymerase III subunit alpha(9)DNA polymerase III(6)PolC-type DNA polymerase III(3) type(3) DNA polymerase III polC-type protein(2) "DNA polymerase III, alpha subunit(1)" DNA-directed DNA polymerase III alpha

subunit(1) "Gram-positive type DNA polymerase III, alpha subunit(1)" OB-fold nucleic acid binding domain protein(1) helix-hairpin-helix motif family protein(1) bacterial DNA polymerase III alpha subunit(1)

379 5 142 3.521126761 2 0.3578 >Cluster 564 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase(35) 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1- carboxylic-acid synthase(12) putative menaquinone biosynthesis bifunctionalprotein(6) 2-succinyl-5-enolpyruvyl-6-hydroxy-3- cyclohexene-1-carboxylic-acid synthase(6) menaquinone biosynthesis protein(4) 2-succinyl-5-enolpyruvyl-6-hydroxy-3- cyclohexene-1- carboxylatesynthase(4) "2-succinyl-6-hydroxy-2,4-cyclohexadiene-1- carboxylic acid synthase/2-oxoglutarate decarboxylase(3)" 2- oxoglutarate decarboxylase(2) "2-succinyl-6-hydroxy-2,4- cyclohexadiene-1-carboxylate synthase(2)" "2-succinyl-6-hydroxy-2, 4- cyclohexadiene-1-carboxylic acid synthase/2-oxoglutarate decarboxylase(1)" putative 2-succinyl-5-enolpyruvyl-6- hydroxy-3-cyclohexene-1-carboxylic-acid synthase(1) "2- succinyl-6-hydroxy-2, 4-cyclohexadiene-1-carboxylic acid synthase(1)"2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase family protein(1) 2-succinyl-5-enolpyruvyl-6- hydroxy-3-cyclohexen e-1-carboxylate synthase(1) "2-succinyl-6- hydroxy-2,4-cyclohexadiene-1-carboxylic acid synthase(1)" Menaquinone biosynthesisprotein(1) "thiaminepyrophosphateenzyme,C-terminal TPP binding domain protein(1)"

380 5 142 3.521126761 2 0.3578 >Cluster 807 cardiolipin synthase(20) phospholipase D(16) Cardiolipin synthetase(12) putative cardiolipin synthetase(7) phospholipase D/Transphosphatidylase(6) Cardiolipin synthase(6) cardiolipin synthetase(5)cardiolipin synthetase 2(2) cardiolipin synthetase (Cardiolipin synthase) (CLsynthase)(2) type 1 cardiolipin synthetase 2(1) Cardiolipin synthetase 2(1) phospholipase D-nucleasedomainprotein(1) hypotheticalprotein(1) MW2011(1)phospholipase D domain protein(1) "cardiolipin synthetase, putative(1)" phospholipase(1)

381 5 142 3.521126761 2 0.3578 >ClusterDNA polymerase III alpha subunit(8)DNA polymerase III polC-type(5)DNA polymerase III alpha chain PolC-1034 cytochrome D ubiquinol oxidase subunit I(33) Cytochrome d ubiquinol oxidase subunit I(10) bacterial Cytochrome Ubiquinol Oxidase family protein(8)putative cytochrome ubiquinol oxidase(6) cytochrome ubiquinol oxidase(4) "cytochrome bd ubiquinol oxidase, subunit I(3)" cytochrome bd-type quinol oxidase subunit 1(2) cytochrome Ubiquinol Oxidase(2) Cytochrome d ubiquinol oxidase subunit 1-like protein(2)cytochrome d ubiquinol oxidase subunitI(2) "cytochrome d ubiquinol oxidase, subunit I, putative(2)" cytochrome bd ubiquinol oxidase subunit I(1) "cytochrome D ubiquinol oxidase, subunit I(1)" MW0968(1) cytochrome D ubiquinol oxidase subunit 1 homolog(1) "cytochrome d ubiquinol oxidase, subunit I(1)" bacterial cytochrome ubiquinol oxidase(1)

382 5 142 3.521126761 2 1324 hypothetical protein(32) Peptidase T(9) peptidase(7) M20/M25/M40 family peptidase(5) peptidase(4) peptidase dimerization domain protein(3) peptidase M20/M25/M40 family protein(3) M42 glutamyl aminopeptidase family protein(3) peptidase T-like protein(2) Tripeptidase T(2)"peptidase T, putative(2)" peptidase T(1) conserved hypotheticalprotein(1) peptidasefamilyM20/M25/M40family protein(1) MW1465(1) probable peptidase T(1)

383 5 142 3.521126761 2 0.3578 >Cluster 1787 peptide ABC transporter ATP-binding protein(30) Oligopeptide transport ATP-binding protein OppD(10) "oligopeptide/dipeptide ABC transporter, ATP-binding, C-terminal domain protein(6)" putative oligopeptide transport ATP-binding protein(6) oligopeptide transport ATP-binding protein OppD(5) "oligopeptide ABC superfamily ATP binding cassette transporter, ABC protein(4)" Oligopeptide transport ATP-binding protein oppD(2) oligopeptide ABC transporter ATP-binding protein OppD(2) oligopeptide/dipeptide ABC transporter(2) "oligopeptide/ dipeptide ABC transporter, ATPase subunit(1)" "oligopeptide/ dipeptide ABC transporter, ATP-binding, domain protein(1)" ABC transporter family protein(1) Oligopeptide transport ATP-binding protein OppD (TC 3.A.1.5.1)(1) oligopeptide transport system ATP- binding protein(1) "oligopeptide ABC transporter, ATP-binding protein(1)" oppD(1) Peptide/nickel transport system ATP-binding protein(1) conserved hypothetical protein(1) oligopeptide transport ATP-binding protein(1) oligopeptide transport system ATP- binding protein OppD homologue(1) "oligopeptide ABC superfamily ATP binding cassette transporter, ABC protein, Opp3D(1)"oligopeptide transport ATP-binding protein oppD(1) "oligopeptide transport system ATP-binding protein oppD, putative(1)"

384 5 142 3.521126761 2 0.3578 >Cluster 2184 type II secretion system protein E(31) hypothetical0.3578 >Cluster putative M20 familyprotein(8) protein(7) ComEA(5)" protein 1(4) like protein(3) protein comGA, putative(2)" conserved hypothetical protein(1) putative type II secretion system protein(1) MW1496(1) probable late competence protein(1) type II/IV secretion system protein(1) late competence protein comGA(1) competence protein ComGA homolog(1) comG operon protein 1. domain protein(1)type II/IV secretion system family "Late competence protein ComGA, access of DNA to "late competence protein, ComGA(4)" ComG operon competence protein ComGA(4) competence ComGAB- putative competence protein ComGA(2) "competence

385 5 142 3.521126761 2 0.3578 >Cluster 2218 glyoxalase(29) Glyoxalase family protein(11) putative dioxygenase(8) glyoxalase/Bleomycin resistance /Dioxygenase superfamily protein(6) Glyoxalase/bleomycin resistance protein/ dioxygenase(5) putative ring-cleaving dioxygenase mhqA(3)conserved hypothetical protein(3) dioxygenase(2) hypothetical protein(2) lactoylglutatione lyase(2) glyoxalase family protein(1) glyoxalase/Bleomycin resistance protein/Dioxygenase superfamily protein(1) glyoxylase family protein(1) possible lactoylglutatione lyase(1) "dioxygenase, putative(1)" glyoxylase(1) probable glyoxylase family protein(1)

386 5 142 3.521126761 2 0.3578 >Cluster 2613 RpiR family transcriptional regulator(34) phosphosugar- binding transcriptional regulator RpiR family protein(8) "Sialic acid utilization regulator, RpiR family(7)" putative transcription regulator(4) "helix-turn-helix domain, rpiR family protein(4)" SIS domain protein(3) RpiR family HTH-type transcriptional regulator(3) transcriptional regulator(3) regulator(2) "transcriptional helix-turn-helix protein RpiR(1) regulator RpiR family(1) "Transcriptional regulator, RpiR family protein(1)" "phosphosugar-binding transcriptional regulator, RpiR family(1)" probable transcriptional regulator(1) RpiR family regulatory protein(1) hypothetical protein(1) conserved hypotheticalprotein(1) "Transcriptionalregulator,RpiRfamily(1)" "RpiR famil transcriptional regulator, putative(1)"

387 5 142 3.521126761 2 0.3578 >Cluster 3084 hypothetical protein(6) chromosomal replication initiator protein DnaA(5) bacterial dnaA family protein(3) conserved hypothetical phage protein(2) Hypothetical protein(1) phi ETA orf 17-like protein(1) chromosomal replication initiator DnaA(1) conserved hypothetical protein(1) "Hypothetical protein, phi-ETA orf17 homolog (1)"RpiR family phosphosugar-binding phosphosugar-

binding transcriptional regulator, RpiR family(1)" MW2236(1) transcription

388 3 142 2.112676056 3 0.371 >Cluster 279 lipase(32) Triacylglycerol lipase(11) lipase 2(9)lipase precursor(9) Lipase 2(5) glycerol ester hydrolase(4) lipase 2 (Glycerol ester hydrolase 2)(3) lipase 2 precursor(3) triacylglycerol lipase(2)"lipase precursor, interruption-N(1)" glycerol ester hydrolase; Lipase precursor(1)truncated triacylglycerol lipase precursor(1) lipase 2 domain protein(1) Lipase(1)

389 3 142 2.112676056 3 0.371 >Cluster 504 multidrug ABC transporter ATP-binding protein(30) Lipid A export ATP-binding/permease protein MsbA(10) ABC transporterATP-binding protein(8) permease protein(7) permease protein(5) permease(3)" multidrug export ATP-binding/permease(2) Multidrug export ATP-binding/permease protein(2) Lipid A export ATP-binding/ permease MsbA(2) ATP-binding ABC transporter(1) lipid A export ATP- binding/permease protein MsbA(1) "Multidrug/protein/lipid ABC transporter family, ATP-binding and permease protein(1)" MW1806(1)"ABC transporter, ATP-binding protein(1)" "ABC superfamily ATP binding cassette transporter, membrane protein(1)" "ABC transporter, ATP-binding/permease protein, putative(1)" ABC transporter homolog(1) ABC transporter transmembrane region family protein(1) "ABC-type multidrug transport system, ATPase and permease component(1)" ABC transporter related(1)

390 3 142 2.112676056 2 0.371 >Cluster 728 amino acid permease(39) cationic amino acid transporter(9)amino acid permease family protein(9) putative amino acidputative multidrug export ATP-binding/Putative multidrug export ATP-binding/"toxin exporting ABC transporter,permease(8) transporter(5) protein(2) permease, putative(1)" probable membrane-embedded amino acidAPC family amino acid-polyamine-organocation amino acid transporter(2)conserved hypothetical aminoacidpermease-associatedregion(1) "aminoacidtransporter(1) Arginine permease(1) MW0539(1)

391 3 142 2.112676056 852 drug:proton antiporter(30) protein(10) putative transport system protein(8) hypotheticalprotein(7) "MFSfamilymajorfacilitatortransporter, multidrug :cation symporter(5)" drug resistance transporter EmrB/ QacA subfamily protein(3)Multidrug ef ux MFS-type transporter MdeA(2)drug resistance transporter(2) EmrB/QacA family drug resistance transporter(1)"EmrB-QacA subfamily drug resistance transporter, putative(1)"Hypothetical protein(1) "drug resistance transporter, EmrB/QacA subfamily(1)" Multidrug effux MFS-typehypothetical protein(1)3 0.371 >Cluster H+ antiporter-2 familytransporter MdeA(1) "drug resistance MFS transporter, drug:H+ antiporter-1 (14 Spanner) (DHA2) family protein(1)" MW2337(1)multidrug resistanc-related transport system protein(1) Multidrug resistance protein B(1) lincomycin resistance protein LmrB(1) "drug resistance MFS transporter, drug:H+ antiporter-2 family(1)" conserved hypothetical protein(1) FIG01107989: hypothetical protein(1)

392 3 142 2.112676056 2 0.371 >Cluster 853 D-alanine--poly(phosphoribitol) ligase subunit 1(22) alanine-phosphoribitol ligase(17) D-alanine-D-alanyl carrier protein ligase(11) D-alanine--poly(phosphoribitol) ligase(10)"D- alanine--poly(phosphoribitol) ligase, subunit 1(7)" "D-alanine-activating enzyme/D-alanine-D-alanyl, dltA protein(4)" D-alanine- activating enzyme(4) D-alanine-poly(phosphoribitol) ligase subunit 1(2) D-alanine--polyphosphoribitol ligase subunit1(1) D-alanine- activating enzyme dltA(1)long-chain-fatty-acid--CoA ligase(1) hypothetical protein(1)

393 3 142 2.112676056 3 0.371 1387 peptidase T(50) Tripeptide aminopeptidase(8) T(6) putative peptidase T(6) M20 family peptidase T(5) aminotripeptidase(3) peptidase T domain protein(1)T, putative(1)" Tripeptidase T(1)

394 3 142 2.112676056 2 0.371 2978 hypothetical protein(47) macro domain protein(13) hypotheticalprotein(7) macrodomain-containing protein(2) "COG2110, Macro domain, possibly ADP-ribose binding module(1)" unknown hypothetical protein(1) "putative phosphatase, high-affinity ADP-ribose binding module(1)" ADP-ribose binding module -like protein(1) putative ADP-ribose binding module protein(1) Macrodomainprotein(1) "Macrodomain,possiblyADP- ribose binding module(1)"ATPase associated with chromosome architecture/replication(1) Appr-1-p processing domain protein(1) protein in Tap1-dppD intergenic region(1) Hypothetical protein(1)

395 3 142 2.112676056 2 0.371 >Cluster 3405 hypothetical protein(35) DNA integration/recombination/ inversion protein(22) conserved hypothetical protein(10) Diacylglucosamine hydrolase like(4) Protein of unknown function DUF208(2) Diacylglucosamine hydrolase like protein(2)Putative cytosolic protein(1) "uncharacterized BCR, COG1636 family protein(1)" adenine nucleotide alpha hydrolases superfamily protein(1) protein of unknown function DUF208(1) Diacylglucosamine hydrolase like-protein(1) ncharacterized conserved protein(1)>ClusterPeptidase"peptidase>Clusterconserved

396 3 142 2.112676056 2 0.371 >Cluster 3416 HAD family hydrolase(30) haloacid dehalogenase-like hydrolase(11) 5'-nucleotidase YjjG(7) "HAD hydrolase, IA, variant 1 family protein(7)" L-2-haloalkanoic acid dehalogenase(5) hydrolase(4) hydrolase of the HAD superfamily(3) HAD-superfamily hydrolase(2) "haloacid dehalogenase-like hydrolase,putative(2)" protein(1) hydrolase(1) 1 family protein(1)" "HAD-superfamily hydrolase, subfamily IA, variant 1(1)" HAD superfamily hydrolase(1)hypothetical protein(1) conserved hypothetical probable haloacid dehalogenase "HAD-superfamily hydrolase, subfamily IA, variantMW0575(1) "HADhydrolase,familyIA,variant1(1)" Hydrolase (HAD superfamily)(1)

397 3 142 2.112676056 2 0.371 >Cluster 4963 peptidase M23B(16) peptidase M23(14) CHAP domain protein(8) Secretory antigen SsaA(8)putative exported protein(7)CHAP domain-containing protein(5) N-acetylmuramoyl-L-alanine amidase(4) putative secretory antigen(3) hypothetical protein(3) secretory antigen SsaA-like protein(2) secretory antigenSsaAhomolog(2) CHAPdomaincontainingprotein(1)secretory antigen ssaA2(1) Secretory antigen precursor SsaA(1) LysM domain protein(1) MW2465(1) secretory antigen SsaA(1)possible secretory antigen(1) "secretory antigen SsaA, putative(1)"

398 3 142 2.112676056 2 0.371 >Cluster 5922 hypothetical protein(38) iraB(24) conserved hypothetical protein(8) IraB(3) IraB protein(2) Hypothetical protein(2) FIG01107931: hypothetical protein(1)

399 3 142 2.112676056 2 0.371 >Cluster 5991 hypothetical protein(62) conserved hypothetical protein(11)Hypothetical protein possible

functionally linked with Alanyl- tRNA synthetase(4) protein of unknown function DUF965(1) hypothetical protein with DUF935(1) protein Stu1959(1) Putative cytosolic protein(1)

400 3 142 2.112676056 3 0.371 >Cluster 6168 hypothetical protein(68) conserved hypothetical protein(7) Hypotheticalprotein(3) FIG01108334:hypotheticalprotein(1)

401 6 142 4.225352113 3 0.3745 >Cluster 1010 isochorismate synthase(41) Menaquinone-specific isochorismate synthase(10) isochorismate synthase family protein(9) putative chorismate binding enzyme(7) menaquinone-specific isochorismate synthase(4) conserved hypotheticalprotein(2) probablemenaquinone-specificisochorismate synthase(1) "isochorismate synthase, putative(1)"Isochorismate synthase(1)MW0926(1) salicylate biosynthesis isochorismate synthase(1)isochorismate synthases family protein(1) hypothetical protein(1)

402 6 142 4.225352113 2 0.3745 >Cluster 1896 hypothetical protein(53) conserved hypothetical protein(9) putativemembraneprotein(7) Hypotheticalprotein(4) Telomeric repeat-binding factor 2(2) telomeric repeat-binding factor 2 family protein(1) probable membrane protein(1) putative lipoprotein with DUF4352(1) Hypothetical protein SAV1800(1)

403 6 142 4.225352113 2 0.3745 >Cluster 2147 diphosphomevalonate decarboxylase(48) mevalonate diphosphate decarboxylase(21) Diphosphomevalonate decarboxylase(10) "mevalonate diphosphate decarboxylase, putative(1)"

404 6 142 4.225352113 3 0.3745 >Cluster 3478 phosphoribosylaminoimidazole-succinocarboxamide synthase(30)phosphoribosylaminoimidazolesuccinocarboxamide synthase(20)Phosphoribosylaminoimidazole-succinocarboxamide synthase(14)putative phosphoribosylaminoimidazole-succinocarboxamide synthase(11)purC(1) "Phosphoribosylaminoimidazole-succinocarboxamide synthase, PurS subunit(1)" phosphoribosylaminoimidazole- succinocarboxamidesynthase PurC(1) Phosphoribosylamidoimidazole-succinocarboxamide synthase(1) phosphoribosylaminoimidazolesuccinocarboxamide synthetase homolog(1)

405 6 142 4.225352113 2 0.3745 >Cluster 3549 GTP pyrophosphokinase(44)hypothetical protein(10) conserved hypotheticalprotein(6) RelA/SpoTdomain-containingprotein(5) GTP-pyrophosphokinase family protein(3) RelA/SpoT domain protein(3)putative GTP pyrophosphokinase(3) GTP-pyrophosphokinase(1) region found in RelA / SpoT proteins family protein(1) MW2427(1)RelA/SpoT-like ppGpp synthetase domain protein(1) RelA / SpoT s family protein(1) GTP pyrophosphokinase ywaC(1)

406 6 142 4.225352113 3851 acetoin utilization protein(43) acetyl-CoA synthetase inhibitor(8)" AcuA(7) putative acetoin utilization protein AcuA(6) acetoin dehydrogenase-like protein(5) Acetoin utilization protein AcuA(3)acetoin dehydrogenase-protein(2) acetoin dehydrogenase homolog(1) "acetoin utilization protein AcuA, putative(1)" acetoin utilization protein acuA(1) Acetoin utilization protein acuA(1) acuA(1) conserved hypothetical protein(1)

407 6 142 4.225352113 2 0.3745 >Cluster 4449 colicin V production protein CvpA(30) Colicin V production protein(11) colicin V production family protein(11) putative membrane protein(7) CvpA family protein(5) colicin V production protein(5) conserved hypothetical protein(4) hypotheticalprotein(3) PutativecolicinVproductionfamily protein(1) possiblecolicinVproductionprotein(1) probable membrane protein(1) "bacteriocin production-related protein, putative(1)"

408 10 142 7.042253521 3 0.382 391 sulfite reductase (43) Sulfite reductase (8) reductase flavoprotein(6)putative sulfite reductase (5) reductase (NADPH) flavoprotein alpha-component(4) Sulfite reductase (NADPH) flavoprotein alpha-component(3) "sulfite reductase (NADPH) flavoprotein, alpha chain(2)" sulfite reductase alpha subunit protein(2) Sulfite reductase flavo protein alpha-component(1) sulfite reductase(1) "sulfite reductase (NADPH) flavoprotein alpha-component, putative(1)" "sulfite reductase (NADPH) flavoprotein, alpha-component(1)" sulfite reductase (NADPH) alpha subunit(1) Sulfite reductase flavoprotein alpha-component(1) putative sulfite reductase(1) Sulfite reductase(1)

409 23 142 16.1971831 8 0.3891 >Cluster 6036 hypothetical protein(75) phage protein(20) Phage protein(10) putative phage protein(5)protein of unknown function DUF1024(4) hypothetical phage protein(4) conserved hypotheticalprotein(3) "Hypotheticalprotein,PVLorf52(2)" hypothetical bacteriophage protein(2) phi PVL ORF 52 analogue(2)phi PVL/orf 52-like protein(2) conserved hypothetical phageprtotein(1) phi PVL orf 52-like protein(1) protein(1) phi PVL ORF 52 like protein(1) relatedprotein(1)

410 32 142 22.53521127 13PVL orf 52-likehypothetical phage-0.3901 >Cluster603 0.3745 >Cluster "Acetyltransferase AcuA, acetoin utilization protein>ClustersulfitesulfiteDNA-directed RNA polymerase subunit beta(45) "DNA- directed RNA polymerase, beta subunit(11)"DNA-directed RNA polymerase beta subunit(10) DNA-directed RNA polymerase beta chain protein(7) RNA polymerase beta chain(2) DNA-directed RNA polymerase beta chain(2) DNA-dependent RNA polymerase beta subunit(1) "RNA polymerase beta chain, putative(1)" "DNA-dependent RNA polymerase, beta subunit(1)"

411 6 142 4.225352113 3 0.4 >Cluster 111 DNA repair protein Rad50(29) hypothetical protein(14) DNA double-strand break repair Rad50 ATPase(10) conserved hypotheticalprotein(8) putativemembraneprotein(7) AAAdomain protein(7) large conserved outer membrane protein(2) DNA double- strand break repair rad50 ATPase(1) Putative membrane associated protein(1) DNA double-strand break repair ATPase(1)

412 6 142 4.225352113 5 0.4 >Cluster 281 penicillin-binding protein(32) penicillin-binding protein 3(22) penicillin-binding protein PBP2B(7) penicillin binding transpeptidase domain protein(7) Cell division protein FtsI (3) penicillin-binding Protein dimerisation domainfamily(1) protein(1)" protein(1) protein FtsI (Peptidoglycan synthetase) / Transpeptidase, Penicillin binding protein transpeptidase domain(1)" Cell division protein FtsI (Peptidoglycan synthetase)(1) Penicillin binding protein transpeptidase(1) Penicillin-binding protein 3(1) penicillin- binding protein transpeptidase(1)"penicillin-binding protein, transpeptidase domain penicillin-binding Protein dimerization domain Cell division protein FtsI(1) "Cell division

413 6 142 4.225352113 2 0.4 >Cluster 377 PTS mannose transporter subunit IIA(32) "Activator of the mannose operon (transcriptional antiterminator), BglG family(8)" PRD domain protein(6) transcriptional antiterminator(5) BglG family transcriptional antiterminator(5) "transcription antiterminator, BglG family(4)" mga helix-turn-helix domain protein(3) transcriptional regulator (antiterminator) (3) "phosphoenolpyruvate-dependent sugar phosphotransferase system, EIIA 2 family protein(2)" transcriptional regulator(2) putative transcriptional regulator ManR(2)MW2561(1) transcriptional antiterminator BglG family protein(1) deoR-like helix-turn-helix domain protein(1) hypothetical protein(1) conserved hypothetical protein(1) "Transcription antiterminator, BglG familyprotein(1)" Hypotheticalprotein(1) probable antiterminator transcriptional regulator(1) "transcriptional antiterminator, BglG family(1)" "Putative transcriptionalantiterminator, BglG family / PTS system, mannitol/fructose-specificIIA component(1)"

414 6 142 4.225352113 4 0.4 >Cluster 665 cholinetransporter(30) glycinebetainetransporter2(8) Glycine betaine transporter OpuD(8) glycine betaine transporter OpuD(7) choline/carnitine/betaine transporter(6) choline transport protein BetT(4) "transporter, betaine/carnitine/choline transporter family protein(4)" glycine betainetransporter(3) "transporter, betaine/carnitine/choline family(1)" putative membrane protein(1) MW2111(1) Glycine betaine transporter(1) "glycine betaine transporter, putative(1)"BCCT family osmoprotectant transporter(1) "osmoprotectant transporter, BCCT family, opuD-like protein, putative(1)" osmoprotectant transporter(1) glycine betaine transporter opuD(1) glycine betaine transporter opuD homolog(1) BCCT family betaine/ carnitine/choline transporter(1)

415 6 142 4.225352113 4 0.4 >Cluster 1031 ammonia permease(32) ammonium transporter family protein(18) Ammoniumtransporter(9) ammoniumtransporter(8) AMT family ammonium or ammonia transporter(5) probabale ammonium transporter(3) probable ammonium transporter(2) "ammonium transporter, Amt family(2)" ammonium transporter NrgA (Membrane protein nrgA) (Protein amtB)(1)

416 6 142 4.225352113 2 0.4 >Cluster 1346 ligase(29) putative membrane protein(7) O-Antigen ligase family protein(7) "Teichuronic acid biosynthesis protein TuaE, putative secreted polysaccharide polymerase(5)" O-antigen polymerase(5) hypothetical protein(5) conserved hypothetical protein(4) putative O-antigen polymerase(3) O-antigen ligase like membrane family protein(3) O-Antigen ligase(2) O-Antigen Polymerase family protein(1) "capsular biosynthesis protein, putative(1)" "Teichuronic acid biosynthesis protein TuaE,putative secreted polysaccharide polymerase(1)" Secreted polysaccharide polymerase(1) MW0105(1) hypothetical membraneprotein(1) "hypothetical protein, capsular polysaccharide synthesis-like protein 14H(1)" probable capsular biosynthesis protein(1)

417 6 142 4.225352113 3 0.4 >Cluster 1684 quinol oxidase subunit 2(27) putative quinol oxidase polypeptide II precursor(10) "cytochrome aa3 quinol oxidase, subunit II(7)" quinol oxidase polypeptide II QoxA(5) AA3-600 quinol oxidase subunit II(5) cytochrome aa3 quinol oxidase subunit II(3) putative quinol oxidase subunit 2(3) quinol oxidase polypeptide II(2)quinol oxidase subunit 2 protein(2) MW0944(1) quinol oxidase polypeptide II QoxA (EC:(1)"quinoloxidase,subunitII(1)" "quinoloxidaseAA3,subunit II(1)" "cytochromeaa3quinoloxidase,subunitII,putative(1)" hypotheticalprotein(1) Quinoloxidasesubunit2(1) Probable quinol oxidase subunit 2(1) Cytochrome c oxidase polypeptide II(1) "quinol oxidase AA3, subunit II, putative(1)" quinol oxidase subunit II(1) "quinol oxidase subunit 2 (Quinol oxidasepolypeptide II) (Quinol oxidase aa3-600, subunit QoxA) (Oxidase aa(3)-600 subunit 2)(1)"

418 6 142 4.225352113 3 0.4 >Cluster 2962 "2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase(46)" putative hydrolase(7) "2-succinyl-6-hydroxy-2,4- cyclohexadiene-1- carboxylate synthase(6)"acyl-CoA thioesterhydrolase(4) hydrolase(2) protein(2) probable hydrolase(1) S33 family peptidase(1) "2-succinyl-6- hydroxy-2,4-cyclohexadiene-1-carboxylate synthase, putative(1)" alpha/beta hydrolase fold family protein(1) alpha/beta hydrolasefold(1) hypotheticalprotein(1) truncated hypothetical protein(1)alpha/beta fold family hydrolase(3) alpha/beta hydrolase(2) conserved hypothetical "hydrolase, alpha/beta hydrolase fold family(1)"

419 6 142 4.225352113 3 0.4 >Cluster 3797 hypothetical protein(33) ABC transporter(20) putative membrane protein(8) conserved hypothetical protein(8) protein co- occurring with transport systems(4) YigZ family protein(2) IMPACT family member YigZ(2) FIG000605: protein co-occurring with transport systems (COG1739)(1) Hypothetical protein(1)hypothetical conserved protein(1) probable membrane protein(1) putative YigZ family protein(1) hypothetical membrane protein(1) protein of unknown function UPF0029(1)

420 6 142 4.225352113 3 0.4 >Cluster 6315 hypothetical protein(33) phage protein(7) conservedhypotheticalprotein(5) Hypotheticalprotein(1) Phage protein(1)

421 16 142 1371 histidinol-phosphate aminotransferase(45) histidinol- phosphate transaminase(11) Biosynthetic Aromatic amino acid aminotransferase beta(8) putative aminotransferase(6) Histidinol- phosphate aminotransferase(5) hypothetical protein(1) "histidinol-phosphate aminotransferase, putative(1)"MW0686(1)

422 17 142 11.97183099 5 0.414 >Cluster 52hypotheticalphageprotein(3) hypotheticalbacteriophageprotein(1)11.26760563 6 0.4024 >Clusterphage minor structural protein(20) hypothetical protein(19) "Structural protein, phage associated(7)" "phage minor structural, N-terminal region domain protein(6)" structural protein(3) putative phage minor structural protein(3) hypothetical phage protein(3) "phage minor structural protein, N- terminal region domain protein(2)" hypothetical bacteriophage protein(1) phi PVL ORF 20 and 21 homologue(1) bacteriophage minor structural protein(1) "phage minor structural protein, N-terminal domain protein(1)" Phage-related protein(1) "putative phage minor structural, N-region domain protein(1)"

423 7 142 4.929577465 5 0.4192 >Cluster 187 phenylalanyl-tRNA synthase subunit beta(18) phenylalanine--tRNA ligase subunit beta(11) Phenylalanyl-tRNA synthetase beta chain(10)putative phenylalanyl-tRNA synthetase beta chain(10) "phenylalanine--tRNA ligase, beta subunit(8)" Phenylalanine--tRNA ligase beta subunit(6)"phenylalanyl-tRNA synthetase, beta subunit(5)" phenylalanyl-tRNA synthetase subunit beta(3) phenylalanyl-tRNA synthetase subunit beta protein(2)Phe-tRNA synthetase beta chain(2) phenylalanyl-tRNA synthetase beta chain(2)phenylalanine--tRNA ligase beta subunit(1)"phenylalanyl-tRNA synthetase, beta subunit PheT(1)"

424 7 142 4.929577465 5 312 DNA mismatch repair protein MutL(63) protein mutL(7) DNA mismatch repair protein(5) MutL family

protein(2) mutL C terminal dimerization domain protein(2) DNA mismatch repair protein mutL. domain protein(2)DNA mismatch repair protein HexB family protein(1) DNA mismatch repair domain protein(1) "DNA mismatch repair protein HexB, putative(1)" MutL C-terminal dimerization domain protein(1)

425 7 142 4.929577465 2 0.4192 >Cluster 360 multidrug MFS transporter(31) putative transport protein(8) Membrane component of multidrug resistance system(7)H+ antiporter-2 family protein(7) EmrB/QacA family drug resistance transporter(3)EmrB/QacA subfamily drug resistance transporter(3) "MFS family major facilitator transporter, multidrug:cation symporter(3)" "drug resistance transporter, EmrB/ QacA subfamily(2)" MFS family major facilitator multidrug:cation transporter(2) Multidrug resistance protein B(2) MFS-type drugefflux transporter(2) drug resistance transporter(2) resistance transporter EmrB/QacA subfamily protein(1) subfamily drug resistance transporter, putative(1)" resistance transporter, EmrB/QacA subfamily, putative(1)" multidrug resistance transporter(1) "drug resistance MFS0.4192 >Cluster DNA mismatch repair DNA mismatch repairdrug"EmrB/QacA"drugprobabletransporter, drug:H+ antiporter-1 (14 Spanner) (DHA2) family protein(1)" "drug resistance MFS transporter, drug:H+ antiporter-2family(1)" MW2273(1) hypotheticalprotein(1)

426 7 142 4.929577465 4 0.4192 >Cluster 2892 spermidine/purescine ABC transporter permease(16) spermidine/ putrescine ABC transporter permease(16) Spermidine Putrescine ABC transporter permease component potC(10) putative ABC transport system permease protein(6) binding--dependent transport system inner membrane component family protein(4)binding-protein-dependent transport system inner membrane component family protein(4)Spermidine/ putrescine transport system permease protein PotC(3) binding- protein-dependent transport systems inner membrane component(3) "spermidine/putrescine ABC transporter, permease protein, putative(2)" inner membrane transporter(2) binding-protein-dependent transport system inner membraneprotein(2)protein(2)"protein(2)spermidinePutrescine ABC transporter permease component potC (TC_3.A.1.11.1)(1)"spermidine/putrescine ABC transporter, permease spermidine/putrescine ABC transporter permease "ABC transporter, permease protein(1)" potC(1)putrescine ABC transporter protein(1) Spermidinespermidine/putrescine ABC transporter homolog(1) Spermidine Putrescine ABC transporter permease component PotC(1) Spermidine/ putrescine transport system permease protein potC(1)

427 18 142 12.67605634 6 0.4253 >Cluster 348 heme transporter IsdB(23)heme uptake protein IsdB(19) iron-regulated heme-iron binding protein(10) Cell surface receptor IsdB for hemoglobin and hemoglobin-haptoglobin complexes(7)

iron-regulated surface determinant protein B(5) iron transport associated domain-containing protein(4) conserved hypotheticalprotein(2) iron-regulatedcellwall-anchoredprotein(1)iron-regulated heme-iron binding protein IsdB(1) Iron- regulated surface determinant protein B(1)LPXTG cell wall anchor domain protein(1) LPXTG cell wall surface anchor protein(1) LPXTG-motif cell wall anchor domain(1) "gram-positive signal peptide,YSIRKfamilydomainprotein(1)" HemeuptakeproteinIsdB(1)neurofilament protein(1) LPXTG-motif cell wall anchor domain protein(1)

428 3 142 2.112676056 3 502 restriction endonuclease subunit M(53) restriction-modification system, M subunit(30)" restriction-modification system, DNA-methyltransferase subunit M(14)"Type I restriction-modification system methyltransferase subunit(5) putative type I restriction enzyme modification protein(5) type I site-specific deoxyribonuclease methyltransferase subunit(4) type I restriction-modification system(4) "Type I restriction-modification system,DNA- methyltransferase subunit M(4)" type I restriction-modification0.4327 >Cluster "type I "Type IsystemsubunitM(4) hypotheticalprotein(3) TypeIrestriction- modification system methylation subunit(2)"type I restriction- modification system, DNA-methyltransferase subunit M(2)" type I restriction enzyme M protein(2) "type I restriction-modification system, methyltransferase subunit(2)" "type I restriction- modification system, subunit M(1)" "type I restriction- modification system, methylase subunit(1)"type I restriction enzyme EcoR124II M protein homolog(1) "type I restriction-modification enzyme, M subunit(1)" "Type I restriction-modification system, DNA-methyltransferase subunit M 1(1)" probable type I site-specific deoxyribonuclease LldI chain(1) "Type I restriction-modification system methyltransferase subunit, HsdM_1(1)" "Type I restriction-modification system methyltransferase subunit, HsdM_2(1)"probable type I site-specific deoxyribonuclease LldI chain hsdM(1) putative restriction enzyme modification protein(1) "Type I restriction-modification system, DNA-methyltransferase subunit M 2(1)" type I site-specific deoxyribonuclease(1) type I restriction-modification system M subunit(1) "restriction-modification system, M subunit(1)" hsdM(1)

429 3 142 2.112676056 2 0.4327 >Cluster 1102 sulfite reductase subunit alpha(31) putative iron- regulated membrane protein(11) pepSY-associated TM helix family protein(8) putative membrane protein(5) sulfite reductase (5) conserved hypothetical protein(4) hypothetical protein(3) propeptide(2) Uncharacterized iron-regulated membrane protein; Iron-uptake factor PiuB(2) Putative Membrane Protein(1) hypothetical membrane protein(1) sulfite reductase(1) PepSY domain protein(1) iron-regulated membrane protein; Iron-uptake factor PiuB(1) Propeptide PepSY amd peptidase M4(1) probable membrane protein(1) Hypothetical protein(1)

430 3 142 2.112676056 3 0.4327 >Cluster 1149 uracil permease(53) Uracil permease(10) putative uracil permease(7) NCS family uracil:cation symporter(3) permease family protein(2) uracil permease (Uracil transporter) (2) putative permease(1) "uracil permease,putative(1)" "uracil permease PyrP, putative(1)" uracil- xanthine permease(1)

431 3 142 2.112676056 3 0.4327 >Cluster 2048 anthranilate phosphoribosyltransferase(61)Anthranilate phosphoribosyltransferase(16) trpD(1)

432 3 142 2.112676056 2 0.4327 >Cluster 2360 urea transporter(43) Eukaryotic-type low-affinity ureatransporter(11) putative membrane protein(5) membrane-bound urea transporter(4) Urea transporter(4) hypothetical protein(3) UT family urea transporter(3) "urea transporter, putative(2)" MW2205(1) probable membrane-bound urea transporter(1)putative urea transporter(1) conserved hypothetical protein(1)

433 3 142 2.112676056 3 0.4327 >Cluster 2676 flavin reductase(30) hypothetical protein(17) conserved hypothetical protein(10) flavin reductase like domain protein(7) putative flavin reductase(5) FMN-binding domain-containing protein(3) "flavin reductase-like, FMN-binding protein(2)" flavoprotein oxygenase DIM6/NTAB family-protein(2) FAD-dependent oxidoreductase(1) "flavin reductase-like, FMN-binding(1)" Hypotheticalprotein(1) FIG01107912:hypotheticalprotein(1) putative flavin reductase family protein(1)

434 3 142 2.112676056 2 0.4327 >Cluster 4019 TetR family transcriptional regulator(42) TetR family regulatory protein(15) "bacterial regulatory

s, tetR family protein(4)" "transcriptional regulator, AcrR(4)" "bacterial regulatory, tetR family protein(3)" hypotheticalprotein(3) "transcriptional regulator, TetR family(2)" regulatory protein TetR(1) regulatory protein TetRfamily(1) family(1)" protein(1)" regulator, TetR family protein(1)" MW2492(1)"Transcriptional regulator, TetR "bacterial regulatory proteins, tetR family conserved hypothetical protein(1) "Transcriptional

435 7 142 4.929577465 3 0.4361 >Cluster 193 preprotein translocase subunit SecA(22) accessory Sec system translocase SecA2(18) Protein export cytoplasm protein SecA2 ATPase RNA helicase(8) preprotein translocase SecA subunit- like protein(6) Sec family Type I general secretory pathway protein SecA(6) Protein translocase subunit SecA 2(5) translocase subunit secA 2 protein(2) "preprotein translocase, SecAsubunit(1)" "preprotein translocase, subunit SecA, putative(1)" MW2570(1) conserved hypothetical protein(1) "preproteintranslocase, SecA subunit, putative(1)" secA Wing and Scaffold domain protein(1) Protein translocase subunit secA(1) SecA DEAD domain protein(1) Protein export cytoplasm protein SecA2 ATPase RNA helicase (TC 3.A.5.1.1)(1) preprotein translocase secA homolog(1) protein translocase subunit secA(1)Protein translocase subunit secA 2(1)

436 7 142 4.929577465 3 0.4361 >Cluster 1340 hypothetical protein(36) aluminum resistance protein(15) Aluminum resistance protein(15) conserved hypothetical protein(4)cystathionine beta-lyase aluminum resistance protein(4) MW1190(1) Aluminium resistance family protein(1) "aluminumresistance protein, putative(1)" aluminium resistance protein(1) possible aluminum (Al2+) resistance protein(1)

437 7 142 4.929577465 2 0.4361 >Cluster 3890 glucosamine-6-phosphate isomerase(34) hypothetical protein(12) glucosamine-6-phosphate isomerase family protein(9)putative membrane protein(7) glucosamine-6-phosphate deaminase(4) putative glucosamine-6-phosphate isomerase(4) Hypotheticalprotein(4) conservedhypothetical protein(3) "glucosamine-6-phosphate isomerase, putative(1)" Hypothetical protein SAV1854(1) probable membrane protein(1)

438 12 142 8.450704225 3 0.4363 >Cluster 1862 serine/threonine protein phosphatase(30) "Serine phosphatase RsbU, regulator of sigma subunit(11)" putative sigma factor sigB regulation protein(9) phosphoserine phosphatase rsbU(4) sigma factor B regulation protein(3) phosphoserine phosphatase RsbU(3) sigmaB regulation protein RsbU(3) Phosphoserine phosphatase rsbU(3) RsbU protein(2) "sigmaB regulation protein RsbU, putative(2)" sigma-B regulation protein serine phosphatase RsbU(1) sigma factor B regulator protein(1) sigmaB regulation protein(1) indirect positive regulator of sigma B(1) stage II sporulation protein E(1) sigma factor sigB regulation protein(1) phosphoserine phosphatase rsbU (Sigma factor sigBregulation protein rsbU)(1) phosphoserine phosphatase RsbU (Sigma factor sigBregulation protein rsbU)(1) Sigma factor sigB regulation protein rsbU(1) Stage II sporulation E family protein(1) sigma factor B regulator(1)

439 20 142 14.08450704 7 0.4465 >Cluster 416 histidine kinase(17) PAS domain-containing sensorhistidine kinase(12) sensor protein kinase WalK(5) kinase protein(5) sensor protein kinase walK(4) sensor signal transduction histidine kinase, WalK(4)" component sensor histidine kinase(4) Sensor protein kinase WalK(3) Two-component sensor kinase SA14-24(3) transcriptional regulatory protein WalK(3) sensor protein(2) two- compornent sensor histidine kinase(2) Sensor protein kinase(2) Two-component sensor kinase(2) sensory box histidine kinase YycG(2)sensor histidine kinase(2) Two-component sensor kinase WalK(1) sensor protein YycG(1) "sensory box histidine kinase VicK,putative(1)" Two-component sensor kinase VicK/WalK(1) component sensor histidine kinase VicK, putative(1)" protein(1) sensor histidine kinase VicK(1)

440 4 142 2.816901408 3 0.4625"two-PAS sensor>Clustersensor"multi-two-177 leucyl-tRNA synthetase(43) leucine--tRNA ligase(21) Leucyl-tRNAsynthetase(11) Leucine--tRNAligase(5) leucyl-rRNA synthetase(1)

441 4 142 2.816901408 4 0.4625 >Cluster 533 terminase(17) phage Terminase family protein(5) terminase large subunit(5) "putative phage terminase, large subunit(4)" putative phage terminase large subunit(3) "Phage terminase, large subunit(2)" phage terminase large subunit(2) phage Terminase(1)"phage terminase, large subunit(1)" "phage terminase, large subunit, putative(1)" Phage terminase large subunit(1)

442 4 142 2.816901408 2 0.4625 >Cluster 842 glutamate synthase(31) "glutamate synthase, smallsubunit(8)" glutamate synthase subunit beta(8) synthase, NADH/NADPH, small subunit domain protein(7)" synthase (NADPH) small subunit(5) Glutamate synthase (5) synthase (NADPH) small chain(3) glutamate synthase (NADPH/NADH) small chain(3) NADH-glutamate synthase small subunit(2) glutamate synthase (2) Glutamate synthase small chain(1) "glutamate synthase, NADH/NADPH, small subunit(1)" Glutamate synthase NADPH small chain(1) glutamate synthase small subunit(1) "NADH- glutamate synthase small subunit, putative(1)"

443 4 142 2.816901408 3 0.4625 >Cluster 1156 GTP-binding protein EngA(21) GTP-binding protein Der(18)ribosome-associated GTPase EngA(12) ribosome biogenesisGTPase Der(7) putative GTPase(7) GTPase Der(6) protein engA(2) small GTP-binding domain protein(1) factor Tu GTP binding domain protein(1) MW1364(1)GTP-bindingelongationsmall GTP-binding protein(1) GTP-binding protein(1) hypotheticalprotein(1) hypotheticalprotein(1)

444 4 142 2.816901408 3 1546 SAM-dependent methyltransferase(32) hypotheticalprotein(8) hypotheticalprotein(8) methyltransferase RlmI(7)putative ribosomal rRNA large subunit methyltransferase(3) methyltransferase domain protein(3) methyltransferase(2) putative SAM-dependent methyltransferase(2)putative SAM-dependent methyltransferases(2) SAM- dependent methyltransferase-protein(2) methyltransferase small domain protein(2) SAM-dependent methyltransferase-like protein(1) S-adenosylmethionine-dependent methyltransferase family protein(1) "methyltransferase, putative(1)" SAM-conserved0.4625 >Cluster conserved LSUm5C1962"glutamateglutamateGlutamatedependent methyltransferasese(1) met-10+ like-family protein(1)protein YwbD(1) Methyltransferase(1) methyltransferase, putative(1)""SAM-dependent

445 4 142 2.816901408 1802 phosphomevalonate kinase(69) kinase(10) "phosphomevalonate kinase, putative(1)"

446 4 142 2.816901408 3 0.4625 >Cluster 2721 hypothetical protein(23) lipoprotein(17) putative lipoprotein(7) staphylococcal tandem lipoprotein(6) tandem lipoprotein(6) Tandem lipoprotein within Pathogenicity island(4) staphylococcus tandem lipoprotein(2) truncated

staphylococcal tandem lipoprotein Lpl2(2) staphylococcus tandem lipoproteins family protein(1) Tandem lipoprotein(1) Tandem lipoproteinwithin Pathogenicity island 2(1) probable lipoprotein(1) hypotheticalprotein(1) hypotheticallipoprotein(1) lipoprotein(1)conservedsa tandem

447 8 142 5.633802817 3 0.4672 266 AraC family transcriptional regulator(33) AraC family regulatory protein(17) transcriptional regulator AraC family protein(4) "bacterial regulatory helix-turn-helix s, AraC family protein(4)" AraC family HTH-type transcriptional regulator(3) AraC family transcription regulator(3) "transcriptional regulator, AraC family(3)" helix-turn-helix domain protein(3) "transcriptional regulator, putative(2)" transcriptionalregulator(2) hypotheticalprotein(2) helix-turn- helix- domain containing protein AraC type(1) araC family transcriptional regulator(1) "bacterial regulatory helix-turn- helix proteins, AraC family protein(1)" MW2301(1) AraC transcriptional regulator(1) "Transcriptional regulator, AraC family protein(1)"

448 8 142 5.633802817 3 0.4672 >Cluster 3782 4'-phosphopantetheinyl transferase(45) 4'- phosphopantetheinyl transferase superfamily protein(16) 4'- phosphopantetheinyl transferase-like protein(4) holo-(3) conserved hypotheticalprotein(3) 4'-phosphopantetheinyltransferasefamily protein(2) putative 4'-phosphopantetheinyl transferase(2) hypotheticalprotein(1) 4-phosphopantetheinyltransferase(1) sfp-type 4-phosphopantetheinyl transferase family protein(1) 4'- phosphopantetheinyl transferase ausB(1) possible holo-(1)

449 14 142 9.85915493 4 0.4688 >Cluster 2277 hypothetical protein(65) conserved hypothetical protein(11)Hypotheticalprotein(2) hypotheticalproteinYpbB(1)3 0.4625 >Cluster Phosphomevalonate>ClusterFIG00743178: hypothetical protein(1)

450 9 142 6.338028169 3 0.4942 >Cluster 585 cholinetransporter(30) glycinebetainetransporter(9) Glycine betaine transporter OpuD(8) glycine betaine transporter 1(8) BCCT family betaine/carnitine/choline transporter(4)glycine betaine transporter OpuD(4) "transporter, betaine/ carnitine/choline transporter family protein(4)" Glycine betaine transporter(4) choline/carnitine/betaine transporter(3) glycine betaine transporter opuD(1) BCCT family osmoprotectant transporter(1) Glycine betaine transporter 1(1) "glycine betaine transporter, putative(1)""transporter, betaine/carnitine/choline family(1)"

451 9 142 6.338028169 3 0.4942 >Cluster 2474 tRNA pseudouridine synthase B(53) putative tRNA pseudouridine synthase B(10) tRNA pseudouridine(55) synthase(7) tRNA pseudouridine 5S synthase(2) tRNA pseudouridine(55) synthase TruB(2) tRNA pseudouridine 55 synthase(1) tRNA-pseudouridine 5S synthase(1) TRNA pseudouridine(55) synthase(1) tRNA pseudouridine synthase B domain protein(1)conserved hypothetical protein(1) pseudouridylate synthase(1)

452 9 142 6.338028169 2 0.4942 >Cluster 5517 hypothetical protein(15) nitrogen fixation protein NifR(1)

453 4 142 2.816901408 2 0.4959 >Cluster 1410 tryptophan synthase subunit beta(38) "tryptophan synthase, beta subunit(15)" Tryptophan synthase beta chain(15)tryptophan synthase beta chain(10) tryptophan synthase beta subunit(1)

454 4 142 2.816901408 3 0.4959 >Cluster 1443 glycosyl transferase family 1(19) glycosyltransferase WbuB(11)capsular polysaccharide synthesis enzyme(8) glycosyl transferases group 1 family protein(7) capsular polysaccharide synthesis enzyme Cap5L(5)capsular polysaccharide biosynthesis protein Cap5L(3) Glycosyltransferase(3) putative glycosyltransferase(3) capsular polysaccharide synthesis enzyme Cap8L(3) Capsular polysaccharide synthesis glycosyltransferase CapL(3) Capsular polysaccharidesynthesisenzymeCap5L(2) glycosyltransferase(2) glycosyltransferase family 1(2) capsular polysaccharide synthesisenzyme CapL(2) "glycosyltransferase, group 1 family protein(1)" glycosyl transferase group 1(1) "cap5L protein/glycosyltransferase, putative(1)" capsular polysaccharide biosynthesis protein glycosyltransferase CapL(1) cap8L(1) Cap8L(1) glycosyltransferase(1)

455 4 142 2.816901408 2 0.4959 >Cluster 1535 formate dehydrogenase(32)NAD-dependent formate dehydrogenase(14) putative D-isomer specific 2-hydroxyacid dehydrogenase(4) "formate dehydrogenase, mitochondrial(4)" 4- phosphoerythronate dehydrogenase(2) formate dehydrogenase (NAD- dependentformatedehydrogenase)(FDH)(1) D-isomerspecific2- hydroxyacid dehydrogenase(1) "formate dehydrogenase, NAD-dependent,putative(1)" D-isomerspecific2-hydroxyaciddehydrogenase NAD-binding(1) Formate dehydrogenase(1) "formate dehydrogenase, (NAD-dependent formate dehydrogenase) (FDH)(1)"

456 4 142 2.816901408 2 0.4959 >Cluster 2303 lipid kinase(31) diacylglycerol kinase(13)conserved hypotheticalprotein(8) hypotheticalprotein(6) Diacylglycerol kinase(6) putative lipid kinase(6) Transcription regulator (contains diacylglycerol kinase catalytic domain)(4)Transcription regulator (2) diacylglycerol kinase catalytic region(1) Diacylglycerol kinase family protein(1) "lipid kinase, putative(1)" diacylglycerol kinase catalytic domain (presumed) family protein(1)

457 4 142 2.816901408 2 0.4959 >Cluster 2963 pantothenate kinase(36) type II pantothenate kinase(17) "Pantothenate kinase type II, eukaryotic(8)" Type II pantothenate kinase(6) hypothetical protein(6) conservedhypothetical protein(3)pantothenate kinase(1)Pantothenate kinase(2) possible "pantothenate kinase, putative(1)"2.816901408 2 0.4959 >Cluster

458 4 142 5159 nitrogen fixation protein NifR(34) hypotheticalprotein(22)protein(4)

459 4 5697 DNA methyltransferase(32)hypothetical protein(22) conserved hypotheticalprotein(9) PaaD-likeprotein(DUF59)involvedinFe-S cluster assembly(4) "putative metal-sulfur cluster biosynthetic enzyme, PaaD(4)" PaaD-like protein involved in Fe-S clusterconserved hypothetical protein(4) Hypothetical Nitrogen regulation protein NIFR3(3)142 2.816901408 2 0.4959 >Clusterassembly(3) MIP18 family protein YitW(3) N-6 Adenine- specific DNA methylase YitW(1) Putative cytosolic protein(1) protein of unknown function DUF59(1) putative n-6 adenine- specific DNA methylase YitW(1) disulfie oxidoreductase(1) PaaD-like protein(1) "aromatic ring hydroxylating enzyme, putative(1)"

460 4 142 2.816901408 2 0.4959 >Cluster 6404 hypothetical protein(69) conserved hypothetical protein(6) Putative cytosolic protein(2)

Hypothetical protein(1) protein of unknown function DUF1250(1) FIG01108414: hypothetical protein(1)

461 5 142 3.521126761 3 Putative surface anchored protein(1)Staphylococcal surface anchored protein(1)

462 5 142 3.521126761 2 927 dihydrolipoamide dehydrogenase(39) dehydrogenase(16) Dihydrolipoamide dehydrogenase of pyruvate dehydrogenase complex(9) dihydrolipoamide dehydrogenase component of pyruvate dehydrogenase E3(7) Dihydrolipoyl dehydrogenase(6) Dihydrolipoamide dehydrogenase(2) dihydrolipoamide dehydrogenase: subunit E3(1) "pyruvate dehydrogenase E3, dihydrolipoamide dehydrogenase component PdhD(1)"

463 5 142 3.521126761 2 0.5433 >Cluster 1051 bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase(32) UDP-N-acetylglucosamine diphosphorylase/glucosamine-1-phosphate N- acetyltransferase(12) putative UDP-N-acetylglucosamine pyrophosphorylase(11) N-acetylglucosamine-1-phosphate uridyltransferase / Glucosamine-1-phosphate N-acetyltransferase(6) UDP-N-acetylglucosamine pyrophosphorylase(6) N- acetylglucosamine-1-phosphate uridyltransferase(5) Glucosamine-1- phosphate acetyltransferase(1) Bifunctional protein glmU(1) UDP-N-acetylglucosamine pyrophosphorylase homologue(1) gcaD(1) bifunctional UDP-N-acetylglucosamine pyrophosphorylase(1)

464 5 142 3.521126761 3 0.5433 >Cluster 1294 argininosuccinate synthase(53) Argininosuccinate synthase(19) putative argininosuccinate synthase(9) "argininosuccinate synthase, putative(1)"0.5433 >Cluster2 Putative0.5433 >Cluster dihydrolipoyl

465 5 142 3.521126761 2 0.5433 >Cluster 1315 cysteine desulfurase(35) putative cysteine desulfurase(10) "Cysteine desulfurase, SufS subfamily(7)" putative selenocysteinelyase(5) "cysteinedesulfurase,SufSsubfamily(4)" Cysteinedesulfurase(3) cysteinedesulfuraseSufSsubfamily protein(3) "aminotransferase, class V superfamily, putative(2)"probable cysteine desulfurase(2) Cysteine sulfinate desulfinase(1) "cysteine desulfurases, SufS subfamily subfamily(1)"SufS subfamily cysteine desulfurase(1) MW0797(1) cysteine desulfurase (Selenocysteine lyase) (Selenocysteine reductase) (Selenocysteine beta-lyase) (SCL)(1) selenocysteine lyase(1) aminotransferase NifS homologue(1) "cysteine desulfurases, SufS subfamily protein(1)" "putative cysteine desulfurase, putative(1)" "cysteine desulfurase, SufS subfamily subfamily(1)"

466 5 142 3.521126761 2 0.5433 >Cluster 1419 "N-acetyl-L,L-diaminopimelate deacetylase(41)" amidohydrolase family protein(8) amidohydrolase(8) putative peptidase(7) putative N-acyl-L-amino acid amidohydrolase(4) N- acyl-L-amino acid amidohydrolase(2) thermostable carboxypeptidase 1(2) peptidase(2) conserved hypothetical protein(2) "peptidase, M20-M25-M40 family(1)" M20D subfamily peptidase(1) hypothetical protein(1) MW0504(1)

467 5 142 3.521126761 2 0.5433 >Cluster 1960 histidine kinase(27) oxygen sensor histidine kinase nreB(9) Two component sensor histidine kinase(6) oxygen sensor histidine kinase NreB(5) two-component sensor histidine kinase(5) putative histidine kinase(4) Oxygen sensor histidine kinase NreB(4) roxygen sensor histidine kinase NreB(3) Oxygen sensor histidine kinase nreB(2) two component sensor histidine kinase(2) MW2314(1) ATP-binding region ATPase domain protein(1)Sensor protein DegS(1) "sensory box histidine kinase, putative(1)" possible sensor histidine kinase(1) "two componentsensor histidine kinase, putative(1)" sensor histidine kinase(1) "histidine kinase-, DNA gyrase B-, and HSP90-like ATPase family protein(1)"Sensor protein NreB(1) Two component system histidine kinase(1) conserved hypothetical protein(1) hypothetical protein(1)

468 5 142 3.521126761 2 0.5433 >Cluster 2120 adenine methyltransferase(30) conserved hypothetical protein(10) Adenine-specific methyltransferase(9) hypotheticalprotein(8) methyltransferasesmalldomainprotein(4) Adenine-specific DNA methylase-like protein(4) adenine-specific DNA methylase-like protein(4) N-6 DNA Methylase family protein(3)Site-specific DNA-methyltransferase(3) adenine-specificDNA methylase(2) adenine-specific DNA methylase-protein(2) "adenine- specific DNA methylase, putative(1)"

469 5 142 3.521126761 2 0.5433 >Cluster 2357 hypothetical protein(68) conserved hypothetical protein(7) Hypotheticalprotein(2) FIG01108619:hypotheticalprotein(1)

470 5 142 3.521126761 2 0.5433 >Cluster 3456 allophanate hydrolase(30)allophanate hydrolase subunit 1 family protein(11) Allophanate hydrolase 2 subunit 1(8) allophanate hydrolase subunit 1(7) hypothetical protein(6) conserved hypothetical protein(6) allophanate hydrolase subunit 1protein(2) MW0679(1) amidolyase(1) hydrolase subunit 1(1) "allophanate hydrolase, subunit 1, putative(1)" Kinase autophosphorylation inhibitor kipI(1) conserved hypothetical protein TIGR00370(1)putative allophanate hydrolase subunit 1(2) Allophanate hydrolase subunit 1(1) urea Urea amidolyase(1) possible allophanate

471 5 142 3.521126761 2 0.5433 >Cluster 3818 hypothetical protein(39) membrane protein(22) putativemembrane protein(8)Hypothetical protein(2)membrane protein(1)

472 5 142 4482 hypothetical protein(58) conserved hypothetical protein(8) base-induced perplasmic lipid-binding protein(4) YceI-like domain-containing protein(2) yceI-like domain protein(1) YceI family protein(1) YceI-like domain protein(1) YceI like family(1)FIG01108092: hypothetical protein(1) Putative exported protein(1) YceI domain protein(1) Hypothetical protein(1)conserved hypothetical protein(6) hypothetical membrane protein(1) probable FIG01108339: hypothetical protein(1)3.521126761 2 0.5433 >Cluster

473 11 142 7.746478873 5 5974 hypothetical protein(7)

474 6 142 4.225352113 3 794 betaine-aldehyde dehydrogenase(32) dehydrogenase(17) betaine aldehyde dehydrogenase(13) putative betaine aldehyde dehydrogenase(7)glycine betaine aldehyde dehydrogenase(6) glycine betaine aldehyde dehydrogenase gbsA(3)0.5569 >Cluster0.5803 >Cluster Betaine aldehydeglycine betaine aldehyde dehydrogenase GbsA(2)

475 6 142 4.225352113 3 0.5803 >Cluster 1254 glycine/betaine ABC transporter ATP-binding protein(34) "Osmotically activated L-carnitine/choline ABC transporter, ATP-binding protein OpuCA(7)"putative glycine betaine/ carnitine/choline transport ATP-binding protein(7) glycine betaine/L- prolinetransportATPbindingsubunit(5) "glycinebetaine/cholineABC superfamily ATP binding cassette transporter, ABC protein(5)"glycine betaine/carnitine/choline ABC transporter(3) glycine betaine/carnitine/choline ABC transporter opuCA(3) Glycine betaine/ carnitine/choline transport ATP-binding protein OpuCA(3) carnitine transport ATP-binding protein OpuCA(3) glycine betaine/carnitine/ choline transport ATP-binding protein opuCA(2) Glycine betaine transport ATP-binding protein(1) putative glycine betaine/carnitine/ cholinetransport ATP-binding protein(1) "amino acid ABC transporter, ATP-bindingprotein(1)" "aminoacidABCtransporter,ATP-bindingprotein, putative(1)" ATPase subunit(1)" binding subunit(1) transporter, ATP-binding protein, putative(1)" betaine-carnitine- choline ABC transporter(1)"glycine betaine/L-proline ABC transporter,glycine betaine/L-proline transport ATP-"glycine betaine/carnitine/choline ABC

476 6 142 4.225352113 3 0.5803 >Cluster 2037 xylose isomerase(31) sugar phosphate isomerase/ epimerase(9) hypothetical protein(9) Inosose isomerase(8) conserved hypothetical protein(8) xylose isomerase-like TIM barrel family protein(4) Xylose isomerase domain-containing protein(3)AP endonuclease 2 C terminus family protein(3) xylose isomerase domain-containing protein TIM barrel(2) "AP endonuclease, family 2(1)" Sugar phosphate isomerase/epimerase(1) Xylose isomerase domain protein TIM barrel(1) IolE protein -like protein(1)

477 6 142 4.225352113 5 0.5803 >Cluster 2269 LytR family transcriptional regulator(30) transcriptional regulator(8) "Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2(6)" putative exported protein(6) transcriptional regulator lytR(4) cell envelope transcriptional attenuator(4) "cell envelope-related function transcriptional attenuator, LytR/CpsA family(3)" cell envelope-related function transcriptional attenuator common domainprotein(3) "transcriptional regulator, putative(3)" hypothetical protein(2) membrane-bound protein lytR(1) probable transcriptional regulator(1) MW2230(1) "Transcriptional regulator, LytR family protein(1)" cell envelope-related transcriptional attenuator(1) cell envelope-like functiontranscriptional attenuator common domain protein(1) LytR family cell envelope-related transcriptional attenuator(1) "Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2 (as in PMID19099556)(1)" Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr(1) membrane-bound protein LytR(1) Transcriptional regulator lytR(1)

478 6 142 4.225352113 2 0.5803 >Cluster 6992 hypothetical protein(13) Phage protein(3) phage protein(3) conserved hypothetical protein(3) hypothetical phage protein(1)

479 13 142 9.154929577 3 0.5835 >Cluster 2242 transporter(30) eamA-like transporter family protein(8) putativemembraneprotein(8) hypotheticalprotein(6) conserved hypotheticalprotein(5) Permeaseofthedrug/metabolitetransporter (DMT) superfamily(5) DMT superfamily drug/metabolite transporter(5) putative inner membrane transporter YicL(2) Drug/metabolite transporter(2) EamA family protein(2) putative integral membrane protein(2) "EamA family transporter, putative(1)" protein of unknown function DUF6 transmembrane(1) fragment of truncated conserved hypothetical protein(1) Hypotheticalprotein(1) "transporter,EamAfamily(1)" "Transporter, drug/metabolite exporter family protein(1)" FIG01108553: hypothetical protein(1) truncated conserved hypothetical protein(1)

480 2 142 1.408450704 2 0.5867 >Cluster 330 hypothetical protein(32) "Phosphoesterase, DHH family protein(11)" putative membrane protein(7) conserved hypotheticalprotein(5) DHHA1domainprotein(5) DHHsubfamily phosphodiesterase(4) DHH family protein(4) putative bifunctional signaling protein/50S ribosomal protein L9(4) diguanylate cyclase and phosphoesterase(2) "phosphoesterase, DHH family protein(2)" phosphoesterase RecJ domain protein(1) DHH subfamily 1 protein(1) DHH superfamily 1 protein(1) "DHH family phosphoesterase, putative(1)"

481 2 142 1.408450704 2 0.5867 >Cluster 388 hypothetical protein(38) Nitric oxide reductase activation protein NorD(8) conserved hypothetical protein(7) cobalamin biosynthesis CobT VWA domain protein(6) von Willebrand factor type A(4) nitric oxide reductase activation protein NorD(4) von Willebrand factor type A domain protein(3)von Willebrand factor domain-containing protein(3) von Willebrand factor(2) nitric oxide reductase activation NorD domain protein(2) "von Willebrand factor, type A(1)" Von Willebrand factor type A domain protein(1) von Willebrand factor type A domain-containingprotein(1) "nitric oxide reductase activation protein, putative(1)"

482 2 142 1.408450704 2 0.5867 >Cluster 522 heme ABC transporter ATP-binding protein(24) Duplicated ATPase component MtsB of energizing module of methionine-regulated ECF transporter(6) ABC transporter ATP-binding protein(6) "heme ABC exporter, ATP-binding protein CcmA(5)" Putative ABC transporter ATP-binding protein(3) ABC transporter family protein(3) "ABC transporter, ATP-binding protein(3)" Cobalt/nickel transport system ATP-binding protein(2) cobalt ABC transporter ATP-binding protein(2) "ABC-type cobalt transport system, ATPase component(2)" cobalt ABC transporter ATPase(2) "putative cobalt ABCtransporter,ATP-bindingprotein(2)" ABCtransporterrelated(1)Duplicated ATPase component MtsB(1) Cobalt transport ATP-binding protein cbiO(1) "Cobalt transport family ABC transporter, ATP-binding protein, putative(1)" hypothetical protein(1) MW2603(1) "ABC transporter, ATP-binding protein, putative(1)" "ABC superfamily ATP-binding cassette transporter, membrane protein(1)" ATP-binding ABC transporter(1)

483 2 142 1.408450704 2 0.5867 >Cluster 541 teichoic acid biosynthesis protein(30) Putative polyribitolphosphotransferase(13) Poly(glycerophosphate) glycerophosphotransferase family protein(7) hypothetical protein(6) putative polyribitolphosphotransferase(3) CDP-Glycerol:Poly(glycerophosphate) glycerophosphotransferase family protein(3) CDP-glycerol:poly(Glycerophosphate) glycerophosphotransferase(2) putative teichoic acid biosynthesis protein B(2) teichoic acid biosynthesis protein B(2) glycosyl glycerophosphate transferase involved in teichoic acid biosynthesis(2)CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase(2) "teichoic acid biosynthesis protein, putative(2)" glycosyl/glycerophosphate transferase(1) conserved hypotheticalprotein(1) putativeCDP-Glycerol:Poly(glycerophosphate)glycerophosphotransferase(1) MW0229(1) putative(1)" conserevd hypothetical protein(1) ribitolphosphotransferase(1)

484 2 142 1.408450704 2 838 PTSglucosetransportersubunitIIBC(30) acetylglucosamine-specific IIBC component(10)" acetylglucosamine-specific IIABC component(8)" component(7) "phosphotransferase system, N-acetylglucosamine-specific IIBC component(4)" specific EIICB component(3) specific IIABC component(2) specific IIA component(2)" specific IIB component / PTS system, N-acetylglucosamine-specific IIC component(2)" PTS system component(2) "phosphotransferase system,PTS system N-acetylglucosamine-PTS system N-acetylglucosamine-

"PTS system, N-acetylglucosamine-"PTS system, N-acetylglucosamine-"tagB protein,CDP-ribitol0.5867 >Cluster "PTSsystem,N- "PTS system, N- PTS system IIBCEIICfamilyprotein(1)" PTSfamilyportercomponentIIBC(1) N- acetylglucosamine-specific IIA component(1) "PTS system, N- acetylglucosamine-specific IIB component / PTS system,N- acetylglucosamine-specific IIC component(1)" "PTS system, N- acetylglucosamine-specific IIA component / PTS system, N- acetylglucosamine-specific IIB component / PTS system, >... at 98.57%(1)" "PTS system, glucose-like IIB component domain protein(1)" PTS system transporter subunit IIBC(1) "PTS system, N-acetylglucosamine-specific IIBC subunit(1)" "PTS system, IIBC components(1)" "pts system, N-acetylglucosamine- specific iibc component(1)" conserved hypothetical protein(1)

485 2 142 1.408450704 1023 cell division protein FtsK(8) protein(5) hypotheticalprotein(4)2 0.5867 >Cluster ftsk/spoiiie family putativecouplingprotein(3) FtsK/SpoIIIE familyAAA-like domain protein(2) protein(2) ftsK/SpoIIIE family protein(2) FtsK/SpoIIIE family cell division protein(2) FIG01108595: hypothetical protein(1) Hypothetical protein(1)

486 2 142 1.408450704 2 0.5867 >Cluster 1197 MFS sugar transporter(28)Sugar efflux transpoter(10) putative sugar efflux transporter(7) major Facilitator Superfamily protein(7) Sugar efflux transporter A(4) "sugar efflux transporter, putative(3)" sugar effluxtransporter(3) "MFS family major facilitator transporter, sugar:cation symporter(2)" MFS family major facilitator transporter(2) major facilitator superfamily permease(2) major facilitator superfamily protein superfamily(1) probable sugar efflux transporter(1) major facilitator superfamily protein(1) hypotheticalprotein(1) conservedhypotheticalprotein(1)major facilitator superfamily MFS_1(1) MW0635(1) "transporter, major facilitator family protein(1)" MFS/sugar transport family protein(1) sugar efflux transpoter(1)

487 2 142 1.408450704 2 0.5867 >Cluster 1278 DNA repair protein(30) ImpB/MucB/SamB family protein(13) DNA polymerase IV(7) impB/mucB/samB family protein(5) ImpB/MucB/SamB family DNA-damage repair protein(5) DNA polymerase V(4) DNA-directed DNA polymerase(3) IMS HHH motif family protein(3) hypotheticalprotein(2) ImpB/MucB/SamBfamilyDNAdamagerepair protein(2) MW1251(1) conserved hypothetical protein(1) "DNA polymerase V, umuC subunit, putative(1)" probable DNA damage repair protein(1) DNA-directed DNA polymerase subunit umuC(1) impB/mucB/samB C-terminal family protein(1) DNA-damage repair protein(1)

488 2 142 1.408450704 2 0.5867 >Cluster 1289 molybdopterin molybdenumtransferase(37) molybdopterinbiosynthesis protein MoeA(9) Molybdopterin biosynthesis protein MoeA(8) Molybdopterin molybdenumtransferase(6) putative molybdenum cofactor biosynthesis protein(6) molybdopterin biosynthesis protein moeA(3) molybdopterin biosynthesis protein(2) molybdenum cofactor synthesis domain protein(2) "molybdopterin biosynthesis MoeA protein, putative(1)" "molybdopterin biosynthesis protein moeA, putative(1)" Molybdopterin biosynthesis proteinA(1) "molybdopterin biosynthesis protein A MoeA, putative(1)" Molybdopterin biosynthesis MoeA protein(1)molybdopterin biosynthesis MoeA protein(1) molybdenum cofactor synthesis domain(1)

489 2 142 1.408450704 2 0.5867 >Cluster 1349 serine hydroxymethyltransferase(52) Serine hydroxymethyltransferase(16) serine hydroxymethyl transferase(6)"serine hydroxymethyltransferase, putative(2)" glycine hydroxymethyltransferase(2) Glycine hydroxymethyltransferase(1)serine hydroxymethyltransferase (Serine methylase)(SHMT)(1)

490 2 142 1.408450704 2 0.5867 >Cluster 1463 nucleotide pyrophosphohydrolase(30) putative tetrapyrrole methyltransferase domain / Nucleoside triphosphate pyrophosphohydrolase MazG(6) tetrapyrrole (Corrin/Porphyrin) Methylases family protein(5) nucleoside triphosphate pyrophosphohydrolase(4) conservedhypotheticalprotein(4) tetrapyrrolemethylasefamilyprotein(4) mazGnucleotide pyrophosphohydrolase domain protein(3) putative tetrapyrrole (corrin/porphyrin) methylases(3) putative tetrapyrrole (corrin/ porphyrin) methylase(3) tetrapyrrole methylase(2)MazG nucleotide pyrophosphohydrolase(2) tetrapyrrole(corrin/porphyrin)methylase(2)possible tetrapyrrole methyltransferase domain / Nucleoside triphosphate pyrophosphohydrolase MazG(2) Putative tetrapyrrole methylase(1) Nucleoside triphosphate pyrophosphohydrolase(1) Uroporphyrin-III C-methyltransferase(1) probable tetrapyrrole methylase(1) possible tetrapyrrole methyltransferase(1)tetrapyrrole methylase/MazG family protein(1)pyrophosphohydrolase domain protein(1)

491 2 142 1.408450704 2 1568 hypothetical protein(12) nuclease(9) protein(4) PD-(D/E)XK nuclease superfamily protein(3)Phage protein(3) hypothetical phage protein(2) conserved phage-associated protein(2)P51-like protein(1)protein(1)

492 2 142conserved hypothetical protein(1) phi APSE phage protein(1) hypothetical bacteriophage1.408450704 2 0.5867 >ClusterMazG nucleotide0.5867 >Cluster putative phage1589 hydrolase(36) hippurate hydrolase(8) "N-acetyl-L,L- diaminopimelate deacetylase(8)" amidohydrolase family protein(8) putative peptidase(8) putative zinc peptidase(4) amidohydrolase(2) peptidase(1) M20D subfamily hippurate hydrolase(1) M20/M25/M40 family peptidase(1) "peptidase, M20 family(1)" conserved hypothetical protein(1) N- acetyldiaminopimelate deacetylase(1)

493 2 142 1.408450704 2 0.5867 >Cluster 1602 queuine tRNA-ribosyltransferase(59) tRNA-guanine transglycosylase(12) Queuine tRNA-ribosyltransferase(7) tRNA guanosine(34) transglycosylase Tgt(3)tRNA-guanine transglycosylase family protein(1)

494 2 142 1.408450704 2 0.5867 >Cluster 1645 DNA-binding protein(14) SAP domain protein(13) SAP domain- containing protein(5) hypothetical protein(3) conserved hypotheticalprotein(1) putativeDNA/RNAbindingprotein(1) putative sAP domain protein(1)

495 2 142 1.408450704 2 0.5867 >Cluster 1761 chorismate mutase(33) bifunctional 3-deoxy-7- phosphoheptulonate synthase/chorismate mutase(10) DAHP synthetase- chorismate mutase(8) Chorismate mutase I / 2-keto-3-deoxy-D- arabino-heptulosonate-7-phosphate synthase I beta(5) protein AroA(5) phospho-2-dehydro-3-deoxyheptonate aldolase(2) AroA(G)- like protein(2) 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase/ chorismate mutase(2) 3-deoxy-7-phosphoheptulonate synthase(2) Chorismate mutase I(2) bifunctional chorismate mutase/3-deoxy-7-phosphoheptulonate synthase(1) bifunctional 3-deoxy-7- phosphoheptulonate synthase(1) "Chorismate mutase I / 2-keto-3- deoxy-D-arabino-heptulosonate-7-phosphate synthase I beta, AroH/AroA I beta(1)" MW1680(1) 3-phosphoshikimate 1- carboxyvinyltransferase(1) bifunctional 3-deoxy-7- phosphoheptulonate synthase/Chorismate mutase I(1) chorismate mutase type II family protein(1) conserved hypothetical protein(1) protein aroA(1) chorismate mutase homolog(1) Chorismate mutase I / 2- keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase I beta #

AroH/AroAI beta(1)

496 2 142 1.408450704 2 0.5867 >Cluster 1764 replication initiation factor family protein(12) replication initiation factor(9) "Transcriptional regulator, Cro/CI family protein transposon-related(3)" Relaxase(2) "Transcriptional regulator, Cro/CI family protein transposon-related protein(1)"conservedhypotheticalprotein(1)hypotheticalprotein(1) possible replication initiation protein(1)

497 2 142 1.408450704 2 0.5867 >Cluster 1817 thioredoxin reductase(31)ferredoxin--NADP reductase(10) Thioredoxin reductase(9) pyridine nucleotide-disulfide oxidoreductase family protein(5) pyridine nucleotide-disulfide oxidoreductase(5) Ferredoxin--NADP reductase(4) pyridine nucleotide-disulphide oxidoreductase family protein(4) FAD- dependent pyridine nucleotide-disulfide oxidoreductase(2) FAD- dependent pyridine nucleotide-disulphideoxidoreductase(1) "thioredoxin reductase, putative(1)" probable thioredoxin reductase(1) Pyridine oxidoreductase(1) MW2294(1) possible thioredoxin-disulfide reductase(1) pyridine nucleotide-disulphide oxidoreductase(1) conserved hypotheticalprotein(1) hypotheticalprotein(1)

498 2 142 1.408450704 2 0.5867 >Cluster 1872 glycosyltransferase(31) teichoic acid biosynthesis protein X(11) Teichoic acid biosynthesis protein X(10) putative glycosyl transferase(6) putative glycosyltransferase tagX(4) Putative glycosyltransferase TagX(3) putative glycosyltransferase TagX(3)putative glycosyltransferase(2) glycosyl transferase tagX(2)teichoic acid biosynthesis enzyme(1) glycosyl transferase family 2 family protein(1) "teichoic acid biosynthesis glycosyltransferase, putative(1)" glycosyltransferase tagX(1) "tagX protein, putative(1)" "glycosyltransferase, group 2 family protein(1)" glycosyl transferase family 2(1)

499 2 142 1.408450704 2 0.5867 >Cluster 1922 mannosyl-glycoprotein endo-beta-N-acetylglucosamidase(8) mannosyl-glycoendo-beta-N-acetylglucosaminidase family protein(5) Secretory antigen SsaA-like protein transposon-related(3) putative cell wall lysis protein(3) putative exported protein(2) hypotheticalprotein(2) transfercomplexproteinTraG(2) Hypotheticalprotein(1) putativemannosyl-glycoendo-beta-N-acetylglucosaminidase domain protein(1) Putative TraG protein(1) putative traG membrane protein(1)

500 2 142 1.408450704 2 0.5867 >Cluster 1996 molybdenum cofactor biosynthesis protein A(41) cyclic pyranopterinphosphatesynthaseMoaA(10) Molybdenumcofactor biosynthesis protein MoaA(7) putative molybdenum cofactor biosynthesis protein A(7)Molybdenum cofactor biosynthesis protein A(4)cyclic pyranopterin phosphate synthase(3) Cyclic pyranopterin monophosphate synthase(3)cyclic pyranopterin monophosphate synthase(3)cyclic pyranopterin monophosphate synthase domainprotein(2)putative(1)"

501 2 2173 gamma-hemolysin subunit B(30) B(17) gamma-hemolysin component B(10) precursor(5) "gamma-hemolysin component C precursor, HlgB(4)" leukocidin-Fsubunit(3) "gammahemolysin,componentB(2)"gamma- hemolysin component B protein(2) beta-channel forming cytolysin(1) gamma-hemolysincomponentBprecursor(1) gammahemolysincomponent B(1) leukocidin f subunit precursor(1) Gamma-hemolysin B subunit HlgB(1) "leukocidin-F subunit (Gamma-hemolysin, H-gamma-I subunit) (1)" "leukocidin F subunit (Gamma-hemolysin, H-gamma-I subunit)(1)"

502 2 142 1.408450704 2 0.5867 >Cluster 2177 ABC transporter substrate-binding protein(30) transport system extracellular binding lipoprotein(9) "Heme ABC type transporter HtsABC, heme-binding protein(8)" periplasmic binding family protein(7) ferrichrome ABC transporter subunit(5) periplasmic binding protein(5) Iron complex transport system substrate-bindingprotein(2) hypotheticalprotein(2) conserved hypotheticalprotein(2) probableirontransportprotein(2)cobalamin Fe3+-siderophores ABC transporter periplasmic protein(2) "Fe3 -citrate ABC transporter, substrate-binding lipoprotein, putative(1)""possible iron (Fe3+) ABC superfamily ATP binding cassette transporter, binding protein(1)" "iron compound ABC transporter, iron compound-binding protein(1)" ferrichrome ABC transporter(1) MW0921(1) Iron(III) dicitrate-binding protein(1)

503 2 142 1.408450704 2 0.5867 >Cluster 2473 pseudouridine synthase(30) ribosomal large subunit pseudouridine synthase D(9) "pseudouridine synthase, RluA family protein(8)" Ribosomal large subunit pseudouridine synthase D(8)putativeRNApseudouridylatesynthase(6) RluDsubfamily ribosomal large subunit pseudouridine synthase(5) conserved hypotheticalprotein(4) "pseudouridinesynthase,RluA family(3)" "ribosomal large subunit pseudouridine synthase, RluD subfamily(2)" pseudouridylate synthase RluD(1) Ribosomal large subunit pseudouridine synthase(1) 23S rRNA pseudouridine(1911/1915/1917) synthase(1) Pseudouridine synthase(1) pseudouridylate synthase(1)

504 2 142 1.408450704 2 0.5867 >Cluster 2900 cytochrome C assembly protein(32) cytochrome C assembly family"molybdopterin cofactor biosynthesis protein A,molybdenum (Mo2+) cofactor biosynthesis protein A(1)142 1.4084507042 0.5867 >Cluster Gamma-hemolysin component gamma-hemolysin component Cprotein(8) putative membrane protein(6) "HemX protein, negative effector of steady-state concentration of glutamyl-tRNA reductase(5)" cytochrome c assembly protein(5) hemX protein(4) HemA concentration negative effector HemX(4) hemA concentration negative effector hemX(4)HemX family protein(2) hemA concentration negative effector(2) "HemX protein, negative effector of steady- state concentration of glutamyl-tRNA reductase hemA(1)" membrane uroporphyrinogen III methylase(1) conserved hypothetical protein(1) hypothetical protein(1) Putative heme export permease(1) Cytochrome c assembly protein(1) possible cytochrome c assembly protein(1) HemA concentration negative effector hemX(1)

505 2 142 1.408450704 2 0.5867 >Cluster 2933 hypothetical protein(46) lipoprotein(3) putative lipoprotein(2) conserved hypothetical protein(2) staphylococcus tandem lipoproteins family protein(1) FIG01107907: hypothetical protein(1) putative membrane protein(1) possible staphylococcal lipoprotein(1) Membrane lipoprotein(1) putative staphylococcal lipoprotein(1) putative sa lipoprotein(1) staphylococcal tandem lipoprotein(1) tandem lipoprotein(1) staphylococcal lipoprotein(1) staphylococcus tandem lipoproteins(1)

506 2 142 1.408450704 2 0.5867 >Cluster 3111 transcriptional regulator(21) HTH-type transcriptional regulator SarU(7) HTH-type transcriptional regulator sarU(5) Transcriptional regulator SarU (Staphylococcal accessory regulator U) (5) Transcriptional regulator SarU(5) staphylococcal accessory regulator U(2) transcriptional regulator SarU(2) staphylococcal accessory regulator family(1) marR familyprotein(1) "staphylococcal accessory regulator A, SarH1(1)" conserved hypothetical protein(1) sarH2(1) "sa accessory regulator A, SarH1(1)" Staphylococcal accessory regulator-like protein (SarU/SarH2)(1) Staphylococcal accessory regulator U(1) "accessory regulator A, SarH1(1)" staphylococcal accessory

regulator A homolog(1) putative staphylococcal accessory regulator(1)

507 2 142 1.408450704 2 0.5867 >Cluster 3218 peptide ABC transporter ATP-binding protein(30) ABC transporter family protein(8) oligopeptide transporter putative ATPase domain(7) "ABC superfamily ATP binding cassette transporter, ABC protein(5)" Nickel transport ATP-binding protein NikE(5) ABC transporter(4) peptide transport system ATP-bindingprotein(3) "peptide ABC transporter, ATP-binding protein(3)" oligopeptide transporter putative ATPase domain protein(3) "oligopeptide transport ATP-binding protein, putative(2)" oligopeptide transporter ATPase subunit(2)Nickel transport ATP-binding protein nikE(2) Oligopeptide transporter putative ATPase domain(1)"oligopeptide ABC transporter, ATPase domain,putative(1)" "ABC transporter, ATP-binding protein(1)" ABCtransporter related(1)domain protein(1)protein, putative(1)"

508 2 142 3306 hypothetical protein(25) membrane protein(16) putative membrane protein(8) ABC-2 transporter family protein(7) ABC-2 type transport system permease protein(6) ABC transporter permease(5) conserved hypothetical protein(4) ABC-2 type transportsystempermease(2) Hypotheticalprotein(1) probable membrane protein(1) "membrane protein, putative(1)" FIG01108144: hypothetical protein(1) PSM ABC-transporter PmtD subunit(1) hypothetical membrane protein(1) Phenol-soluble modulin export ABC transporter permease PmtD(1)

509 2 142 1.408450704 2 0.5867 >Cluster 3590 N-(5'-phosphoribosyl)anthranilate isomerase(40) N- (5'phosphoribosyl)anthranilate (PRA) isomerase family protein(8) Phosphoribosylanthranilate isomerase(7) N- (5'phosphoribosyl)anthranilate isomerase(6) phosphoribosylanthranilate isomerase(4) N- (5'phosphoribosyl)anthranilate (PRA) isomerase(3) transcription termination/antitermination factor NusG(2)phosphoriborylanthranilate isomerase(2) hypothetical protein(2) "N- (5'phosphoribosyl)anthranilate isomerase, putative(1)" N-5'-phosphoriborylanthranilate isomerase(1) N-5'-phosphoribosyl- anthranilate isomerase(1)N-(5'phosphoribosyl)anthranilate (PRA)isomerase(1) Phosphoribosyl anthranilate isomerase(1)

510 2 142 1.408450704 2 0.5867 >Cluster 3645 nitrate reductase(28) "respiratory nitrate reductase, gamma subunit(17)" putative nitrate reductase gamma chain(10) Respiratory nitrate reductase gamma chain(10) nitrate reductase gamma chain(5) respiratory nitrate reductase subunit gamma(4) Nitrate reductase gamma chain(3) respiratory nitrate reductase gamma subunit protein(2) nitrate reductase gamma subunit(1)

511 2 142 1.408450704 2 0.5867 >Cluster 3891 hypothetical protein(55) conserved hypothetical protein(10)DUF1801 domain-containing protein(4) "bacteriocin- protection, YdeI/OmpD-Associated family protein(3)" Hypotheticalprotein(1) DomainofunknownfunctionDUF1801(1) domain of unknown function (DU1801) family protein(1) hypothetical protein with DUF419(1) 60 kda chaperonin groel(1)Oligopeptide transporter putative ATPase"peptide ABC transporter, ATP-binding1.408450704 2 0.5867 >Cluster

512 2 142 1.408450704 2 0.5867 >Cluster 3899 dephospho-CoA kinase(50) Dephospho-CoA kinase(16) putative dephospho-CoA kinase(11) conserved hypothetical protein(2) dephospho- CoA kinase domain protein(1) probable dephospho-CoA kinase(1)

513 2 142 1.408450704 2 5239 camphor resistance protein CrcB(33) protein(9) crcB-like family protein(7) ProteinCrcB2(3) hypotheticalprotein(2) crcBprotein(2) camphor resistance protein CrcB2(2) camphor resistance protein CrcB_1(2) conserved hypothetical protein(1) Putative fluoride ion transporter CrcB(1) putative protein CrcB(1) protein crcB(1) putative Protein CrcB homolog 2(1) camphor resistance protein(1) Protein crcB 2(1) Putative fluoride ion transporter CrcB 2(1) CrcB family protein(1) probable membrane protein(1) Camphor resistance CrcB protein(1) camphor resistance protein CrcB1(1) crcB family protein(1)

514 2 142 1.408450704 2 0.5867 >Cluster 5302 general stress protein(32) "RNA binding protein, containsribosomalproteinS1domain(9)" S1RNAbindingdomain protein(8) S1 RNA-binding domain-containing protein(5) S1RNA-binding domain protein(4) hypothetical protein(3) hypotheticalprotein(3) Generalstressprotein13(3) RNA binding domain(3) general stress protein 13(3) RNA binding domain protein(3) S1-type RNA-binding domainconserved putativeS1 putative S1"S1 RNA binding domain protein, putative(1)"RNA binding S1 domain protein(1)

515 2 5510 pathogenicity island protein(25) hypothetical protein(5) hypothetical protein in superantigen-encoding pathogenicity islands SaPI(3) putative mobile element-associated protein(2) Hypothetical protein in superantigen-encoding pathogenicity islands SaPI(1) hypothetical mobile element-associated protein(1) putative SAV0795-like protein in superantigen-encoding pathogenicity islands SaPI(1) bovine pathogenicity island protein Orf10(1)protein(1)MW0837(1)142 1.408450704 2 0.5867 >Clusterpathogenicity island protein ORF10(1)protein(1)

516 6 142 4.225352113 1333 acyl-CoA dehydrogenase(41) Butyryl-CoA dehydrogenase(6) "acyl-CoA dehydrogenase, C-terminal domain protein(5)" conserved hypothetical protein(4) acyl-CoA0.5867 >Cluster CrcB-like CrcB protein(5)mobile element-associated3 0.5993 >Cluster hypothetical protein(7)dehydrogenase-related protein(3) "Acyl-CoA dehydrogenase, short-chain"acyl-CoA dehydrogenase, N-terminal domain "acyl-CoA dehydrogenase, middle domain protein(2)" butyryl-CoA dehydrogenase(1) butyryl-CoAspecific(3)" protein(3)" MW2203(1) dehydrogenase homolog(1) Acyl-CoA dehydrogenase type 2domain(1)protein(1)"

517 6 1382 malate dehydrogenase(32) NAD-dependent malic enzyme(11) NADP-dependent malic enzyme(9) "malic enzyme, NAD binding domain protein(7)" malate dehydrogenase (oxaloacetate-decarboxylating) (NADP(+))(5) putative NAD-dependent malic enzyme 4(3) "NADP-dependent malic enzyme, putative(3)" NAD-dependent malic enzyme 4(3) NAD-dependent malic enzyme (NAD-ME)(2) Malate dehydrogenase (oxaloacetate-decarboxylating)(1) NADP-dependent malic enzyme (NADP-ME). domain protein(1) "NADP-dependent malic enzyme, malate dehydrogenase(1)" MW1645(1) glutamate/Leucine/"acyl-CoA dehydrogenase, domain"acyl-CoA dehydrogenase, putative(1)"142 4.225352113 4 0.5993 >ClusterPhenylalanine/Valine dehydrogenase family protein(1)dehydrogenase homolog(1)

518 6 142 4.225352113 2 0.5993 3779 GTP pyrophosphokinase(56) hypothetical protein(5) domain protein(3) GTP-pyrophosphokinase family protein(3) GTP pyrophosphokinase YjbM(3)RelA/SpoT domain-containing protein(2) conserved hypothetical protein(2) GTP pyrophosphokinase RelA1(1) GTP diphosphokinase(1) GTP pyrophosphokinase yjbM(1) "GTP pyrophosphokinase, putative(1)" RelA / SpoT s family protein(1) RelA/SpoT family protein(1) region found in RelA / SpoT proteins family protein(1)

519 14 142 9.85915493 6 0.6035 >Cluster 761 malate:quinone oxidoreductase(32) putative malate:quinone oxidoreductase 2(14) Malate:quinone oxidoreductase(12) malate dehydrogenase(7) malate dehydrogenase (acceptor)(5)malate:quinone oxidoreductase 2(4) Malate:quinone oxidoreductase 2(2) malate:quinone-oxidoreductase(1) malate dehydrogenase (quinone)(1) probable malate:quinone oxidoreductase(1) Probable malate:quinone oxidoreductase 2(1)malate/quinone oxidoreductase(1) malate--quinone oxidoreductase(1)

520 7 142 4.929577465 2 0.6101 >Cluster79 carbamoyl phosphate synthase large subunit(34) Carbamoyl-phosphate synthase large chain(16) "putative carbamoyl- phosphate synthase, pyrimidine-specific, large chain(8)" "carbamoyl- phosphate synthase, large subunit(7)" carbamoyl-phosphate synthasemalate>ClusterRelA/SpoTlarge chain(7) carbamoyl-phosphate synthase large subunit protein(2) "putative carbamoyl-phosphate synthase,pyrimidine- specific, large chain(2)""carbamoyl-phosphate synthase (glutamine- hydrolyzing), large subunit(1)" carbamoyl-phosphate synthase pyrimidine-specific large chain (Carbamoyl-phosphate synthetase ammonia chain)(1) carbamoyl-phosphate synthase small chain(1) "carbamoyl-phosphate synthase, large subunit PyrAB(1)" "carbamoyl-phosphate synthase, pyrimidine-specific, large chain(1)"

521 7 142 4.929577465 2 0.6101 >Cluster 572 histidine kinase(30) alkaline phosphatase synthesis sensor protein(12) sensory box protein(8) alkaline phosphatase synthesis sensor protein PhoR(6) Phosphate regulon sensor protein PhoR (SphS)(5) Phosphate regulon sensor protein PhoR(4) sensor histidine kinase(3) sensory box histidine kinase PhoR(2) "histidine kinase-, DNA gyrase B-, and HSP90-like ATPase family protein(2)" Alkaline phosphatase synthesis sensor protein phoR(2) multi-sensor signal transduction histidinekinase(2) "sensory box histidine kinase PhoR, putative(1)" "Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase domain protein(1)"PAS sensor protein(1) PAS domain S-box protein(1) PhosphateregulonsensorproteinphoR(1) Phosphate regulon sensor protein PhoR SphS(1)

522 7 142 4.929577465 2 0.6101 >Cluster 2107 cation transporter(34) zinc resistance protein(8) Zn(II) and Co(II) transmembrane diffusion facilitator(7) "cadmium, cobaltandzinc/H(+)-K(+)antiporter(7)" cationdiffusionfacilitator family transporter(6) cation efflux family protein(4) "cation efflux system protein, CDF family(2)" cobalt-zinc-cadmium resistance protein czcD(2) zinc transporter zitB(1) "cation efflux family protein, putative(1)" zinc transporter ZitB(1) Cobalt-zinc-cadmium resistance protein czcD(1) Cobalt-zinc-cadmium resistance protein CzcD(1) "cation-efflux system membrane protein, putative(1)" cation-efflux system membrane protein(1) czrB(1) CDF family cation diffusion facilitator CzrB(1) cation- efflux system membrane protein homolog(1)

523 7 142 4.929577465 2 0.6101 >Cluster 5757 hypothetical protein(35) conserved hypothetical protein(7) Phage protein(7) phage protein(4) hypothetical phage protein(3) phi SLT orf 99-like protein(3) hypothetical phage-related protein(3) putative phage protein(3)"Hypothetical protein, SLT orf99(1)" hypothetical bacteriophage protein(1) conserved hypothetical phage protein(1)

524 7 142 4.929577465 2 0.6229 >Cluster1975 iron ABC transporter substrate-binding protein(30) lipoprotein(11) Iron compound ABC uptake transporter substrate- bindingprotein(8) hypotheticalprotein(6) ironcomplex transport system substrate-binding protein(4) transferrin receptor(4) ABC-type siderophore binding protein(4) periplasmic binding protein(3) ferrichrome ABC transporter lipoprotein(2) cobalamin Fe3+-siderophores ABC transporter periplasmic protein(2) Ferric anguibactin-binding protein(1) conserved hypothetical protein(1) MW0698(1) "iron compound ABC transporter, siderophore-binding lipoprotein, putative(1)" periplasmic binding family protein(1) "iron (Fe+3) ABC superfamily ATP binding cassette transporter, binding protein(1)"

525 7 142 4.929577465 5 0.6229 >Cluster 1978 lactate dehydrogenase(29)D-lactate dehydrogenase(21) D- isomer specific 2-hydroxyacid dehydrogenase(10) D-isomer specific 2-hydroxyacid dehydrogenase family protein(6) "D-lactate dehydrogenase, putative(2)" D-lactate dehydrogenase (D-LDH) (D- specific D-2-hydroxyacid dehydrogenase)(2)(R)-2-hydroxyacid dehydrogenase(1) D-isomer specific 2-hydroxyacid dehydrogenase NAD- binding(1) probable D-specific D-2-hydroxyacid dehydrogenase(1)MW2480(1) hypotheticalprotein(1) D-specificD-2-hydroxyacid dehydrogenase ddh(1) D-specific D-2-hydroxyacid dehydrogenase ddh homolog(1) D-2-hydroxyacid dehydrogenase(1) D- specific D-2-hydroxyacid dehydrogenase-like protein(1)

526 7 142 4.929577465 3 0.6229 >Cluster3133 Exotoxin 6(7) superantigen-like protein 11(7) exotoxin(5) exotoxin 6(3) Exotoxin(2) conserved hypothetical protein(2) staphylococcal exotoxin 11(1) set16(1) staphylococcal enterotoxin- like toxin(1) staphylococcal exotoxin(1)

527 2 142 1.408450704 2 0.6377 >Cluster 443 elongationfactor4(30) GTP-bindingproteinLepA(21) Translation elongation factor LepA(10) putative GTP-binding protein(7) Elongation factor 4(6) GTP-binding protein lepA(3)GTP-binding protein(2) conserved hypothetical protein(1) "elongation factor Tu GTP binding domain-containing protein, putative(1)" GTP binding protein protein(1) GTP-binding LepA family protein(1)

528 2 142 1.408450704 2 0.6377 >Cluster 584 peptide ABC transporter substrate-binding protein(29) transport system extracellular binding lipoprotein(11) "Oligopeptide ABC transporter, periplasmichypothetical protein(21) superantigen-like protein(20)oligopeptide-binding protein OppA(6)" "bacterial extracellular solute-binding, 5 Middle family protein(4)" "oligopeptide ABC superfamily ATP binding cassette transporter, binding protein(4)" "bacterial extracellular solute-binding s, 5 Middle family protein(3)" 4-phytase(3) peptide/nickel transport system substrate-binding protein(2) oligopeptide transport systemsubstrate-bindingproteinOppA(2) putativephagehead-tail adaptor(1) "Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein oppA(1)" "bacterial extracellular solute-binding proteins, family 5 Middle family protein(1)"conserved hypotheticalprotein(1) hypotheticalprotein(1) oligopeptideABC transporter oligopeptide-binding protein(1)MW0872(1) "oligopeptide ABC transporter, oligopeptide-binding protein(1)" "Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1) (1)" "oligopeptide ABC superfamily ATP binding cassette transporter, binding protein, Opp3A(1)" oligopeptide binding protein(1) Oligopeptide-binding proteinoppA(1) "oligopeptide ABC transporter, substrate-binding protein, putative(1)" "oligopeptide ABC transporter, substrate-binding lipoprotein oppA, putative(1)"

529 2 142 1.408450704 2 0.6377 >Cluster 769 hypothetical protein(30) membrane protein(22) putative membrane protein(16) conserved hypothetical protein(3) truncated hypotheticalprotein(2) "membraneprotein,putative(2)" Glucosyltransferase (side chain biosynthesis)(1) hypothetical membraneprotein(1) Hypotheticalprotein(1) putativemembrane protein(pseudogene)(1) FIG01107966:hypotheticalprotein(1) probable membrane protein(1)

530 2 142 1.408450704 2 0.6377 >Cluster 914 transposase(35) hypothetical protein(15) conserved hypotheticalprotein(7) putativemembraneprotein(6) LXGdomain of WXG superfamily protein(6) Ribonuclease(2) truncated hypotheticalprotein(2) membraneprotein(2) probablemembrane protein(1) unnamed protein product(1) Phage protein(1) transposase family protein(1) hypothetical fig|282458.1.peg.573 homolog(1) Hypothetical protein(1)

531 2 142 1.408450704 2 0.6377 >Cluster 975 GntR family transcriptional regulator(34) GntR family regulatory protein(8) putative transcriptional regulator of pyridoxine metabolism(7) "bacterial regulatory s, gntR family protein(4)" aminotransferase class I and II family protein(2) putative GntR family transcriptional regulator(2) "transcriptional regulator, GntR family(2)" "putative transcriptional regulator, GntR family(2)" Predicted transcriptional regulator of pyridoxinemetabolism(2) transcriptional regulator of GntR family(2) transcriptional regulator(2) gntR family regulatory protein(1) "transcription regulator, GntR family(1)" "bacterial regulatory, gntR family protein(1)" gntR family transcriptional regulator(1) conserved hypothetical protein(1) hypothetical protein(1) regulatory protein GntR HTH(1) "bacterial regulatory proteins, gntR family protein(1)"MW0473(1)protein(1)"

532 2 1509 amidohydrolase(38) putative peptidase(8) amidohydrolase family protein(6) HmrA protein involved in methicillin resistance / amidohydrolase of M40 family(5) "peptidase, M20/ M25/M40 family(4)" Aminopeptidase(3) HmrA protein(3)"transcriptional regulator, gntR family"Transcriptional regulator, GntR family(1)"142 1.408450704 2 0.6377 >Clusteramidase(1)peptidase(1)putative(1)"resistance / amidohydrolase related protein, HmrA(1)"hmrA protein(1) M20D subfamily "aminobenzoyl-glutamate utilization protein B, "amidohydrolase, putative(1)" "methicillinhypotheticalprotein(1) family peptidase(1) amidohydrolase(1) domain protein(1)

533 2 142 1526 ABC transporter permease(9) "ABC transporter, permease protein(6)" permease family protein(1) Macrolide export ATP-binding/permease protein MacB(1) hypothetical protein(1) putative permease protein(1) protein of unknown function DUF214(1) truncated hypothetical protein(1) probable ATP- binding ABC transporter(1) MW0174(1)

534 2 142 1.408450704 2 0.6377 >Cluster 1544 glycosyl transferase family 1(30) galactosyl transferase(9) Exopolysaccharide biosynthesis glycosyltransferase EpsF(8) glycosyl transferases group 1 family protein(5) "glycosyl transferase, group 1 family protein(4)" glycosyltransferase Family 4 family protein(3) Glycosyltransferase(3) glycoside hydrolase family protein(3) glycosyltransferase(2) glycosyltransferasegroup1 family protein(2) group 1 glycosyl transferase(2) "Beta-D- Glcpalpha-1,6-galactosyltransferase(1)" "glycosyltransferase,group 1 family protein(1)" glycosyl transferase group 1(1) MW0104(1)"capsular glycosyltransferase, putative(1)" hypotheticalprotein(1) conservedhypotheticalprotein(1) glycosyltransferase(1) probable capsular glycosyltransferase(1)

535 2 142 1.408450704 2 0.6377 >Cluster 2014 leucotoxin LukD(35) Leukotoxin LukD(7) lukF-PV(6) Panton-Valentine leukocidin chain F precursor(4) Leucotoxi conservedhypotheticalprotein(1)M20/M25/M40 putative n-acyl-L-amino acid hmrA(1) N-acyl-L-amino acid amidohydrolase HmrA(1) probable amidase(1)1.408450704 2 0.6377 >ClusterLukDv(4) leukocidin LukD precursor(3) leucotoxin LukDv(3) beta-channel forming cytolysin(2) panton-Valentine leukocidin chain F protein(2) leukotoxin LukD(2) leukocidin LukD(2) Gamma-hemolysin component B(1) "leukotoxin, LukD(1)" Leukocidin F subunit lukD(1) leukotoxin(1) gamma-hemolysin component B precursor(1) leukotoxin D subunit(1) leukotoxin F-subunit(1) leukocidinD-subunit(1) gamma-hemolysincomponent B(1) "leukocidin-F subunit (Gamma-hemolysin, H-gamma-I subunit)(1)"Panton-ValentineleukocidinsubunitF(1) LukF-PV(1)

536 2 142 1.408450704 2 0.6377 >Cluster 2026 iron ABC transporter permease(30) FecCD transport family protein(7) Iron compound ABC uptake transporter permease protein(7) fecCD transport family protein(6) iron complex transport system permease protein(6) "iron (Fe+3) ABC superfamily ATP binding cassette transporter, membrane protein(5)" ttg start(2) cobalamin Fe3+-siderophores ABC transporter permease(2)FecCD family ferrichrome transporter permease(2) iron ABC superfamily ATP binding cassette transporter(2) enterochelin uptake permease(1) transport system permease protein(1) Ferric anguibactin transport system permease protein fatD(1) "iron compound ABC transporter, permease protein(1)" ttg start domain protein(1) "iron chelate uptake ABC transporter, FeCT family, permeaseprotein(1)" hypotheticalprotein(1) conserved hypotheticalprotein(1) MW0695(1) "ironcompoundABC transporter, permease protein, putative(1)" ferrichrome ABC transporterpermease(1) "ironcompoundABCuptaketransporter, permease protein(1)"

537 2 142 1.408450704 2 0.6377 >Cluster 2200 biotin--acetyl-CoA-carboxylase ligase(28) biotin--(10) bifunctional biotin-(acetyl-CoA carboxylase) ligase / biotin operonrepressor(6) bifunctional bifunctional protein(3) ligase(2) operon-related protein and biotin-acetyl-CoA-carboxylase(1)"BirA bifunctional protein, putative(1)" Biotin--protein ligase / Biotinoperonrepressor(1) hypotheticalprotein(1) truncated hypotheticalprotein(1) BirAfamilytranscriptionalBiotin operon repressor / Biotin-protein ligase(5) biotin--(3) Bifunctional protein BirA(3) BirA protein (3) Biotin-protein ligase(3) BirA bifunctionalbiotin-(2) biotin-acetyl-CoA-carboxylase birA bifunctional protein(2) bifunctional biotinregulator(1) "bifunctional biotin operon-related protein / biotin- acetyl-CoA-carboxylase, putative(1)" Biotin-protein ligase / Biotin operon repressor(1) HTH domain protein(1) Biotin operon repressor(1) BirA bifunctional protein includes biotin operon repressor biotin--acetyl-CoA-carboxylase synthetase(1)

538 2 142 1.408450704 2 0.6377 >Cluster 2713 Replication protein Rep(4) plasmid replication initiation protein(3) replication protein Rep(2) initiatorReplication family protein(1)

539 2 142 1.408450704 2 0.6377 >Cluster 2763 multidrug ABC transporter ATP-binding protein(33) ABC transporter ATP-binding protein(13) ABC transporter familyprotein(8) component(7)" ABC protein(5)" protein(3) transporter(2) probable ATP-binding ABC transporter(1) MW0327(1) "ABC transporter, ATP-binding protein, putative(1)" hypothetical protein(1) 50S ribosome-binding GTPase family protein(1) ABC transporter related(1)"ABC-type multidrug transport system, ATPase "ABC superfamily ATP binding cassette transporter, putative lantibiotic transport ATP-binding "ABC transporter, ATP-binding protein(3)" ABC "ABC transporter ATP-binding protein, putative(1)"

540 2 142 1.408450704 2 0.6377 >Cluster 3449 transaldolase(45) Transaldolase(11) putative transaldolase(10) putative translaldolase(8) translaldolase(2) conserved hypothetical protein(1) hypotheticalprotein(1) "transaldolase,putative(1)" probable transaldolase(1) MW1721(1)

541 2 142 1.408450704 2 0.6377 >Cluster 3966 hypothetical protein(52) putative membrane protein(12) conserved hypothetical protein(5) conserved hypothetical membrane protein(2) Hypotheticalprotein(2) hypotheticalproteinwith DUF2179(1) hypothetical membrane protein(1) FIG01225759: hypotheticalprotein(1) probablemembraneprotein(1) UPF0316 protein SA1727(1) protein_1892(1) Integral membrane protein(1)

542 2 142 1.408450704 4016 transcriptional regulator(17) Two-component response regulator(9) response regulator(5) LuxR family DNA-binding response regulator(4)two-component response regulator(4) DNA-binding response regulator(4) "bacterial regulatory, luxR family protein(3)" Transcriptional regulatory protein desR(3)"bacterial regulatory s, luxR family protein(3)" two component transcriptional regulator(2) response regulator receiver domain protein(1) MW1209(1) response regulator receiver(1) conserved hypothetical protein(1) hypothetical protein(1)

543 2 142 1.408450704 2 0.6377 >Cluster 4354 nitroreductase(37) nitroreductase family protein(28)2 0.6377 >Cluster response regulator(9) two-component systemPutative nitroreductase family protein(4) conserved hypothetical protein(4) nitroreductase-like protein(3) Putative nitroreductase family protein SACOL0874(2)Nitroreductase family protein(1) possible nitroreductase(1)

544 2 142 1.408450704 2 0.6377 >Cluster 4639 beta-lactamase(13) MBL fold metallo-hydrolase(9) beta-lactamase superfamily domain protein(8) hypothetical protein(5) conserved hypothetical protein(5) Metallo-beta- lactamase superfamily domain protein in prophage(4)hypothetical phage protein(3) Beta-lactamase superfamily domainprotein(2)hydrolase(1)

545 8 569 recombinase RecB(16) cassette chromosome recombinase B(13) Cassette chromosome recombinase B(7) ogr/Delta-like zinc finger family protein(4) site-specific recombinase(3) Site- specificrecombinase(1) cassettechromosomerecombinaseB3(1) Resolvase domain(1) cassette chromosome recombinase B1 domain protein(1) "resolvase, N terminal domain protein(1)"

546 8 142 5.633802817 4 0.6433 >Cluster 750 dehydrosqualene desaturase(37) squalene synthase(15) Dehydrosqualene desaturase(8) phytoene desaturase family protein(8) "Dehydrosqualene desaturase (Diapophytoene desaturase) (4,4'-diapophytoene desaturase)(3)" Phytoene dehydrogenase(2) "Staphyloxanthin biosynthesis protein CrtN (dehydrosqualene dehydrogenase), truncated(2)" "Dehydrosqualene desaturase (Diapophytoene desaturase) (4,4-diapophytoene desaturase) (1)" Zeta-phytoene desaturase(1) "squalene synthase, putative(1)" "dehydrosqualene desaturase (Diapophytoene desaturase)(4,4'-diapophytoene desaturase)(1)" Squalene/phytoene synthase(1)

547 9 142 6.338028169 4 0.6611 >Cluster 1725 superantigen-like protein(17) hypothetical protein(17) Exotoxin 8(8) superantigen-like protein 11(6) exotoxin(4) Exotoxin(2) "staphylococcal/Streptococcal toxin, beta-grasp domain protein(1)" set18(1) staphylococcal enterotoxin-like toxin(1) staphylococcal exotoxin 9(1) staphylococcal exotoxin(1)exotoxin 8(1) toxin beta-grasp domain protein(1) conserved hypothetical protein(1)phiPVL ORF044-like protein(1) Metal-dependent phage protein(1)142 5.633802817 2 0.6433 >Cluster

548 9 142 6.338028169 2 0.671 >Cluster 928 6-phosphogluconate dehydrogenase(38) "6-phosphogluconate dehydrogenase, decarboxylating(26)" "phosphogluconate dehydrogenase (NADP(+)-dependent, decarboxylating)(6)" "6- phosphogluconate dehydrogenase,decarboxylating(4)" "6-phosphogluconate dehydrogenase (NADP(+)-dependent, decarboxylating)(3)" phosphogluconate dehydrogenase (decarboxylating)(2) phosphogluconate dehydrogenase(2)

549 10 142 7.042253521 2 0.684 >Cluster 1754 spermidine/putrescine ABC transporter ATP-binding protein(29) putative ABC transport ATP-binding protein(6) Putrescinetransport ATP-binding protein PotA(6) Spermidine/putrescine import ATP-binding protein PotA(6) putrescine/spermidine ABC transporter ATPase(4) ABC transporter family protein(4) spermidine/ putrescine import ATP-binding protein PotA(4) spermidine/ putrescine ABC transporter ATPase(2) spermidine/putrescine import ATP-binding protein potA(2) spermidine/putrescine ABC transporter(2) "spermidine/putrescine ABC transporter, ATP-binding protein PotA(1)" "ABC transporter, ATP-binding protein(1)" spermidine/ putrescine import ATP-binding PotA domain protein(1) spermidine/ putrescine ABC transporter ATP-binding subunit(1) Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)(1) Spermidine/ putrescine import ATP-binding protein potA(1) ABC transporter domain protein(1) Spermidine/putrescine transport ATP-binding protein potA(1) spermidine/putrescine import ATP-binding protein(1)ABC transporter related(1) "spermidine/putrescine ABC superfamily ATP binding cassette transporter, ABCprotein(1)" protein(1)" protein(1) potA(1) potA(1)

550 3"spermidine/putrescine ABC transporter, ATP-bindingspermidine/putrescine ATP-binding ABC transporterSpermidine Putrescine transport ATP-binding protein142 2.112676056 2 0.7009 >Cluster 451 acetyltransferase(31) Putative O-acetyltransferase(10) O- acetyltransferase OatA(7)O-acetyltransferase(6) putative membrane protein(6) acyltransferase(4) hypothetical protein(3) acyltransferase family protein(2) conserved hypothetical protein(2) Putative O-acetyltransferase SAS0844(2) O-acetyltransferase OatA domain protein(2) "lipopolysaccharide modification acyltransferase, putative(1)" MW0856(1) putative O- acetyltransferase family protein(1) Lipopolysaccharide modification acyltransferase(1) putative O-acetyltransferase protein(1) Acyltransferase family protein(1) acyltransferase 3(1)

551 3 142 2.112676056 3 0.7009 >Cluster 1699 YibE/F-like protein(24) hypothetical protein(20) yibE/F-likefamily protein(11) conserved hypothetical protein(7) putative membrane protein(6) YibE/F family protein(4) Hypothetical protein(1) Putative membrane spanning protein(1) probable membrane protein(1) Membrane protein(1) "membrane protein, putative(1)" "hypothetical protein, YibE/F-like protein(1)" hypothetical membrane protein(1) FIG01107858: hypothetical protein(1)

552 3 142 2.112676056 2 0.7009 >Cluster 4173 heptaprenyl pyrophosphate synthase subunit A(30) hypothetical protein(22) Heptaprenyl diphosphate synthase component I(10) heptaprenyl diphosphate synthase (HEPPP synthase) subunit 1 family protein(7) heptaprenyl diphosphate synthase subunit 1(4)conserved hypothetical protein(3) hyothetical protein(2) MW1361(1) putative staphylococcal protein(1)

553 3 142 2.112676056 2 0.7009 >Cluster 4224 hypothetical protein(57) conserved hypothetical protein(11)Cysteinyl-tRNA synthetase related protein(9) hypothetical protein with DUF402(1) Putative cytosolic protein(1) protein LMOf2365_1711(1) protein of unknown function DUF402(1)

554 12 142 8.450704225 5 0.7066 >Cluster 1322 phosphoribosylamine--glycine ligase(44) Phosphoribosylamine--glycine ligase(15) phosphoribosylamine--glycine ligase PurD(6) putative phosphoribosylamine--glycine ligase(6) phosphoribosylamine-glycine ligase(6) phosphoribosylamine--glycine ligase (GARS) (Glycinamideribonucleotide synthetase) (Phosphoribosylglycinamide synthetase)(1) Phosphoribosylamine-glycineligase(1)protein(1)"protein(1)"

555 14 188 DNA gyrase subunit A(28) "DNA topoisomerase IV, A subunit(14)" Topoisomerase IV subunit A(10) topoisomerase IV subunit A(10) DNA topoisomerase 4 subunit A(8) DNA topoisomerase IV subunit A(5) DNA topoisomerase (ATP-hydrolyzing) ParC(4) DNA topoisomerase (ATP-hydrolyzing) ParC / grlA(1)

556 3 142 2.112676056 3 0.7302 >Cluster 547 dihydroxy-acid dehydratase(51) Dihydroxy-acid dehydratase(15) putative dihydroxy-acid dehydratase(11) dihydroxy-"phosphoribosylglycinamide synthetase, C domain"phosphoribosylglycinamide synthetase, N domain142 9.85915493 7 0.7293 >Clusteracid dehydratase 1(2)

557 3 142 2.112676056 3 0.7302 >Cluster 631 membrane protein(16) hypothetical protein(14) putative sodium:sulfate symporter protein(10) Sodium/di- and tricarboxylate cotransporter(7) anion transporter family protein(7)na+ symporter family protein(6) DASS family divalent anion:sodium (Na+) symporter(4) anion transporter(4) conserved hypotheticalprotein(3) "Transporter,DivalentAnion:SodiumSymporter family protein(2)" "transporter, divalent anion:Na+ symporter (DASS) family protein(1)"probable membrane transport protein(1) divalent anion symporter(1) "transporter, DASS family(1)" Sodium/di-and tricarboxylate cotransporter(1) Na+ symporter family protein(1) "sodium:anion symporter protein, putative(1)"

558 3 142 2.112676056 3 0.7302 >Cluster 760 amidophosphoribosyltransferase(49) Amidophosphoribosyltransferase(17) putative amidophosphoribosyltransferase precursor(10) phosphoribosylpyrophosphate amidotransferase(2) amidophosphoribosyltransferase PurF(1) phosphoribosylpyrophosphate amidotransferase PurF(1) amidophosphoribosyltransferase precursor(1)

559 3 142 2.112676056 3 0.7302 >Cluster 1328 3-oxoacyl-(25) 3-oxoacyl-ACP synthase(21) beta- ketoacyl-(9) beta-ketoacyl-acyl-carrier-protein synthase II(8) 3- oxoacyl-(acyl carrier protein) synthase II(4) "3-oxoacyl-(acyl- carrier-protein) synthase, KASII(3)" 3-oxoacyl-synthase II(2) 3-oxoacyl-(acyl-carrier-protein) synthase II(2) "3-oxoacyl- synthase, putative(1)" 3-oxoacyl-acyl-carrier-protein(1) Beta- ketoacyl synthase(1) 3-oxoacyl- synthase(1) "3-oxoacyl-acyl- carrier-protein synthase, KASII(1)" 3-oxoacyl-ACP synthase II(1)conserved hypothetical protein(1)

560 3 142 2.112676056 3 0.7302 >Cluster 1445 integrase(25) phage integrase family protein(4) Phage integrase(3) putative phage integrase(3) "pathogenicity island protein, integrase(3)" phage integrase(2)bacteriophage integrase(1) "site-specific recombinase, phage integrase family(1)"

561 3 142 2.112676056 3 0.7302 >Cluster 3140 RNA polymerase sigma-B factor(36) RNA polymerase sigma factorSigB(35) sigma factor B(3) RNA polymerase sigma-B factor protein(2) "sigma factor B, putative(1)" "RNA polymerase sigma factor, sigma-70 family protein(1)" RNA polymerase sigma-B type(1) RNA polymerase sigma factor B(1) RNA polymerase subunit sigma(1) DNA-directed RNA polymerase sigma subunit FliA(1)

562 3 142 2.112676056 2 0.7302 >Cluster 4046 potassium-transporting ATPase subunit C(32) Potassium- transporting ATPase C chain(14) "K+-transporting ATPase, C subunit(10)" putative potassium-transporting ATPase C chain(6) P- ATPase superfamily P-type ATPase potassium (K+) transporter subunit C(5) "potassium-transporting ATPase, C subunit(3)" probable potassium-transportingATPaseCchain(2) K+-transportingATPase subunit C(2) potassium-transporting ATPase subunit C 1(2) potassium-transportingATPaseCchain(1) "potassium-transporting ATPase, C(1)" Potassium-transporting ATPase C chain (TC 3.A.3.7.1) (1)

563 4 142 2.816901408 2 0.7402 >Cluster 2066 proline dehydrogenase(51)proline dehydrogenase family protein(7) Proline dehydrogenase (Proline oxidase)(6)Proline dehydrogenase(5) Proline dehydrogenase 2(2) proline oxidase(2)L-proline dehydrogenase(2) proline dehydrohenasehomolog(1)oxidase(1)

564 4 5297 hypothetical protein(41) cytosolic protein(22) conserved hypothetical protein(13) putative DUF3055-containing protein(1) hypothetical protein BH3432(1) Hypothetical protein(1) Putative cytosolic protein(1)

565 4 142 2.816901408 3 0.757 >Cluster 249 hypothetical protein(49) conserved hypothetical protein(5) hypothetical protein within a prophage(3) Hypothetical protein(2) conserved hypothetical phage protein(1)

566 4 142 2.816901408 2 0.757 >Cluster 2464 dimethyladenosine transferase(29) 16S rRNA methyltransferase(27) ribosomal RNA adenine dimethylase(6) SSU rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))- dimethyltransferase(4)Ribosomal RNA small subunit methyltransferase A(3) 16S rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))-dimethyltransferase(3)proline dehydrogenase 1(1) probable proline MW1707(1) conserved hypothetical protein(1)142 2.816901408 2 0.7402 >ClusterDimethyladenosine transferase(2) SSU rRNA (adenine(1518)-N(6)/ adenine(1519)-N(6))-dimethyltransferase(2)ksgA(1) possible dimethyladenosine transferase(1)

567 4 142 2.816901408 2 0.757 >Cluster 2505 ferrichrome ABC transporter substrate-binding protein(30) periplasmic binding family protein(8) Ferrichrome-binding periplasmic protein precursor(8) periplasmic binding protein(7) putative ferrichrome-binding lipoprotein precursor(7) "iron (Fe +3) ABC superfamily ATP binding cassette transporter, bindingprotein(5)"protein(3)protein(2)protein(2)"receptor 1(1)protein(1)Fe3 -hydroxamate transport system, periplasmic component, putative(1)"Iron complex transport system substrate-binding iron-siderophore ABC transporter periplasmic "iron compound ABC transporter, iron compound-binding Ferrichrome-binding protein(1) ferric hydroxamate hypothetical protein(1) conserved hypothetical probable ferrichrome-binding protein(1) "ABC-typeFerrichrome-binding periplasmic protein precursor (TC 3.A. 1.14.3)(1) hydroxamate siderophore binding lipoprotein(1)

568 4 142 2.816901408 2 0.757 2920 enterotoxin(34) enterotoxin family protein(19) carboxylate--amineligase(9) putativeenterotoxin(6) type E(5) Enterotoxin(5) hypothetical protein(4) enterotoxin P(2) "enterotoxin type A, putative(1)" "staphylococcal/ Streptococcal toxin, beta-grasp domain protein(1)" MW1552(1)enterotoxin type D(1)domain protein(1)"enterotoxin homolog(1)enterotoxin type A(1)toxin beta-grasp domain protein(1)"staphylococcal/Streptococcal toxin, OB-fold "toxin, OB-fold domain protein(1)" enterotoxin type A(1) Putative "toxin, beta-grasp domain protein(1)"

569 4 142 2.816901408 3 0.757 >Cluster 3031 indole-3-glycerol phosphate synthase(49) Indole-3-glycerol phosphate synthase(15) indole-3-glycerol phosphate synthase family protein(7) indole-3-glycerol-phosphate synthase(5) "indole-3- glycerol phosphate synthase, putative(2)" indole-3-glycerol phosphate synthase (IGPS)(1) Indole-3-glycerol-phosphate synthase(1)

570 4 142 2.816901408 3 0.757 >Cluster 4692 methionine sulfoxide reductase A(37) Peptide methionine sulfoxidereductaseMsrA(11) hypotheticalprotein(8) peptide methionine sulfoxide reductase family protein(8) "methionine sulfoxide reductase A, MsrA(3)" Peptide methionine sulfoxide reductase(3) peptide methionine sulfoxide reductase(2) possible peptide-methionine-(S)-S-oxide reductase(1) peptide methionine>Clusterenterotoxintoxin(2)sulfoxide reductase msrA/msrB domain protein(1) MW2580(1) peptide methionine sulfoxide reductase MsrA/msrB(1) "peptide methionine sulfoxide reductase, putative(1)" putative peptide- methionine (S)-S-oxide reductase(1) Methionine sulfoxide reductase A(1) methionine-S-sulfoxide reductase(1)

571 5 142 3.521126761 2 0.7676 >Cluster 550 ATPase(26) Potassium-transporting ATPase A chain(14) "K +-transporting ATPase, A subunit(10)" P-ATPase superfamily P-type ATPase potassium (K+) transporter subunit A(6) putative potassium- transporting ATPase a chain(6) potassium-transporting ATPase subunit KdpA(3) potassium-transporting ATPase subunit A(3)"potassium- transporting ATPase, A subunit(3)" probable potassium- transporting ATPase A chain(2) potassium-transporting ATPase subunit A 1(2) "potassium-transporting ATPase, A(1)" Potassium-transporting ATPase A chain (TC 3.A.3.7.1)(1) potassium- transporting ATPase A subunit(1) potassium-transporting ATPase A chain(1)

572 5 142 3.521126761 2 0.7676 >Cluster 1085 30S ribosomal protein S12 methylthiotransferase(19) tRNA (N(6)-L-threonylcarbamoyladenosine(37)-C(2))- methylthiotransferase MtaB(11) conserved hypothetical protein(9) hypotheticalprotein(7) "MiaBfamilyprotein,possiblyinvolvedin tRNA or rRNA modification(5)" tRNA-t(6)A37 methylthiotransferase(5) "radical SAM methylthiotransferase, MiaB/RimO family protein(4)" Threonylcarbamoyladenosine tRNA methylthiotransferase MtaB(3) tRNA modifying enzyme miaB-like protein(3) threonylcarbamoyladenosine tRNA methylthiotransferase MtaB(3) radical SAM domain-containing protein(2) RNA modification enzyme(2) radical SAM domain protein(2) "RNA modification enzyme,MiaBfamily(1)" possible2-methylthioadeninesynthase(1)TRNA 2-methylthioadenosine synthase -like protein(1) "RNA modification enzyme, MiaB family protein(1)" Adenine synthetase(1) tRNA methylthiotransferase YqeV(1) "2- methylthioadenine synthetase, putative(1)"

573 5 142 3.521126761 2 0.7676 >Cluster 1608 hypothetical protein(22) membrane protein(16) putative membrane protein(8) "phosphotransferase system, EIIC family protein(6)" "membrane protein, putative(5)" Membrane protein(5)toxin regulatorly-like protein(4) conserved hypothetical protein(3) regulatory protein(3) regulatory protein- protein(2) MW2491(1) Regulatory protein(1) hypothetical membrane protein(1) probable membrane-embedded regulatory protein(1) Putative membrane spanning protein(1) "membrane-localized toxin regulator, putative(1)"

574 5 142 3.521126761 2 0.7676 >Cluster 2130 peptide ABC transporter ATP-binding protein(28) Oligopeptide transport ATP-binding protein OppD(7) putative oligopeptide transport ATP-binding protein(6) "oligopeptide/ dipeptide ABC transporter, ATP-binding, C-terminal domain protein(6)" "oligopeptide ABC superfamily ATP binding cassette transporter, ABC protein(4)" ABC transporter(2) peptide/ nickel transport system ATP-binding protein(2) Oligopeptide transport ATP-binding protein oppD(2) "oligopeptide ABC transporter ATP-binding protein oppF, putative(1)" putative peptide ABC transporter ATP-binding protein y4tR(1) oligopeptide ABC transporter ATP-binding protein(1) "oligopeptide ABC transporter, ATP-binding protein, putative(1)" "ABC transporter, ATP-bindingprotein(1)" MW0874(1) oligopeptidetransportATP- binding protein(1) conserved hypotheticalprotein(1) protein(1)" 3.A.1.5.1)(1) homologue(1) domain protein(1)" "oligopeptide/dipeptide ABC transporter, ATPase subunit(1)""oligopeptide ABC transporter, ATP-bindingOligopeptide transport ATP-binding protein OppD (TColigopeptide ABC transporter ATP-binding protein"oligopeptide/dipeptide ABC transporter, ATP-binding,

575 5 142 3.521126761 2 0.7676 >Cluster 2459 recombinase(17) "recombinase, phage RecT family protein(8)"putative phage-related DNA recombination protein(3) phage-related DNA recombination protein(2)hypothetical protein(2) hypothetical phage protein(2) DNA Recombination protein RecT(1) RecT protein(1) possible recombination protein RecT(1) Recombinational DNA repair protein RecT (prophage associated)(1) DNAsingle-strand annealing proteins(1)protein(1)

576 5 142 3.521126761 3956 succinate dehydrogenase(30) cytochrome b558 subunit(9) putative succinate dehydrogenase cytochrome b558(7) "succinate dehydrogenase, cytochrome b556subunit(7)" cytochrome cytochrome subunit(4) b subunit(3) "succinate dehydrogenase, cytochrome b558 subunit(2)""succinate dehydrogenase (or fumarate reductase) b subunit, b558 family(5)" succinate dehydrogenase b-558(5) succinate dehydrogenase cytochrome b558conserved hypothetical2 0.7676 >Cluster Succinate dehydrogenasesuccinate dehydrogenase/fumarate reductase cytochromesuccinate dehydrogenase subunit C protein(2) "succinate dehydrogenase cytochrome b-558 subunit, putative(1)" succinate dehydrogenase cytochrome B558 subunit (Cytochrome b-558)(1)Succinate dehydrogenase cytochrome b558(1) "putative succinate dehydrogenase, cytochrome b556 subunit(1)" succinate dehydrogenase cytochrome b558 subunit (Cytochrome b-558)(1) "Succinate:quinone oxidoreductase Type B subfamily 1, transmembrane subunit SdhC(1)"

577 5 142 3.521126761 2 0.7778 >Cluster 641 zinc ABC transporter substrate-binding protein(30) putative solute binding lipoprotein(8) putative zinc-binding lipoprotein ZinT(6) "ABC superfamily ATP binding cassette transporter, binding protein(5)" periplasmic solute binding family protein(4)yodA lipocalin-like domain protein(4) zinc-binding lipoprotein(3) Zn-binding lipoprotein adcA-like protein(3) Zinctransport system substrate-binding protein(3) Zn-binding lipoprotein adcA-protein(2) Candidate zinc-binding lipoprotein ZinT(2) "ABC transporter, substrate-

binding protein(2)" conserved hypotheticalprotein(2) hypotheticalprotein(2) probablezinc- binding lipoprotein(1) zinc-binding lipoprotein AdcA(1) MW2328(1)"metal-bindinglipoprotein,putative(1)" Ribulose-phosphate 3-epimerase(1)

578 5 142 3.521126761 4 0.7778 >Cluster 1038 hypothetical protein(34) yycH family protein(11) YycH protein(8) putative exported protein(6) conserved hypothetical protein(5) two-component regulator YycH(4) two- component system walKR regulator(2) Two-component system regulatory protein(2) sensor protein kinase walK(1) YycH like protein(1) hypothetical protein YycH(1) "YycH domain signaltransduction protein, putative(1)" yycH(1) FIG011501: YycH protein(1)

579 5 142 3.521126761 1083 PTS beta-glucoside transporter subunit IIBC(27) PTS system sugar-specific permease component family protein(9) putative membrane protein(5) ascorbate-specific PTS system enzyme IIC(5)PTS system ascorbate-specific IIC component(4) membrane protein(4) Ascorbate-specific permease IIC component ulaA(3) PTSascorbate transporter subunit IIC(3) specific transporter subunit IIC(3) Putative integral membrane protein(2) probable membrane protein(1) "PTS system, L-ascorbate specific IIC component ulaA(1)" "putative sugar-specific permease, SgaT/ UlaA(1)" "hypothetical protein, transport like-protein SgaT, Putative integral membrane protein(1)" putative sugar-specific permease SgaT/UlaA(1) MW0306(1) ascorbate-specific PTS system enzyme(1) PTS family porter component IIC(1) conserved hypotheticalprotein(1) "sugar-specificpermease,SgaT/UlaAfamily protein(1)"

580 5 142 3.521126761 2 0.7778 >Cluster 1860 sorbitol dehydrogenase(39) Galactitol-1-phosphate 5- dehydrogenase(8) putative zinc-binding dehydrogenase(7) zinc- binding dehydrogenase family protein(4) zinc-binding alcohol dehydrogenase(4) L-iditol 2-dehydrogenase(3) Sorbitol dehydrogenase(3) D-arabitol-phosphate dehydrogenase(3) arabitol-Putative membrane proteinYycH family protein(1)3 0.7778 >ClusterPTS system ascorbate-hypothetical protein(3)PTS system component(2)phosphate dehydrogenase(1) "zinc-binding sorbitol dehydrogenase, putative(1)" "sorbitol dehydrogenase, putative(1)" putative chlorophyll synthesis pathway protein BchC(1) Alcohol dehydrogenase GroES domain protein(1) probable zinc-binding sorbitol dehydrogenase(1) sorbitol dehydrogenase (L- iditol 2-dehydrogenase)(Glucitol dehydrogenase)(1)

581 5 142 3.521126761 3 0.7778 >Cluster 2009 hypothetical protein(33) chitinase(22) putative membrane protein(9) conserved hypothetical protein(6) membrane protein(2)"chitin synthesis regulation, resistance to Congo red family protein(2)" conserved hypothetical membrane protein(2) Hypotheticalprotein(2) hypotheticalmembraneprotein(1)FIG01108899: hypothetical protein(1)

582 5 142 3.521126761 2068 choloylglycine hydrolase(34)probable membrane protein(1)2 0.7778 >ClusterCholoylglycine hydrolase(9)"linear amide C-N hydrolase, choloylglycine hydrolase familyprotein(8)" V acylase(6) amidase(2) amidase(1) hydrolase(1) amide C-N hydrolases, choloylglycine hydrolase family protein(1)" Choloylglycinehydrolase-likeprotein(1) conservedhypothetical protein(1) hypotheticalprotein(1) "choloylglycinehydrolase, putative(1)"putative choloylglycine hydrolase(7) penicillin choloylglycine hydrolase family protein(3) penicillin Penicillin acylase(2) C59 family penicillin probable penicillin-related choloylglycine Penicillin amidase(1) MW0251(1) "linear

583 5 142 3.521126761 2 0.7778 >Cluster 3535 hypothetical protein(17) phage protein(6) Hypothetical protein(2) hypothetical phage-related protein(1) putative phage protein(1) protein of unknown function DUF968(1) putative virulence associated protein(1) "Hypothetical protein, PV83 orf19(1)" "Hypothetical protein, PV83 orf19-like protein(1)" phi PV83 orf 19-like protein(1)

584 6 142 4.225352113 2 1427 ornithine--oxo-acid aminotransferase(27) transaminase(21) ornithine aminotransferase(12) aminotransferase(9) Ornithine aminotransferase 2(4) Acetylornithine aminotransferase 2(3) acetylornithine aminotransferase 1(2) "ornithine aminotransferase, putative(2)" ornithine-oxo-acid transaminase(1)

585 6 142 4.225352113 3 0.7944 >Cluster0.7944 >Cluster ornithine--oxo-acid Ornithine1755 ribosomal RNA large subunit methyltransferase N(22) Ribosomal RNA large subunit methyltransferase N(12) 23S rRNA (adenine(2503)-C2)-methyltransferase(11) conservedhypothetical protein(6) 23S rRNA methyltransferase(5) putative dual- specificity RNA methyltransferase RlmN(4) "Ribosomal RNA large subunit methyltransferase N, Rlmn(4)" "radical SAM enzyme, Cfr family(3)"23S rRNA m2A2503 methyltransferase(3) hypothetical protein(3) 23S rRNA (adenine(2503)-C(2))-methyltransferase RlmN(3) radical SAM superfamily protein(1) "ribosomal RNA large subunit methyltransferase N, putative(1)" 23S rRNA (adenine(2503)-C(2))-methyltransferase(1) putative ribosomal RNA large subunit methyltransferase N domain protein(1) "Ribosomal RNA large subunit methyltransferase N, LSU rRNA m2A2503(1)" possible Fe-S-cluster redox enzyme(1) Ribosomal RNA large subunit methyltransferase N ## LSU rRNA m2A2503(1)

586 6 142 4.225352113 2 0.7944 >Cluster2572dehydrosqualene synthase(41) Dehydrosqualene synthase(10) squalenedesaturase(10) squalene/phytoenesynthase(5)synthase(4) squalene synthase(2) squalene/phytoene family protein(1) Staphyloxanthin biosynthesis proteinPhytoene synthase CrtM (dehydrosqualene synthase)(1) crtM(1) "dehydrosqualene synthase (Diapophytoene synthase) (4,4'-diapophytoene synthase) (DAP synthase)(1)" "squalene desaturase, putative(1)" Squalene/ phytoene synthase(1)

587 6 142 4.225352113 2 0.8043 >Cluster 1150 homoserine dehydrogenase(50) Homoserine dehydrogenase(11)putative homoserine dehydrogenase(10) homoserine dehydrogenase family protein(5) "homoserine dehydrogenase, NAD binding domain protein(3)" "homoserine dehydrogenase, putative(1)"

588 6 142 4.225352113 3 0.8043 >Cluster 1178 branched-chain alpha-keto acid dehydrogenase subunit E2(34) Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex(10) dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex(8) dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex(7) Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex(6) dihydrolipoyllysine-residue acetyltransferase component of pyruvatedehydrogenase complex(4) dihydrolipoamide S- acetyltransferase component of pyruvate dehydrogenase complex E2(3) dihydrolipoyllysineacetyltransferase(2) pyruvatedehydrogenase complex dihydrolipoyllysine-residue acetyltransferase(2) Dihydrolipoyllysine-residue succinyltransferase(1) "pyruvate dehydrogenase complex E2, dihydrolipoamide S-acetyltransferase component PdhC(1)" "dihydrolipoamide S-acetyltransferasecomponent of pyruvate dehydrogenase complex E2, putative(1)"dihydrolipoyllysine-residue acetyltransferase(1)dihydrolipoyllysine-residue acetyltransferase

component ofpyruvatedehydrogenase complex (E2)(Dihydrolipoamideacetyltransfera>(1)

589 6 142 4.225352113 2 0.8043 >Cluster 3643 peptide ABC transporter ATP-binding protein(29) ABC transporter ATP-binding protein(21) ABC transporter family protein(8) "ABC superfamily ATP binding cassette transporter, binding protein(5)" "ABC transporter, ATP-binding protein(3)" putative hemin import ATP-binding protein HrtA(3) ABC transporter(2)ABC transporter ATPase(2)hypothetical protein(2) lipoprotein-releasing system ATP-binding protein LolD(1) MW0286(1)probable ATP-binding ABC transporter(1) "ABC transporter, ATP-binding protein, putative(1)" ABC transporter related(1)

590 7 142 4.929577465 3 0.8081 >Cluster 3595 DNA repair protein RadC(36) hypothetical protein(28) DNA repair RadC family protein(8) DNA repair protein radC(4) "putative DNA repair protein, RadC(2)" radC-like JAB domain protein(1) DNA repair protein RadC-like protein(1) conserved hypothetical protein(1) DNA repair protein(1) DNA repair protein homolog(1) DNA repair protein RadC family protein(1) MW1604(1) DNA repair protein RadC homolog(1) truncated DNA repair protein RadC(1) "DNA repair protein RadC, putative(1)"

591 7 142 4.929577465 5 0.8081 >Cluster 5157 hypothetical protein(38) putative lipoprotein(14) lipoprotein(14) conserved hypothetical protein(5) "lipoprotein, putative(3)" Hypothetical protein(2) possible lipoprotein(1) probablelipoprotein(1) FIG01108076:hypotheticalprotein(1)

592 32 142 22.53521127 16 947 transposase(131) IS1181 transposase(16) protein(15) transposase family protein(14)0.812 >Cluster Mobile element transposase IS204/IS1001/IS1096/IS1165 family protein(8) transposase ISL3 family protein(8) for IS1181(5) hypothetical protein(3) Transposase for Insertion sequence IS1181(2) transposase for insertion sequence IS1181(2) "transposase, putative(2)" putativetransposase(2) TransposaseforinsertionsequenceIS1181(1)

593 7 142 4.929577465 3 0.8149 >Clusterhelix-turn-helix domain of Transposase(6) transposase Transposase for IS1181(2)222 penicillin-binding protein(30) penicillin-binding protein 1(30) penicillin binding transpeptidase domain protein(4) Cell division protein FtsI (Peptidoglycan synthetase)(4) PASTA domain protein(3) penicillin-binding Protein dimerization domain protein(2) Cell division protein FtsI (2) penicillin- binding protein 2B (PBP-2B)(2) Cell division protein FtsI(1) Peptidoglycan glycosyltransferase(1) penicillin-binding protein 1 (Peptidoglycan synthetase)(1) "penicillin-binding protein, transpeptidase domain protein(1)" "Division specific D,D- transpeptidase(1)"

594 7 142 4.929577465 4 0.8149 >Cluster 528 prolyl-tRNA synthetase(43) proline--tRNA ligase(22) "Prolyl-tRNA synthetase, bacterial type(6)"Prolyl-tRNA synthetase(4)Proline--tRNA ligase(4) ligase(2) hypothetical protein(1)

595 7 142 4.929577465 3 1672 NADH-dependent flavin oxidoreductase(37) oxidoreductase / NADH oxidase family protein(10) dependent flavin oxidoreductase(8) flavin oxidoreductase / NADH oxidase family protein(6)putative NADH-dependent flavin oxidoreductase(3) NADH:flavin oxidoreductase/NADH oxidase(3)Oye family NADH-dependent flavin oxidoreductase(2) flavin oxidoreductase(2) NADH:flavin oxidoreductase / NADH oxidase familyprotein(1) Putative NADH-dependent flavin oxidoreductase yqiG(1)flavin reductase(1) MW0838(1) probable NADH- dependent flavin oxidoreductase(1) "Oye family NADH-dependent flavin oxidoreductase, putative(1)" hypotheticalprotein(1)protein(1)""oxidoreductase, FAD/FMN-binding"FMN oxidoreductase, putative(1)"142 5.633802817 4 0.8195 >Cluster

596 8 789 gamma-aminobutyrate permease(30) lysine-specific permease(17)Lysine-specific permease(13) amino acid permease family protein(8) APC family amino acid-polyamine-organocation transporter(5) amino acid permease(4) amino acid permease- associated region(1) putative amino-acid permease(1) conserved hypotheticalprotein(1) lysinespecificpermease(1)

597 8 142 5.633802817 3 0.8243 >Cluster 3002 hypothetical protein(67) putative lipoprotein(7) lipoprotein(5) tandem lipoprotein(4) conserved hypothetical protein(4) Membranelipoprotein(3) putativestaphylococcalproline-tRNA0.8149 >Cluster NADH:flavin Putative NADH-tandem lipoprotein(3) staphylococcal tandem lipoprotein(2)FIG01107840: hypothetical protein(2)putative tandem lipoprotein(2)"lipoprotein, putative(2)"putative sa tandemstaphylococcus tandemlipoprotein(2) Hypothetical protein(1) lipoproteins family protein(1) putative exported protein(1) staphylococcus tandem lipoproteins(1)

598 9 142 6.338028169 4 0.8325 >Cluster 3498 preprotein translocase subunit TatB(26) twin arginine- targeting protein translocase TatC(13) Sec-independent periplasmic protein translocase(7) putative Sec-independent protein translocase protein(6) conserved hypothetical protein(4) Twin-arginine translocation protein TatCd(4) Twin-arginine translocation protein TatC(3) Sec-independent protein translocase protein TatCy(3) sec-independent protein translocase(2) MttB family protein(1) hypotheticalprotein(1) Putativesec-independentproteintranslocase protein(1) twin-arginine protein translocase protein subunit C(1) Sec-independent protein translocase protein tatC(1)sec-independent protein translocase protein(1)translocase protein (TatC) family protein(1)arginine targeting transporter TatC(1)

599 9 142 6.338028169 6 type VII secretion protein EssC(24)sec-independentTat family twin0.8377 >Cluster40cell division protein FtsK(22) "FtsK/SpoIIIE family protein, putative secretionsystem component EssC/YukA(7)" protein EssC(4) conserved hypotheticalprotein(3) putativemembraneprotein(2) DNA segregation ATPase-like protein(2) cell division protein FtsK/ SpoIIIE domain-containig protein(2) diarrheal toxin(2)essC(2) "FtsK/SpoIIIE family protein, putative EssC component of Type VII secretion system(1)" Protein essC(1) virulence protein EssC(1) MW0263(1) Diarrheal toxin(1) probable DNA segregation ATPase and related protein(1) Virulence Protein essC(1)DNA segregation ATPase and related proteins(1)ftsK/SpoIIIE family protein(1) cell division protein FtsK/SpoIIIE(1)

600 10 142 7.042253521 4 0.8399 >Cluster 319 DNA ligase(29) NAD-dependent DNA ligase LigA(16) "DNA ligase, NAD-dependent(14)" aromatic ring-opening dioxygenase LigA(8) DNA ligase (NAD(+)) LigA(6) DNA ligase (polydeoxyribonucleotide syntase)(4) NAD-dependent DNA ligase(2)DNA ligase (NAD+)(1) DNA ligase (NAD(+))(1)

601 11 142 7.746478873 2 0.8493 >Cluster 1177 GTPase ObgE(24) Spo0B-associated GTP-binding protein(16) GTPase Obg(10) GTP-binding

protein Obg(9) obg family GTPase CgtA(7) GTP-binding protein Obg/CgtA(4) GTPase obg(3) GTPase(2)GTP-binding protein(2) Obg family GTPase CgtA(1)COG0536: GTP-binding protein Obg(1) Spo0B-related GTP-binding protein(1)GTP-binding protein CgtA (probably involved in DNA repair)(1)

602 13 142 9.154929577 4 0.8591 >Cluster 1647 "4,4'-diaponeurosporenoate glycosyltransferase(54)" putative glycosyl transferase(7) "glycosyl transferase, group 2 family protein(4)" glycosyl transferase family protein(3) conserved hypothetical protein(3) "glycosyl transferase, putative(1)"

glycosyl transferase family 2(1) "glycosyltransferase, group 2 family protein(1)" Staphyloxanthin biosynthesis protein CrtQ(1)glycosyltransferase(1) glycosyl transferase family 2 family protein(1) "4,4-diaponeurosporenoate glycosyltransferase(1)" hypothetical protein(1)

603 18 142 12.67605634 7 0.8813 >Cluster16 adhesin(26) putative cell-wall-anchored protein SasA(LPXTG motif)(6) serine-rich adhesin for platelets(4) serine/ threonine-rich antigen(4)serine-threonine rich protein(3) LPXTG- motif-containing protein(3) LPXTG-motif cell wall surface anchor family protein(2) conserved hypothetical protein(2) serine-rich adhesinforplatelet(2) Predictedcell-wall-anchoredproteinSasA (LPXTG motif)(2) putative serine rich repeat containing protein(2) serine-rich platelet adhesin(2) serine-rich adhesin for platelets (Staphylococcus aureussurface protein A)(1) serine-rich repeat- containing protein(1) MW2575(1) Serine-rich adhesin for platelets precursor protein A(1) LPXTG-motif cell wall anchor domain protein(1) LPXTG cell wall surface anchor family protein(1) hypotheticalprotein(1) LPXTG-motifcellwallanchordomain(1) cell-wall-anchored protein SasA(1) Serine-rich adhesin for platelets SraP(1) putative cell wall-anchored protein(1)

604 18 142 12.67605634 8 0.8813 >Cluster 653 peptigoglycan-binding protein LysM(20) immunoglobulin G- binding protein A(8) immunoglobulin G binding protein A(6) immunoglobulin G binding protein A precursor(6) YSIRK signal domain/LPXTG anchor domain surface protein(4) "Protein A, von Willebrand factor binding protein Spa(4)" hypothetical protein(3) Immunoglobulin G-binding protein A(3) signal peptide protein(2) Immunoglobulin G binding protein A precursor(2) LPXTG-motif cell wall anchor domain(2) LPXTG-motif cell wall anchor domain protein(1) "Gram-positive signal peptide protein, YSIRK family(1)" immunoGlobulin g binding protein a(1) IMMUNOGLOBULIN G BINDING PROTEIN A PRECURSOR(1) protein A(1) truncated protein A(1) "Protein A, von Willebrand factor binding protein Spa; Immunoglobulin G binding protein A precursor(1)" immunoglobulin G binding protein A precursor (protein A)(1)

605 5 142 3.521126761 dynamin family protein(35)small GTP-binding domain protein(5) protein(5) Uncharacterized protein Bsub YpbR(4) putative dynamin family protein(4)putative GTPase(3) 50S ribosome- binding GTPase family protein(2) GTPase(2) GTPase of unknown function family protein(1) Dynamin family protein(1)"dynamin family protein, putative(1)" hypothetical protein with DLP_2domain(1) protein Bsub YpbR(1) Putative GTPase (dynamin-related)(1) dynamin family(1)

606 2 142 1.408450704 123 excinuclease ABC subunit A(40) A(10) ABC-ATPase UvrA(9) UvrABC system protein A(8) excision endonuclease subunit UvrA(5) "excinuclease ABC, A subunit(5)" exinuclease ABC subunit A(3)

607 3 142 2.112676056 2 1 >Cluster 127 restriction endonuclease subunit R(30) type-1 restriction enzyme R protein(9) putative type I restriction enzyme(7) "Type I restriction-modification system, restriction subunit R(7)" Type-1 restriction enzyme R protein(6) type-I restriction-modification system restriction endonuclease subunit(5) probable type I restriction enzyme restriction chain(2) "Type I restriction-modification system,restriction subunit R(2)" "type I restriction-modification enzyme, R subunit(2)" type I site- specific restriction-modification system restrictionsubunit(2) family(2)" subunit(1) protein(1)" putative(1)" subfamily,putative(1)" typeIrestrictionenzymerestriction chain(1) type I site-specific deoxyribonuclease restriction subunit(1)3 1 >Cluster 70 hypothetical protein(13) conserved hypotheticalseptin family protein(1)Hypothetical protein(1)2 1 >Cluster Excinuclease ABC subunit"type I site-specific deoxyribonuclease, HsdRType I restriction-modification system restriction"type I site-specific deoxyribonuclease, HsdR family"type I site-specific deoxyribonuclease, HsdR family,"type I site-specific deoxyribonuclease, HsdR family

608 5 142 3.521126761 3 1 >Cluster 338 PTSfructosetransportersubunitIIC(30) fructosespecificpermease(12) component(7) component(6)" system, fructose-specific IIB component / PTS system, fructose- specific IIC >(4)" "PTS system, fructose subfamily, IIA component domain protein(3)" "Pts system, fructose-specific iiabc component(3)" PTS system D-fructose-specific transporter subunit IIABC(2) PTS system fructose-specific IIABC component(2) "PTS system, fructose-specific IIA component(2)" hypotheticalPTS system fructose-specific EIIABC"PTS transport system, fructose-specific IIABC"PTS system, fructose-specific IIA component / PTSprotein(1) PTS family fructose/mannitol (fru) porter component IIABC(1) "PTS system, fructose subfamily, IIC subunit(1)" fructose- specific IIA component(1)"PTS system, fructose-specific IIABC component(1)" "fructose-specific PTS transport system, IIABC component, putative(1)" IIABC fructose/xylitol-PTS(1) "fructose specific permease, putative(1)" "PTS system, fructose-specific IIA component / PTS system, fructose-specific IIB component / PTS system,fructose-specific IIC c>(1)" putative membrane protein(1) "phosphoenolpyruvate-dependent sugar phosphotransferase system, EIIA 2(1)"

609 5 142 3.521126761 5 1 >Cluster 456 regulatory protein BlaR1(10) Beta-lactamase regulatory sensor-transducer BlaR1(10) beta-lactam sensor/signal transducer BlaR1(9) beta-lactamase(9) beta-lactamase regulatory protein(8)blaR1 peptidase M56 family protein(3) regulatory protein blaR1(2) penicillin binding transpeptidase domain protein(2) bla regulatorproteinBlaR1(1) hypotheticalprotein(1) regulatory BlaR1domainprotein(1) beta-lactamaseregulatoryproteinBlaR1(1) putative membrane protein(1) Regulatory protein BlaR1(1) Beta-lactamase regulator protein(1)

610 9 142 6.338028169 3 1 >Cluster 542 septation ring formation regulator EzrA(44) Septation ring formation regulator EzrA(17) putative septation ring formation regulator(6) septation ring formation regulator(4) conserved hypotheticalprotein(2) SeptationringformationregulatorezrA(2) septation ring formation regulator ezrA(2)"septation ring formation regulator, EzrA family protein(2)" hypothetical protein(1) MW1660(1) possible septation ring formation regulator(1)

611 2 142 1.408450704 2 1 >Cluster 613 ABC transporter ATP-binding protein(51) ABC transporter family protein(9) ABC transporter ATP-binding protein uup(9)ABC transporter(3) "ABC transporter, ATP-binding protein(2)" "ABC transporter, ATP-binding protein, putative(2)" ABC transporter ATPase(2) ABC superfamily ATP binding cassette transporter binding protein(1) ABC transporter related(1) ABC transporter homolog(1) hypothetical protein(1) MW1280(1) ATP-binding ABC transporter protein(1)

612 2 142 1.408450704 2 1 >Cluster 647 glycerol kinase(66) Glycerol kinase(14)

613 5 142 3.521126761 2 1 >Cluster 657 C4-dicarboxylate ABC transporter(31) "sodium-dependent transporter (huNaDC-1), putative(6)" sodium-dependent dicarboxylate transporter sdcS(6) sodium-dependent dicarboxylate transporter SdcS(5) putative sodium:sulfate symporter(5)DASS family divalent anion:sodium (Na+) symporter(5) Sodium- dependent dicarboxylate transporter SdcS(4) sodium-dependent transporter(3) Sodium-dependent dicarboxylate transporter(2) sodium:sulfatesymporter(2) hypotheticalprotein(2) Sodium- dependent dicarboxylate transporter sdcS(1) probable sodium- sulfate symport protein(1) MW1857(1) sodium-dependent dicarboxylate transporter SdcS (Na(+)/dicarboxylatesymporter)(1) "transporter, divalent anion:Na+ symporter (DASS) family protein(1)" "sodium-dependent transporter huNaDC-1, putative(1)" "sodium-dependent transporter (huNaDC-1),putative(1)" "sodium-dependentdicarboxylatetransporter sdcS, putative(1)" anion transporter(1)

614 3 142 2.112676056 3 1 >Cluster 689 proline:sodium symporter PutP(27) sodium/proline symporter(16)high affinity proline permease(14) Sodium/proline symporter(8) SSS family solute:sodium (Na+)/proline symporter(5)Proline/sodium symporter PutP(4) Proline/sodium symporter PutP Propionate/sodium symporter(2) proline uptake protein(1) Proline/sodium symporter PutP (TC 2.A.21.2.1) @ Propionate/sodium symporter(1) Proline/sodium symporter PutP / Propionate/sodium symporter(1) sodium:proline symporter(1) "Proline/sodium symporter PutP, Propionate/sodium symporter(1)"

615 5 142 3.521126761 2 1 >Cluster 724 alkyl hydroperoxide reductase subunit F(32) NADH dehydrogenase(18) Alkyl hydroperoxide reductase protein F(9) Alkyl hydroperoxide reductase subunit F(6)"alkyl hydroperoxide reductase, F subunit(4)" "alkyl hydroperoxide reductase, large subunit(4)" alkyl hydroperoxide reductase subunit Fprotein(2) "alkyl hydroperoxide reductase, subunit F(1)" peroxiredoxin subunit F(1) Peroxiredoxin reductase AhpF subunit(1) FAD-dependent pyridine nucleotide-disulphide oxidoreductase(1) "alkyl hydroperoxide reductase, subunit F, putative(1)"

616 11 142 7.746478873 3 1 >Cluster 739 "2', 3'-cyclic nucleotide 2'-phosphodiesterase(18)" bifunctional metallophosphatase/5'-nucleotidase(12) 5' nucleotidase family protein(12) "5'-nucleotidase, C-terminal domain protein(8)" "2',3'-cyclic-nucleotide 2'-phosphodiesterase(8)" putative 5'-nucleotidase(6) "2,3-cyclic-nucleotide 2-phosphodiesterase(3)" 5'-Nucleotidase domain-containing protein(2) UDP-sugar hydrolase(2) conserved hypothetical protein(1)possible5'-nucleotidase(1) hypotheticalprotein(1) "2,3- cyclic-nucleotide 2'phosphodiesterase(1)" MW0905(1) "5'-nucleotidase, domain protein(1)" 5'-Nucleotidase domain protein(1) 5'- nucleotidase(1) "5'-nucleotidase/2',3'-cyclic phosphodiesterase- related esterase(1)" probable 5' nucleotidase(1)

617 2 142 1.408450704 2 1 >Cluster 871 multidrug MFS transporter(32) H+ antiporter-2 family protein(10) drug resistance transporter EmrB/QacA subfamily protein(7) "Drug resistance transporter, EmrB/QacA subfamily(6)"putative transport protein(5) Lincomycin resistance protein lmrS(3) drug resistance transporter(2) "drug resistance MFS transporter, drug:H+ antiporter-1 (14 Spanner) (DHA2) family protein(1)" probablemultidrugresistanceprotein(1) Multidrug resistanceproteinB(1) lincomycinresistanceproteinLmrB(1) EmrB/QacA family drug resistance transporter(1) "EmrB-QacA subfamily drug resistance transporter, putative(1)" Putative multidrug resistance transporter(1) "drug resistance transporter, EmrB/QacA subfamily(1)" "drug resistance MFS transporter, drug:H+ antiporter-2 family(1)" conserved hypotheticalprotein(1) MFSfamilymajorfacilitatortransporter(1)hypotheticalprotein(1) MW2093(1)

618 4 142 2.816901408 4 1 >Cluster 886 trehalose permease IIC protein(29) "PTS system, trehalose-specific IIBC component(11)" "sugar-specific PTS transport system, IIBC component(7)" "PTS system, trehalose- specific IIB component / PTS system, trehalose-specific IICcomponent(6)" component(5) component(3)" phosphoenolpyruvate-dependent, trehalose-specific(2)" PTS system trehalose-specificEIIBCcomponent(2) PTSsystemcomponent(2) PTS family trehalose (tre) porter component IIBC(1) "pts system, trehalose-specific iibc component(1)" PTS enzyme II(1) PTS ascorbate transporter subunit IIA(1) "PTS system, trehalose- specific IIBC subunit(1)"conserved hypothetical protein(1) phosphoenolpyruvate-dependent and trehalose-specific PTS enzymeII(1) "PTS system, IIBC components(1)" 157

619 2 142 1.408450704 2 1 >ClusterPTS system trehalose-specific IIBC "PTS system, trehalose-specific IIB "PTS system, IIBC component(3)" "PTS enzyme II,918 helicase DnaB(30) membrane attachment protein(15) attachmentfamilyprotein(8) initiation and membrane attachment protein(5) chromosome replication initiation and membrane attachment protein(5) replicative DNA helicase loader DnaB(2) Replication initiation and membrane attachment protein(1) chromosome replication initiation/chromosome replication initiation/ replication initiation and membrane HelicaseloaderDnaB(8) replicationmembraneattachment protein(1) replication initiation and membrane attachment protein (DnaB) family protein(1) "replication initiation and membrane attachment protein, DnaB/DnaD family(1)" chromosome replication initiation(1) hypothetical protein(1) conserved hypothetical protein(1) replicative DNA helicase(1)

620 2 142 1.408450704 2 1 >Cluster 1117 guanine permease(30) xanthine/uracil permeases family protein(12) Hypoxanthine/guanine permease PbuG(7) guanine/ hypoxanthine permease pbuG(7) permease family protein(5) xanthine/uracil permease family protein(3)Guanine/hypoxanthine permease PbuG(3) conserved hypothetical protein(3) Xanthine/uracil/ thiamine/ascorbate permease family protein(2) xanthine/uracil/ vitamin C permease(2) Guanine-hypoxanthine permease(1) Xanthine/ uracil/vitamin C permease(1) NCS2 family nucleobase:cationsymporter-2(1)protein(1)permease(1)

621 3 1198 preprotein translocase subunit SecY(33) "preprotein translocase, SecY subunit(11)" preprotein translocase SecY subunit(10) Preprotein translocase secY subunit(8) Protein translocase subunit SecY(6) preprotein translocase subunit secY(3) Preprotein translocase secY subunit (TC 3.A.5.1.1)(2) Sec family Type I general secretory pathway preprotein translocase SecY(2)Sec family Type I general secretory pathway preprotein translocase SecY_1(2) "preprotein translocase, SecY subunit, putative(1)" Sec family Type I general secretory pathway preprotein

translocase SecY1(1) preprotein translocase subunit(1) Protein translocase subunit secY(1)

622 2 142 1.408450704 2 1 >Cluster 1252 dihydrolipoamide succinyltransferase(35) dihydrolipoamide succinyltransferase E2 component of 2-oxoglutarate dehydrogenase complex(12) Dihydrolipoamide succinyltransferase component (E2) of 2-oxoglutarate dehydrogenase complex(9)Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex(6) "dihydrolipoyllysine-residue succinyltransferase, E2 component of oxoglutarate dehydrogenase (succinyl-transferring) complex(5)" dihydrolipoyllysine-residue succinyltransferase(5) dihydrolipoyllysine succinyl transferase(2) "2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase(1)"Dihydrolipoamide succinyltransferase component E2 of 2- oxoglutarate dehydrogenase complex(1) dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex(1) biotin-lipoyl like family protein(1) "2-xanthine/uracil/thiamine/ascorbate permease family inner membrane protein YicO(1) putative Xanthine/uracil/vitamin C permease family protein(1)142 2.112676056 3 1 >Clusteroxoglutarate dehydrogenase, E2 subunit, dihydrolipoamide succinyltransferase(1)" "dihydrolipoamidesuccinyltransferase, putative(1)" 2-oxoacid dehydrogenases acyltransferase family protein(1)

623 2 142 1.408450704 2 1 >Cluster 1268 UDP-N-acetylglucosamine 1-carboxyvinyltransferase(57) putative UDP-N-acetylglucosamine 1-carboxyvinyltransferase(11) UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1(11) UDP-N- acetylglucosamine 1-carboxyvinyl transferase 1(1)

624 2 142 1.408450704 2 1329 methicillin resistance protein(28) protein(11) FmhC protein of FemAB family(9) protein(7) FemAB family peptidoglycan biosynthesis protein(5) FmhC protein(3) acetyltransferase domainprotein(3) "peptidoglycan biosynthesis, FemAB family protein(2)" methicillin resistance protein FmhC(2)aminoacyltransferase FemA (Factor essential forexpression of methicillin resistance A)(1) endopeptidase resistance protein(1)aminoacyltransferase FemA(1) methicillin resistance- relatedproteinFmhC(1) Methicillinresistanceprotein(1)conserved hypotheticalprotein(1) UDP-N-acetylmuramoylheptapeptide-glycineL- seryltransferase(1) endopeptidase resistance protein FmhC(1)

625 2 142 1.408450704 2 1 >Cluster 1330 hypothetical protein(37) tetratricopeptide repeat family protein(10) "TPR-repeat-containing protein, putative component of Menaquinone-cytochrome C reductase(8)" conserved hypothetical protein(7) putative PEP-CTERM system TPR-repeat lipoprotein(4)TPR repeat-containing protein(3) tetratricopeptide TPR_2 repeat protein(2) "TPR-repeat-containing protein, component of menaquinone-cytochrome C reductase(2)" Tetratricopeptide repeat family protein(2) Menaquinone-cytochrome C reductase(1) "anaphase-promoting complex, cyclosome, subunit 3 family protein(1)" Tetratricopeptide TPR_2 repeat protein(1) tetratricopeptide repeat protein(1) TPR domain protein(1) tetratricopeptide repeat domain protein(1)

626 5 142 3.521126761 4 1 >Cluster 1351 N-glycosyltransferase(21)"poly-beta-1,6 N-acetyl-D- glucosamine synthase(19)"Polysaccharide intercellular adhesin (PIA) biosynthesis N-glycosyltransferase IcaA(6)intercellular adhesion protein A(5) glucosaminyltransferase(5) intercellular adhesion protein icaA(3) "Poly-beta-1,6-N-acetyl-D-glucosamine synthase(3)" biofilm PIA synthesis N-glycosyltransferase icaA(2)1 >Cluster FemAB family femAB familypolysaccharide intercellular adhesin (PIA) biosynthesis N- glycosyltransferase IcaA(2) "poly-beta-1,6-N-acetyl-D-glucosamine synthase domain protein(1)" N-glucosaminyltransferase IcaA protein(1) glycosyl transferase family 2(1) N- glycosyltransferase IcaA(1) glycosyl transferase group 2 family protein(1) biofilm PIA synthesis N-glycosyltransferase icaA (Intercellular adhesion protein A)(1) Intercellular adhesion protein A(1) N-acetylglucosaminyltransferase icaA(1) N- glycosyltransferase PgaC(1) "intercellular adhesion protein A, putative(1)" chitin synthase(1) intercellular adhesion protein A glucosaminyltransferase(1) "poly-beta-1,6-N-acetyl-D- glucosamine synthase(1)" intercellular adhesion protein IcaA(1) glycosyl transferase 2 family protein(1)

627 2 142 1.408450704 2 1 >Cluster1423 preprotein translocase subunit SecY(27) system protein translocase subunit SecY2(13) system translocase SecY2(9) preprotein translocase SecY subunit- like protein(7) Preprotein translocase SecY2 subunit(7) Accessory Sec system protein translocase subunit SecY2(3) conserved hypotheticalprotein(2) translocasesubunitsecY(2) Protein translocase subunit secY(1) eubacterial secY family protein(1)Preprotein translocase SecY2 subunit (TC 3.A.5.1.1)(1) MW2574(1) hypotheticalprotein(1) "translocase,putative(1)"Sec family Type I general secretory pathway protein SecY(1) probable membrane protein(1) preprotein translocase SecY subunit- likeprotein(1) "preprotein translocase subunit SecY, putative(1)" SecY-related translocase(1)

628 2 142 1.408450704 2 1 >Cluster 1777 hypothetical protein(29) membrane protein(18) putative membrane protein(9) putative permease(5) conserved hypotheticalprotein(5) Putativemembranespanningprotein(4) AI-2E family transporter(3) hypothetical membrane protein(1) FIG01108417: hypothetical protein(1) putative DUF20-containing protein(1) probable membrane protein(1) "domain of unknown function, putative(1)" protein of unknown function UPF0118(1) Hypothetical protein(1)

629 3 142 2.112676056 2 1 >Cluster 1779 branched-chain amino acid aminotransferase(40) branched chain amino acid aminotransferase(10) Branched-chain amino acid aminotransferase(10) putative branched-chain-amino-acid aminotransferase(6) putative aminotransferase(6) branched-chain-amino-acid aminotransferase(3) branched-chain-amino-acid transaminase(2) Probable branched-chain-amino-acid aminotransferase(1) ilvE(1) branched-chain amino acid aminotroansferase homologue(1)accessory Secaccessory Sec

630 5 142 3.521126761 2 1 >Cluster 1816 diaminohydroxyphosphoribosylaminopyrimidine deaminase(30) riboflavinbiosynthesisproteinRibD(17) bifunctionalriboflavin biosynthesisprotein(8) Diaminohydroxyphosphoribosylaminopyrimidine deaminase(7) riboflavin specific deaminase(6) Diaminohydroxyphosphoribosylaminopyrimidine deaminase / 5-amino-6-(5- phosphoribosylamino)uracil reductase(5) bifunctional pyrimidine reductase / riboflavin biosynthesis protein(4) Riboflavin biosynthesis protein RibD(3)

631 2 142 1.408450704 2 1 >Cluster 1844 conjugal transfer protein(8) hypothetical protein(7) conjugative transposon TcpC family protein(6) putative membrane protein(2) conserved hypothetical protein(2) "conserved hypothetical protein, transposon-related protein(2)" Hypotheticalprotein(1) hypotheticalproteintransposon-related(1) transposon-related protein(1)

632 2 142 1.408450704 2 1 >Cluster 1893 nickel transporter NixA(30) HoxN/HupN/NixA family nickel/cobalt transporter(10) putative nickel

transport protein(8)NiCoT family nickel (Ni2+)-cobalt (Co2+) transporter(5) high-affinity nickel-transport protein nixA(4) "transition metal uptake transporter, Ni2+-Co2+ transporter family protein(4)" High-affinity nickel-transport protein nixA(3) high-affinity nickel-transporter(3) High-affinity nickel transport protein(2) high-affinity nickel-transport protein(2) probable high-affinity nickel-transport protein(1) "high affinity nickel transporter, putative(1)" high-affinity nickel transport protein(1) "transition metal uptake transporter, Ni2+-Co2+ transporter (NiCoT) family(1)"high-affinity nickel transporter(1)affinity nickel-transporter NixA(1)

633 2 142 1.408450704 2016 ornithine carbamoyltransferase(49) putative ornithine carbamoyltransferase(10) Ornithine carbamoyltransferase(10) "Ornithine carbamoyltransferase, catabolic(6)" ornithine transcarbamoylase(2) catabolic ornithine carbamoyltransferase(1) "ornithinecarbamoyltransferase, catabolic(1)"

634 2 142 1.408450704 2 1 >Cluster 2022 mannose-6-phosphate isomerase(39) "mannose-6-phosphate isomerase,classI(15)" putativemannose-6-phosphateisomerase(9) Mannose-6-phosphate isomerase(6) "Mannose-6-phosphate isomerase,MW2617(1) high- hypothetical protein(1)2 1 >Clusterclass I(4)" mannose-6 phosphate isomelase pmi(2) truncated mannose-6-phosphate isomerase(2) mannose-6 phosphate isomerase pmi(2)conserved hypothetical protein(1) hypothetical protein(1) MW2067(1)

635 5 142 3.521126761 2 1 >Cluster 2119 L-asparaginase(34) asparaginase(22) asparaginase family protein(8) putative L-asparaginase(7) probable L- asparaginase(2) Alpha/beta hydrolase fold-3 domain protein(2) Alpha/beta hydrolase fold-3 domain-containing protein(2) "L- asparaginase, type II(1)""L-asparaginase, putative(1)" Asparaginase/glutaminase(1)

636 2 142 1.408450704 2 1 >Cluster 2149 2-oxoisovalerate dehydrogenase(30) 2-oxoisovalerate dehydrogenase subunit beta(9) 2-oxoisovalerate dehydrogenase betasubunit(9) component, component, dehydrogenase E1(4) Branched-chain alpha-keto acid dehydrogenase E1 component beta chain(3) "transketolase, C-terminal domain protein(3)" branched-chain alpha-keto acid dehydrogenase E1 component protein(2) "2-oxoisovalerate dehydrogenase, E1 component, beta subunit, putative(1)" 2-oxoisovalerate dehydrogenase (acylating) beta subunit(1) Transketolase central region(1) "Transketolase, pyridine binding domain protein(1)"2-oxoisovalerate dehydrogenase subunit beta (branched-chainalpha-keto acid dehydrogenase e1 component beta chain) (bckdhe1-be>(1)

637 5 142 3.521126761 2 1 >Cluster 2152 replication initiator A family protein(4) replication protein(3) Replication protein(3) truncated putative replication initiation protein Rep(3) "Replication initiator A, N- terminal(2)" replication initiation protein Rep(1) putative"Branched-chain alpha-keto acid dehydrogenase, E1 beta subunit(8)" "2-oxoisovalerate dehydrogenase, E1 beta subunit(7)" branched-chain alpha-keto acidreplication-associated protein RepA(1) protein(1) hypothetical protein(1)

638 3 142 2.112676056 2244 gamma-hemolysin subunit A(30) C(17) gamma-hemolysin component C(14) precursor(6) "gamma-hemolysin component C precursor, HlgC(4)" gamma-hemolysin component C protein(2) leukocidin-S subunit(2) leukocidin/Hemolysin toxin family protein(1) beta-channel forming cytolysin(1) "leukocidin s subunit precursor, putative(1)"Gamma-hemolysin C subunit HlgC(1) gamma hemolysin component C(1) "gamma hemolysin, component C(1)"replication initiation2 1 >Cluster Gamma-hemolysin component gamma-hemolysin component C

639 4 142 2.816901408 3 1 >Cluster 2248 2-ketogluconate reductase(19) D-isomer specific 2- hydroxyacid dehydrogenase(14) D-glycerate dehydrogenase(12) glyoxylatereductase(8) Glyoxylatereductase/Glyoxylatereductase/ Hydroxypyruvatereductase(6) Glyoxylatereductase(5) NADP oxidoreductase coenzyme F420-dependent family protein(3) glyoxylate reductase (Glycolate reductase)(2) Glyoxylate reductase (NADP+)(1) hypotheticalprotein(1) dehydrogenase(1)glycerate dehydrogenase(1) 4-phosphoerythronate dehydrogenase(1) D-isomer specific 2-hydroxyacid dehydrogenase NAD-binding(1) "D-isomer specific 2-hydroxyacid dehydrogenase, NAD binding domain protein(1)"D-isomer specific 2-hydroxyacid dehydrogenase family protein(1) glycerate dehydrogenase-like protein(1) MW0812(1)

640 2 142 1.408450704 2 1 >Cluster 2263 hypothetical protein(29) putative lipoprotein(11) lipoprotein(11) conserved hypothetical protein(4) hypothetical lipoprotein(2) Hypothetical protein(2) FIG01107997: hypothetical protein(1)

641 3 142 2.112676056 2 1 >Cluster 2370 nucleoside hydrolase(30) Inosine-uridine preferring nucleoside hydrolase(13) inosine-uridine preferring nucleoside hydrolase family protein(7) putative inosine-uridine preferring nucleosidehydrolase(7) inosine/uridine-preferringnucleoside hydrolase(6) purine nucleosidase(3) putative inosine-uridine preferring nucleosidehydrolase(2) pyrimidine-specific ribonucleoside hydrolase rihA(2) inosine-uridine preferring nucleoside hydrolase(2) conserved hypothetical protein(2) "inosine-uridine preferring nucleoside hydrolase family protein, putative(1)"Purine nucleosidase(1) probable inosine-uridine preferring nucleoside hydrolase family protein(1) pyrimidine-specific ribonucleoside hydrolase RihA (Cytidine/uridine-specific hydrolase)(1)MW0219(1) hypothetical protein(1)

642 4 142 2.816901408 3 1 >Cluster 2441 hypothetical protein(33) 3-beta hydroxysteroid dehydrogenase(31) conserved hypothetical protein(7) Accessory signaltransductionproteinGraX/ApsX(4) NADH(P)-bindingfamily protein(4) Hypotheticalprotein(1) "NAD-dependentepimerase/ dehydratase, putative(1)"FIG01107875: hypothetical protein(1)

643 3 142 2.112676056 2 1 >Cluster2984 phosphotransferase(30) aminoglycoside phosphotransferase family protein(8) phosphotransferase enzyme family protein(7)conserved hypothetical protein(7) phosphotransferase family protein(6) hypotheticalprotein(6) choline/ethanolaminekinase family protein(3) putative phosphotransferase(3) choline kinase(2) putative choline kinase involved in lipopolysaccharide biosynthesis(2) kanamycin kinase(2) phosphotransferase enzyme family(1) possible kanamycin kinase(1)Phosphotransferase enzyme family protein(1)aminoglycoside phosphotransferase(1)phosphotransferase, putative(1)"

644 4 142 2.816901408 3248 metal-dependent hydrolase(30)"aminoglycoside2 1 >Clustercyclase family protein(13) Metal-dependent hydrolase/cyclase(9) conservedhypotheticalprotein(8) hypotheticalprotein(6) putativecyclase enzyme(5) "cyclase, putative(3)" putative cyclase

protein(3)cyclase(2) putative cyclase(1) Polyketide cyclase family protein(1)

645 6 142 4.225352113 3287 transcriptional regulator(32) staphylococcal accessory regulator Y(4) protein(3) staphylococcal accessory regulator family(3) accessoryregulatorSarY(3) Hypotheticalprotein(3) accessory regulator Y sarY(1) Hypothetical protein SA2091(1) marR family protein(1) Staphylococcal accessory regulator-like protein (SarY)(1) conserved hypothetical protein(1) accessory regulator Y(1) "staphylococcal accessory regulator Y, putative(1)"accessory Y(1) accessory regulator family(1) sa accessory regulator Y(1)

646 4 142 2.816901408 2 1 >Cluster 3525 methionine ABC transporter permease(30) Methionine ABC transporter permease protein(9) ABC transporter permease protein(9)methionine import system permease protein metI(4) ABC transporterpermease(4) "ABCtransporter,permeaseprotein(3)" methionine import system permease protein MetP(3) binding-protein- dependent transport systems inner membrane component(3) conserved hypothetical protein(3) inner membrane transporter(2) methionine import system permease protein MetI(2) hypothetical protein(2) binding-protein-dependent transport system inner membrane protein(2) "ABCtransporter,permease,putative(1)" "ABC superfamily ATP binding cassette transporter, membrane protein(1)" Methionine import system permease protein MetP(1) D-methionine transport system permease protein metI(1) probable ABC transporter permease protein(1)

647 2 142 1.408450704 2 1 >Cluster3 1 >Cluster hypothetical protein(21) accessory regulator-like3577 thiamine-phosphate pyrophosphorylase(39) Thiamin-phosphate pyrophosphorylase(10) putative thiamine-phosphate pyrophosphorylase(10) thiamine-phosphate diphosphorylase(6) Thiamine-phosphate synthase(4) thiamine-phosphate synthase(3) Thiamine-phosphate pyrophosphorylase(2) thiamin phosphate synthase(2) thiamine phosphate synthase(1) "thiamin monophosphate synthase, chain B(1)" thiamine-phosphate pyrophosphorylase (TMPpyrophosphorylase) (TMP-PPase) (Thiamine- phosphate synthase)(1)

648 4 142 2.816901408 3 1 >Cluster 3737 uracil-DNA glycosylase(53) putative uracil-DNA glycosylase(10) Uracil-DNA glycosylase(8)"Uracil-DNA glycosylase, family 1(8)" "uracil-DNA glycosylase, putative(1)"

649 3 142 2.112676056 2 3882 hypothetical protein(45) lipoprotein(13) lipoprotein(10) conserved hypothetical protein(6) protein(3) "lipoprotein, putative(2)" protein SAV1801(1) probable lipoprotein(1)

650 3 142 2.112676056 2 3948 precorrin-2 dehydrogenase(39) Siroheme synthase / Precorrin-2 oxidase(8) "siroheme synthase, N-terminal domain protein(7)" hypotheticalprotein(6) sirohemesynthase(6) Precorrin-2 dehydrogenase(4) conserved hypothetical protein(3) sirohemesynthase/precorrin-2oxidase(2) possiblesirohydrochlorin ferrochelatase(1) MW2539(1) "siroheme synthase, putative(1)" "siroheme synthase, domain protein(1)"

651 2 142 1.408450704 2 1 >Cluster 4151 hypothetical protein(45) Substrate-specific component MtsA of methionine-regulated ECF transporter(7) conserved hypothetical protein(6) putative membrane protein(3) "ECF-type riboflavin transporter, S component family protein(2)" probable membrane protein(1) ECF-type riboflavin transporter S component domain protein(1) membrane protein(1) conserved hypotehticalprotein(1) proteinofunknownfunctionDUF1393(1)

652 2 142 1.408450704 2 1 >Cluster 4178 hypothetical protein(28) membrane protein(21) putative membrane protein(16) conserved hypothetical protein(5)1 >Cluster putative Hypothetical Hypothetical1 >ClusterHypotheticalprotein(3) Putativemembranespanningprotein(3) hypothetical membrane protein(1) Hypothetical protein SAV2327(1) probable membrane protein(1) "membrane protein, putative(1)"

653 2 142 1.408450704 2 1 >Cluster 4480 galactose-6-phosphate isomerase(27) galactose-6- phosphate isomerase LacB subunit(15) "galactose-6-phosphate isomerase, LacB subunit(12)" "Galactose-6-phosphate isomerase, LacB subunit(9)" galactose-6-phosphate isomerase subunit LacB(5) Galactose-6-phosphate isomerase subunit LacB(4) Galactose-6- phosphate isomerase subunit lacB(2) galactose-6-phosphate isomerase subunit lacB(2)"galactose-6-phosphate isomerase, subunit LacB(1)"galactose-6-phosphate isomerase subunit(1)Galactose-6- phosphate isomerase lacB subunit(1)

654 2 142 1.408450704 2 1 >Cluster 4560 hypothetical protein(29) Putative cytosolic protein(2) "Repetitive hypothetical protein near ESAT cluster, SA0282 homolog(1)" conserved hypothetical protein(1) conserved hypothetical family protein(1)

Appendix B

newid	Cohort	baseline_age	sex	race	ethnic	pancreat	F508genotype	fev1pct

Clin-AMT0011	Clin	15.7	female	white	unknown	insufficient	heteroz	56.0
Clin-AMT0098	Clin	10.1	male	white	non-hispanic	insufficient	homoz	112.0
Clin-AMT0099	Clin	8.6	female	white	non-hispanic	insufficient	heteroz	99.0
Clin-AMT0125	Clin	16.2	female	white	non-hispanic	insufficient	homoz	40.0
Clin-AMT0134	Clin	18.3	male	white	non-hispanic	insufficient	homoz	82.0
Clin-AMT0140	Clin	17.0	male	white	non-hispanic	insufficient	homoz	60.0
Clin-AMT0155	Clin	9.2	male	white	non-hispanic	insufficient	homoz	80.0
Clin-AMT0167	Clin	16.7	female	white	non-hispanic	insufficient	homoz	86.0
Clin-AMT0172	Clin	15.9	female	white	unknown	insufficient	homoz	91.0
Clin-AMT0177	Clin	16.5	male	white	non-hispanic	insufficient	homoz	99.0
Clin-AMT0188	Clin	13.3	female	white	non-hispanic	insufficient	homoz	83.0
Clin-AMT0341	Clin	1.3	female	white	non-hispanic	insufficient	homoz	
Clin-AMT0413	Clin	15.4	male	white	non-hispanic	insufficient	homoz	98.0
Clin-AMT0456	Clin	16.3	female	white	non-hispanic	insufficient	homoz	72.0
Clin-AMT0463	Clin	10.7	female	white	non-hispanic	insufficient	homoz	97.0
Clin-AMT0495	Clin	10.8	female	unknown	non-hispanic	insufficient	heteroz	116.0
Clin-AMT0504	Clin	3.9	male	white	non-hispanic	insufficient	other	
Clin-AMT0506	Clin	5.9	male	white	non-hispanic	insufficient	heteroz	83.0
Clin-AMT0507	Clin	6.4	female	white	non-hispanic	insufficient	heteroz	112.0
Mult-1	Mult	9.1	female	white	non-hispanic	insufficient	homoz	93.7
Mult-5	Mult	10.4	male	white	non-hispanic	insufficient	other	70.9
Mult-7	Mult	13.0	male	white	non-hispanic	insufficient	heteroz	107.4
Mult-10	Mult	14.4	male	white	non-hispanic	insufficient	homoz	54.5
Mult-21	Mult	11.8	female	white	non-hispanic	insufficient	heteroz	41.6
Mult-25	Mult	14.3	male	white	non-hispanic	sufficient	heteroz	111.5
Mult-37	Mult	14.0	female	unknown	hispanic	insufficient	heteroz	101.9
Mult-39	Mult	11.1	male	black	non-hispanic	insufficient	heteroz	100.6
Mult-101	Mult	6.7	female	white	non-hispanic	insufficient	heteroz	95.0

Mult-102	Mult	11.2	female	white	non-hispanic	insufficient	homoz	80.6
Mult-113	Mult	10.0	male	white	non-hispanic	insufficient	other	109.9
Mult-115	Mult	7.3	male	white	non-hispanic	insufficient	homoz	33.4
Mult-225	Mult	11.3	female	white	non-hispanic	insufficient	heteroz	80.3
Mult-234	Mult	15.8	female	white	non-hispanic	insufficient	heteroz	76.5
Mult-241	Mult	13.5	female	white	non-hispanic	insufficient	homoz	95.9
Mult-242	Mult	15.3	female	white	non-hispanic	insufficient	heteroz	91.4
Mult-243	Mult	8.2	male	white	non-hispanic	insufficient	heteroz	116.7
Mult-249	Mult	15.5	female	white	non-hispanic	insufficient	heteroz	113.5
Mult-301	Mult	16.0	male	white	non-hispanic	insufficient	homoz	97.4
Mult-339	Mult	10.8	female	white	non-hispanic	insufficient	heteroz	107.8
Mult-414	Mult	16.8	female	white	non-hispanic	insufficient	homoz	92.8
Mult-415	Mult	14.7	male	white	non-hispanic	insufficient	homoz	89.0
Mult-426	Mult	15.5	female	white	non-hispanic	insufficient	homoz	76.1
PASA-2	PASA	8.4	female	white	non-hispanic	insufficient	homoz	73.8
PASA-11	PASA	9.4	female	white	non-hispanic	insufficient	homoz	71.0
PASA-12	PASA	11.7	female	white	non-hispanic	insufficient	homoz	62.0
PASA-13	PASA	15.2	male	white	hispanic	insufficient	heteroz	56.3
PASA-20	PASA	8.7	female	white	non-hispanic	insufficient	heteroz	90.0
PASA-24	PASA	13.3	male	white	non-hispanic	insufficient	homoz	68.5
PASA-30	PASA	11.9	female	white	non-hispanic	insufficient	other	67.3
PASA-31	PASA	15.0	female	white	non-hispanic	insufficient	heteroz	33.5
PASA-32	PASA	9.2	female	white	non-hispanic	insufficient	homoz	106.3
PASA-37	PASA	7.1	female	white	non-hispanic	insufficient	homoz	108.3
PASA-38	PASA	13.4	male	white	non-hispanic	insufficient	homoz	96.6
PASA-39	PASA	15.9	female	white	non-hispanic	sufficient	heteroz	109.5
PASA-41	PASA	11.8	male	white	non-hispanic	insufficient	homoz	78.2

PASA-46	PASA	14.2	male	white	non-hispanic	insufficient	homoz	94.4
PASA-47	PASA	11.6	female	white	non-hispanic	insufficient	heteroz	66.1
PASA-49	PASA	5.5	female	white	non-hispanic	insufficient	homoz	
PASA-52	PASA	11.0	female	white	non-hispanic	insufficient	heteroz	89.8
PASA-54	PASA	2.4	female	white	non-hispanic	insufficient	other	
PASA-62	PASA	6.9	male	white	non-hispanic	insufficient	homoz	100.2
PASA-75	PASA	6.8	female	white	non-hispanic	insufficient	homoz	88.8
PASA-79	PASA	16.6	female	white	non-hispanic	insufficient	homoz	60.4
PASA-87	PASA	16.8	male	white	non-hispanic	insufficient	homoz	75.4
PASA-93	PASA	9.9	female	white	non-hispanic	insufficient	other	83.3
PASA-94	PASA	14.8	female	white	non-hispanic	insufficient	heteroz	86.6