Bacterial Phenotypes of the Equatorial Pacific in the Presence of Different Phytoplankton Metabolites

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Abstract:

Metabolites are compounds released by phytoplankton, and are major sources of carbon and other essential nutrients for heterotrophic bacteria living in the pelagic ocean, which form the base of marine food webs via the recycling of nutrients. The goal of my experiment was to determine how the prevalence of different phytoplankton metabolites can influence heterotrophic bacterial community composition, with a focus on the equatorial Pacific region. My hypothesis was that different bacteria would show increased growth on media enriched with certain metabolites, and decreased growth on other media enriched with other metabolites. This was achieved by collecting seawater samples from three locations along a transect of the equator, which were then concentrated and plated on agar plates enriched with some of the more common phytoplankton metabolites: Glycine Betaine (GBT), Dimethylsulfoniopropionate (DMSP), Spermidine, Glucosamine, and Sucrose. A complete nutrient option (1/2 YTSS) was used as a control. These plates were assessed for bacterial growth via Colony Forming Units (CFU's) after a 5-day incubation period. My results showed a northward trend of increasing phenotypic diversity, as well as higher numbers of CFU's in locations north of the equator. There was also significant variation amongst the different metabolites in regard to the species of bacteria (based on colony phenotype) that grew and the number of CFU's present. There were some consistent phenotypes across all media types. Based on these results, the metabolites present in the water column may be an important determining factor in the composition of the heterotrophic bacterial community.

Plain Language Summary:

The equatorial Pacific is well-studied due to the equatorial current and associated upwelling which provides ample nutrients for life, but there is a lack of research on the bacterial

community in this region. These bacteria often get nutrients by metabolizing and transforming compounds found in the water column released by phytoplankton, with different compounds being released by different species of phytoplankton. These compounds are known as metabolites. My research focused on how metabolites in the equatorial Pacific influence the bacterial community, which has important implications for marine ecosystems. For my experiment, I cultivated bacteria on media enriched with some of these metabolites to determine if they can lead to the growth of different types of bacteria. I found that there are bacteria that will preferentially grow on certain metabolites and do not appear to grow on others. There were also some bacteria that will utilize a variety of these metabolites for substrate. These bacteria hold important roles in biogeochemical cycling throughout the ocean by recycling some of the nutrients that they take up. My results offer a glimpse at these organisms, hopefully providing some information on how bacteria interact with the environment around them.

Introduction:

Marine phytoplankton are responsible for nearly half of the world's photosynthesis (Fu et. al, 2020). During the course of this process, phytoplankton produce a variety of organic chemical compounds via metabolic processes—known as metabolites. Many of them are categorized as organic carbon compounds and contain essential elements for life like sulfur and nitrogen (Heal et al., 2021). The production of these metabolites varies with specific phytoplankton species, with some species producing different metabolites than other species and vice versa (Durham et al., 2022). These metabolites are released to the surrounding ocean via processes such as exudation (excretion from cells), leakage (healthy cells leak a fraction of internal metabolites), and cell lysis or disintegration (Moran at al., 2022). This manifests itself in a pool of dissolved organic carbon (DOC) totaling nearly 660 Petagrams (10^{15} grams) of carbon, roughly equivalent to inorganic carbon contained in the Earth's atmosphere (Ferrer-Gonzalez et al., 2021). These compounds serve an important role as substrates for marine heterotrophic bacteria, which are responsible for processing approximately half of all metabolites produced during photosynthesis over the course of one year (Fu et al., 2020). Specifically, a set of core bioactive metabolites formed by phytoplankton are in high demand for use as substrate, with turnover time spanning hours to days. These heterotrophic marine bacteria consume these compounds and incorporate elements into their own structure for growth (Moran et al., 2022). Bacteria respire during this process, releasing CO_2 to the surrounding ocean and leading to elevated CO_2 levels which influence air-sea exchange (i.e. the exchange of gas particles between the ocean and the atmosphere).

The communities of phytoplankton that produce these metabolites can vary with changing conditions such as nutrient availability, as the source of nutrients available can

determine which species of phytoplankton can grow (Zhong et al., 2022). ENSO, or El Nino Southern Oscillation, can cause alterations in global currents and upwelling, resulting in oligotrophic regions where there would typically be higher nutrient levels. One such region affected in this way is the equatorial Pacific, where the upwelling and westward movement of nutrient-rich water is halted during El Nino, resulting in decreased nutrient levels and less primary production (Pennington et al., 2006). This results in a temporarily oligotrophic zone, which can shift the phytoplankton community composition and thus the metabolites produced. Furthermore, different nutrient conditions like low nitrogen or sulfur may cause the same species to produce different metabolites (Van Tol & Armbrust, 2021). Logically, a shift in the types of metabolites prevalent would lead to a shift in the community composition of marine heterotrophic bacteria, as their substate sources would change and lead to favorable substrate conditions for bacteria that can metabolize these new sources of organic compounds. Despite this perceived connection, research on the connection between variations in conditions and heterotrophic bacterial communities is lacking, and the relationship between the two is not well understood.

A better understanding of how varying conditions alter metabolite production and associated marine heterotrophic bacterial communities could provide valuable insights on ocean biogeochemical cycling. Specifically, shifts in these communities could have impacts on CO_2 levels produced by the ocean, leading to impacts on global climate. These bacterial communities also form the base of many marine food webs and are responsible for the recycling of major nutrients, which can have a cascading effect on other trophic levels in ways that may be economically important like fisheries. My goal was to address this gap in knowledge by characterizing how heterotrophic bacterial communities in the surface waters of the equatorial

Pacific behave metabolically under conditions like El Nino. A better understanding of these shifts in bacterial communities and metabolisms can inform us on previously overlooked impacts of El Nino and other climate patterns on biogeochemical cycling and Earth's climate. My hypothesis was that the communities of marine heterotrophic bacteria found in surface waters of the equatorial Pacific will have bacteria that preferentially metabolize different phytoplankton metabolites. This would be proven by certain bacteria having increased prevalence on media enriched with certain metabolites, and a lack of growth on media enriched with other metabolites. My project focused on the heterotrophic bacteria associated with the phycosphere, or the diffusive boundary layer immediately surrounding phytoplankton cells, where the concentration of these metabolites is the highest (Seymour et al., 2017).

Methods:

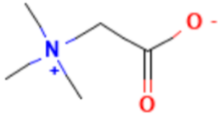
Prior to the cruise, I prepared agar plates for use in the lab in Seattle, WA. These plates were made in 0.5L batches; the general recipe was 50 mL of Anhydrous Salts, 50 mL of Hydrous salts, 400 mL of Milli-Q Water, 8.0 g of bacteriological agar, and the associated nutrients (Table 1). The type of agar used as a media in these plates was a standardized agar dilution similar to those used in Rodrigues & Carvalho (2022). All plate batches were autoclaved to kill any potential microbial contaminants and then poured, with approximately 25 mL being used for each plate. After pouring, the plates were allowed to solidify for 24 hours and then packaged in sterile plastic sleeves for transport to the cruise.

Table 1. Overview of media/nutrient concentrations. This table contains the amounts and concentrations of nutrients/metabolites in the agar plates used for the incubations. All other components are detailed in the generalized recipe from the above paragraph.

Media Name	Ingredient	Amount (mg)	Concentration (mg/mL)	Amount per plate (mg)	Concentration (mM/0.5L)
1/2 YTSS	Yeast Extract	1000	2	50	--
	Tryptone	625	1.25	31.25	--
DMSP	DMSP	100	0.2	5	1.49
Glucosamine	Glucosamine	442	0.884	22.1	4.93
Spermidine	Spermidine	290	0.58	14.5	3.99
GBT	GBT	234	0.468	11.7	3.99
Sucrose	Sucrose	684	1.368	34.2	4.00

These nutrient options were selected to capture the diversity of the bacterial community in this region, as well as focus on key phytoplankton metabolites. The first media–½ YTSS–is a generalized enriched media with all essential nutrients, and is a commonly used media in culturing marine bacteria (Salgado et al., 2014). This was utilized to create agar plates permissive to bacterial growth regardless of differences in metabolic processes that would serve as a control. Dimethylsulfoniopropionate (DMSP) is an organosulfur compound and phytoplankton metabolite that is abundant in marine ecosystems, and is an important source of

carbon and sulfur to marine bacteria (Wirth et al., 2020) (Figure 1). Glycine Betaine (GBT) is also abundant in these marine environments and is an important substrate source for marine bacteria (Boysen et al., 2022) (Figure 1).



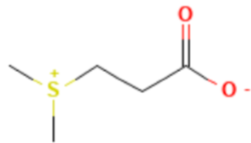


Figure 1. Glycine Betaine (top) and Dimethylsulfoniopropionate (bottom). Both share similar chemical structure, and comparing them as substrate may inform us on differential preference for sulfur vs. nitrogen-containing metabolites in marine heterotrophic bacteria.
(Source: PubChem, NIH)

Sucrose is a carbohydrate that is abundant in marine environments, and a metabolite commonly formed by cyanobacteria and other algae (Kolman et al., 2015) (Figure 2). Glucosamine is an amino sugar and serves important roles in bacterial cell structures (Riemann & Azam, 2002) (Figure 2). Specifically, I used N-acetyl-Glucosamine, an acetylated version of glucosamine that is common in the marine environment. Spermidine is a polyamine and common marine metabolite that is released by phytoplankton like the dinoflagellate *Alexandrium tamarense* (Fu et. al, 2020) (Figure 2).

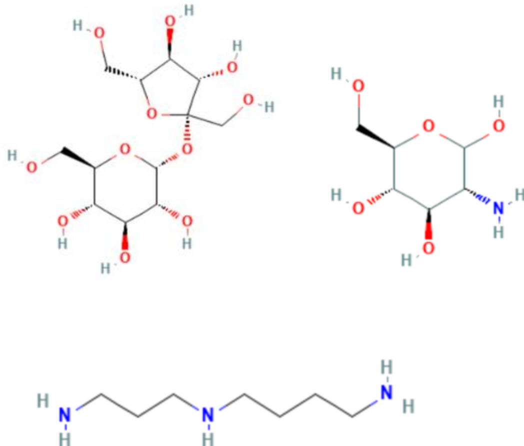


Figure 2. Sucrose (top left) vs. Glucosamine (top right) vs Spermidine (bottom)
Both Sucrose and Glucosamine are based around a sugar structure, except Glucosamine has replaced a hydroxyl group (-OH) with an -NH_2 group. Spermidine is especially high in nitrogen.
(Source: PubChem, NIH)

My sampling took place during a cruise onboard the R/V Thomas G. Thompson from December 29th to January 11th of 2024. The cruise started in Pago Pago, American Samoa and collected samples between 5° S and 5° N and along the 167° W longitude line at 12 different sampling locations combined with a geological survey off the coast of American Samoa at 14.0°11.80'S & 169°35.64'W. I took samples from 3 stations at a depth of 40 meters during the cruise: Station 5 (3.0°S, 167°W), Station 9 (0.0°S & 167°W), and Station 13 (3.0°N, 167°W) (Figure 3). These sites were chosen to create an accurate approximation of the equatorial region by including a sampling station north, south, and directly on the equator.

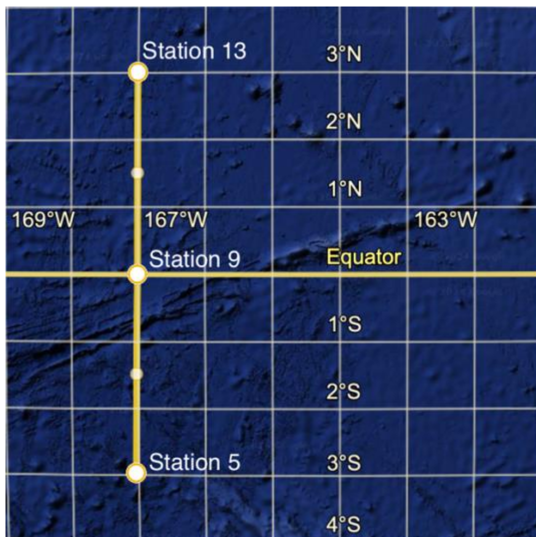


Figure 3. Sampling Locations and Cruise Details.
Station 5, 9, and 13 are where seawater samples were taken from a depth of 40m for microbial culturing and analysis.
(Source: Google Earth)

At each station, I collected 1.0 liter(L) of water from a depth of 40 meters(m) using a CTD equipped with 10 L Niskin bottles. Special consideration was taken to avoid contact with non-target microbes present on plastic tubing, any surfaces of the Niskin bottle, and other potential sources of contamination. I wore an N-95 mask and nitrile gloves during the collection of samples and plating, and there was frequent sterilization of surfaces via 70% ethanol throughout the entire process. To further avoid potential contamination with non-target microbes, a 1.0 L plastic bottle was pre-washed and rinsed 3 times with DI water and 3 times with water from 40 m depth. After rinsing, this bottle was filled to the maximum volume allowed with

seawater from 40 m depth. This seawater was then filtered via a vacuum filtration system, where the contents of 0.5 L of the 40 m seawater were concentrated onto a 47 mm diameter 0.2 µm filter. This was done to concentrate the bacteria collected and allow for greater amounts of bacteria to be cultured, and resulted in a small volume of concentrated bacteria suspended in seawater and on the filter.

During filtration, special measures were taken to ensure a clean environment with air flow being restricted in the immediate vicinity and a covering being placed on the sample during the filtration process to prevent particles in the air from falling in. The filter system was sterilized via rinsing with 70% ethanol prior to the addition of the seawater sample to ensure that no contaminants entered the sample. Post-sterilization, I added 0.5 L of the 40 m seawater to the vacuum filtration system. After the 0.5 L of seawater was fully filtered, I shut off the vacuum and broke the seal of the vacuum filtration system to prevent residual vacuum from drawing more water in. I then added 15-20 mL of seawater from the unfiltered 40 m sample bottle via a pipette and resuspended any bacteria by mixing the water and rinsing the filter with the pipette. There was typically still some residual vacuum, resulting in 12-13 mL of concentrate. This concentrate was then transferred via pipette to a 15 mL falcon tube. After this, the 47 mm 0.2 µm filter was removed from the filtration system using sterile tweezers and added to the falcon tube. The falcon tube was then vortexed for 30 seconds to resuspend any bacteria on the filter that was missed during the pipette resuspension process.

To store concentrate for future use, 1.6 mL of the resulting concentrate was added to a 2 mL cryovial containing 0.4 mL of 100% glycerol stock and frozen at -80°C for later culturing/genomic analysis. The rest of the concentrate was used for plating on media enriched with different metabolites, with 200 µL being used for each plate. The concentrated bacteria

from each station were streaked onto 18 plates: 3 plates of ½ YTSS, 3 plates of DMSP-enriched agar, 3 plates of GBT-enriched agar, 3 plates of sucrose-enriched agar, 3 plates of spermidine-enriched agar, and 3 plates of glucosamine-enriched agar. During the cruise, these plates were then allowed to incubate at room temperature for a 120-hour period in the absence of light to remove any photoautotrophic bacteria. Following the incubation, the plates were examined for bacterial colonies. Pictures of plates were taken, and if colonies were present, colony morphology and characteristics were recorded. Some colonies of interest identified based on their metabolism (i.e. increased growth on a specific metabolite), morphology, and similarity/dissimilarity compared to other colonies were selected for additional isolation/culturing and shipped back to the lab. Once back in the lab, Colony Forming Units (CFUs) were counted from the pictures taken post-incubation. A CFU can be defined as an isolated colony in this case, or specifically the single bacterial cell that was the founding member of the bacterial colony.

Results:

There was bacterial growth across all sampling locations and all nutrients, with variation in the colony phenotypes present between the media types (Table 2). Of all media types, ½ YTSS showed the most phenotypic diversity. The rest of the media types displayed fewer phenotypes, with most displaying 2-3. There was an increasing trend of phenotypic diversity on the ½ YTSS media as we headed in a northward direction, but other media types were relatively consistent (Figure 4).

Table 2. Phenotypic diversity across media types and stations. Each letter represents a unique bacterial phenotype. Some of the phenotypes were similar, but differed in one aspect (size, color, etc.) to the point where their identities could be questioned, with some types being denoted with an * being especially hard to discern.

Station Number	Nutrient type	Phenotypic traits
Station 5	1/2 YTSS	type A, type B, type C, type D: large translucent colonies w/solid center, type E: large solid white colones, type M: medium (>1mm) translucent colonies
	Spermidine	type A: small pinpoint white colonies, type B: small pinpoint translucent colonies
	Glucosamine	type A, type B
	Sucrose	type A, type B, type M
	GBT	type A, type B, type N: translucent "patchy" colonies
	DMSP	type A, type B, type C: medium (>1mm) white colonies
Station 9	1/2 YTSS	type A, type B, type C, type D, type E, type F: medium (>1mm) pale pink colonies, type G: medium (>1mm) pale yellow colonies
	Spermidine	type A, type B
	Glucosamine	type A, type B, type C, type H: large "sheets" of translucent bacteria
	Sucrose	type A, type B, type C*
	GBT	type A, type B, type N
	DMSP	type A, type B
Station 13	1/2 YTSS	type A, type B, type C, type D, type E, type F, type I: Large bright pink colonies, type J: Large bright yellow colonies, type K: Tranlsucent edges with solid "snowflake" pattern white centers, type M
	Spermidine	type A, type B, type C
	Glucosamine	type A, type B, type C
	Sucrose	type A, type B, type C
	GBT	type A, type B, type L: small, pale blue colonies (potentially type N)
	DMSP	type A, type B, type C

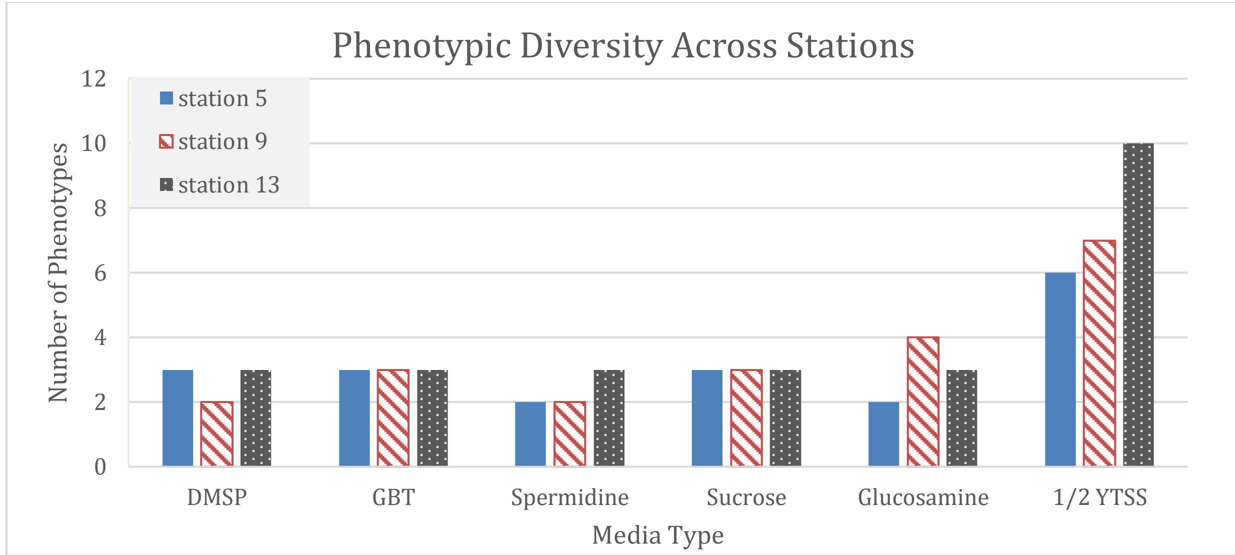


Figure 4. Phenotype diversity at each station. This is based on observed phenotypic differences across all media types at each station described in table 2.

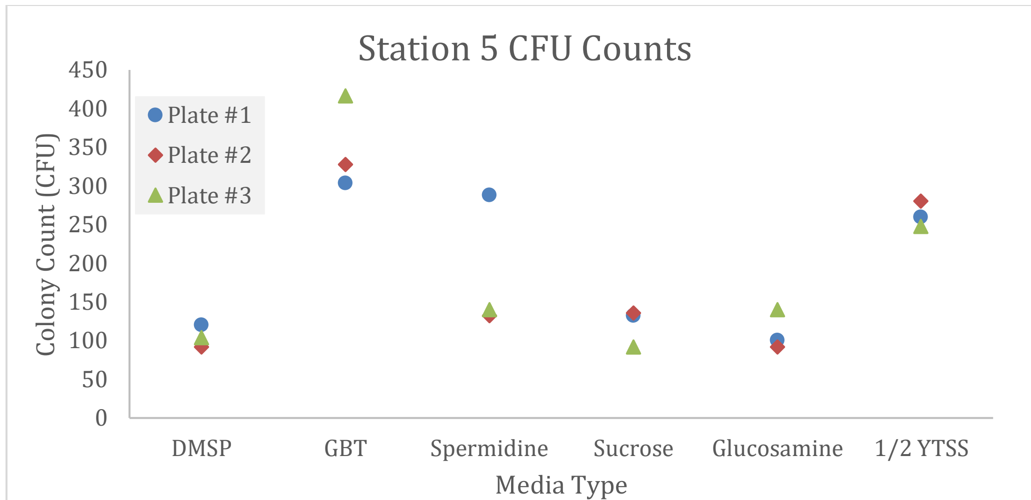
The CFU's also reflected much of the phenotypic diversity mentioned above, with some outliers in regard to CFU counts (Table 3, Table 4). Most plates showed a relatively consistent pattern of the raw numbers of CFU per plate (Figure 5). In some cases, there were consistent amounts of CFU's in 1-2 of the plates, with the third plate having significantly more or less CFU's; this was evident in Spermidine at Station 5 and GBT at Station 9 (Figure 5).

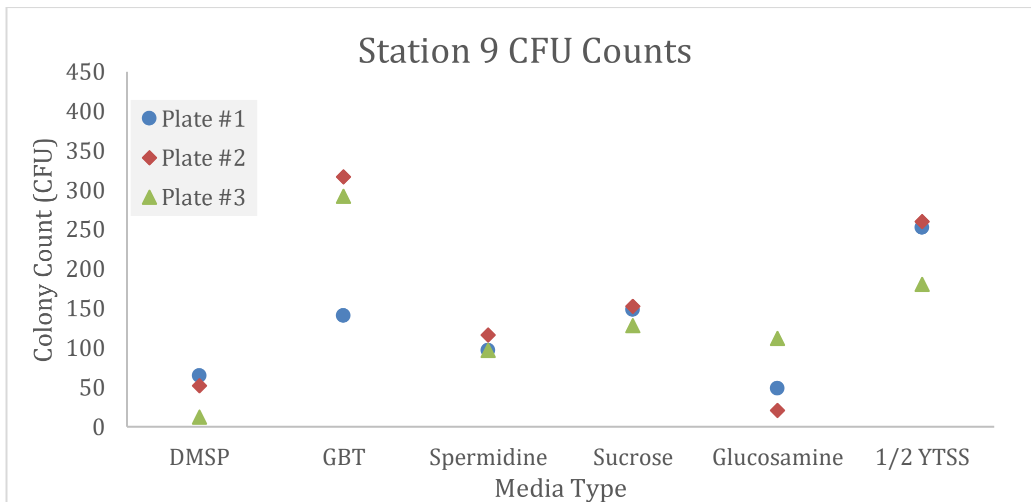
Table 3. CFU's across media types and stations. The CFU's were calculated by counting a quadrant (i.e. quarter) of the bacterial plate and multiplying by 4.

Station Number	Plate Number	Growth by Nutrient Type (CFU's)					
		DMSP	GBT	Spermidine	Sucrose	Glucosamine	1/2 YTSS
Station 5	Plate #1	120	304	288	132	100	260
	Plate #2	92	328	132	136	92	280
	Plate #3	104	416	140	92	140	248
Station 9	Plate #1	64	140	96	148	48	252
	Plate #2	52	316	116	152	20	260
	Plate #3	12	292	96	128	112	180
Station 13	Plate #1	96	156	124	140	144	272
	Plate #2	84	140	152	124	148	228
	Plate #3	112	160	176	132	192	204

Table 4. Individual colony type counts via CFU's. In this table, the CFU's were calculated by counting a quadrant of the bacterial plate, multiplying by 4, and then taking an average of the counts across the three replicates for each media type at each station. The phenotypes are described in table 2.

Colony type	Media Type								
	DMSP			GBT			Spermidine		
	Station 5	Station 9	Station 13	Station 5	Station 9	Station 13	Station 5	Station 9	Station 13
A	68	12	33	65	49	19	93	64	71
B	36	31	52	65	73	75	93	37	60
C	3	0	12	0	0	0	0	0	20
D	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0
I	0	0	0	0	0	0	0	0	0
J	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	0	0	0	0
L	0	0	0	0	0	59	0	0	0
M	0	0	0	0	0	0	0	0	0
N	0	0	0	219	127	0	0	0	0
Colony type	Media Type								
	Sucrose			Glucosamine			1/2 YTSS		
	Station 5	Station 9	Station 13	Station 5	Station 9	Station 13	Station 5	Station 9	Station 13
A	60	79	79	77	31	96	53	35	37
B	51	53	35	33	15	47	83	41	41
C	0	11	19	0	4	19	48	11	32
D	0	0	0	0	0	0	41	25	16
E	0	0	0	0	0	0	20	23	51
F	0	0	0	0	0	0	0	3	4
G	0	0	0	0	0	0	0	3	0
H	0	0	0	0	11	0	0	0	0
I	0	0	0	0	0	0	0	0	4
J	0	0	0	0	0	0	0	0	5
K	0	0	0	0	0	0	0	0	13
L	0	0	0	0	0	0	0	0	0
M	9	0	0	0	0	0	19	0	31
N	0	0	0	0	0	0	0	0	0





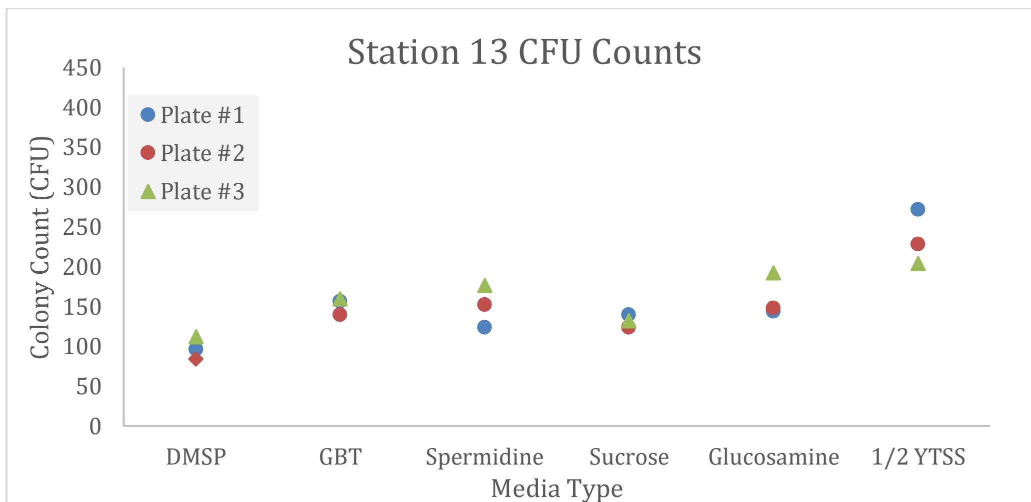


Figure 5. CFU variation among stations. This is a comparison of the number of CFU's observed on each plate at each station across all media types.

When the CFU counts and phenotype classification were combined, a pattern emerged in most media types (excluding ½ YTSS). This typically presented itself in 1-2 phenotypes with similar numbers of CFU's and then an occasional outlier phenotype that was unlike the rest and much lower or much higher in CFU's (Figure 6). This was observed in GBT especially. In the case of ½ YTSS, there were still a few dominant phenotypes, but there were significantly more outlier populations with low CFU counts and unique phenotypic traits. There were some consistent phenotypes present across multiple media types or present in all media types, such as type-A and type-B. Other types seemed to only be present in one media type and/or at one station in particular.

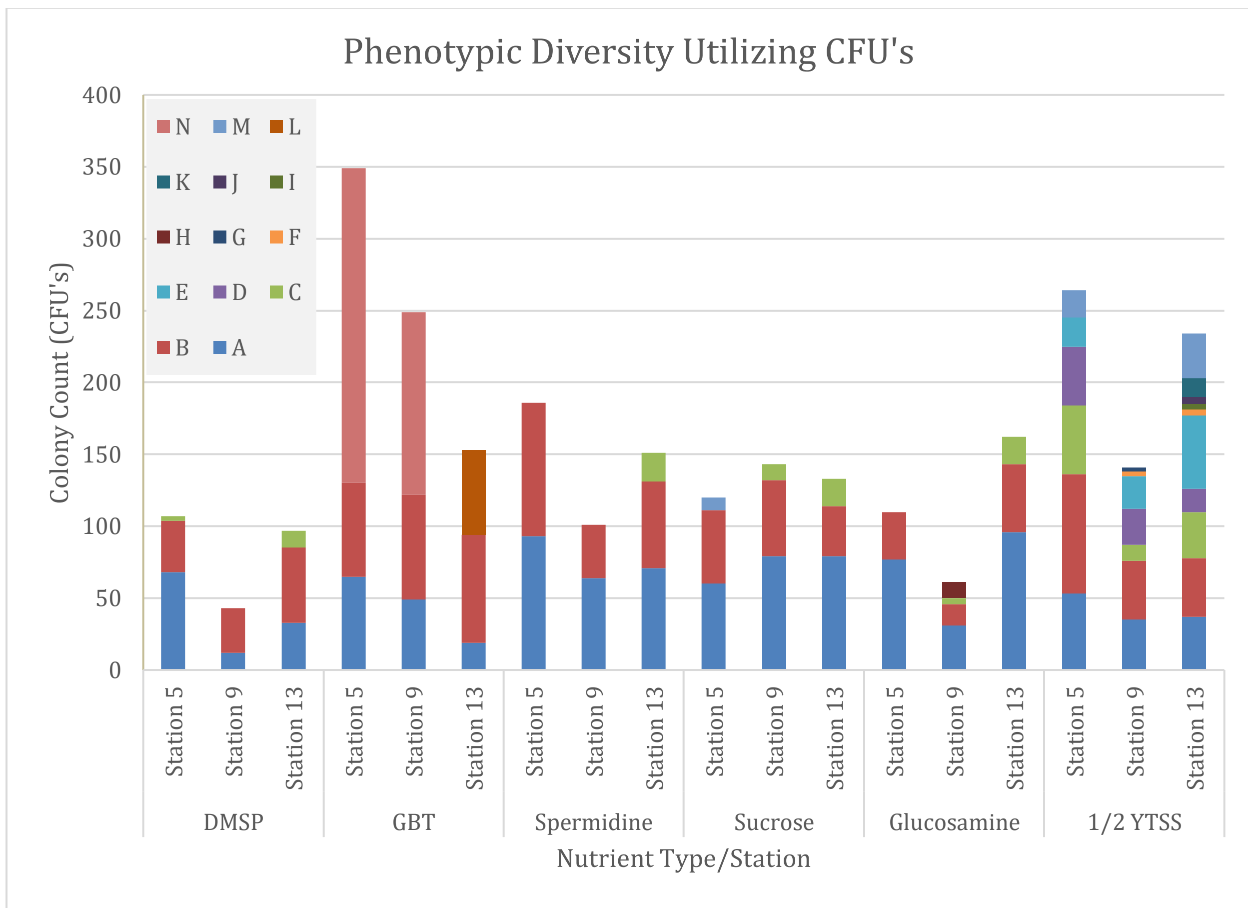


Figure 6. Phenotypic variation by CFU's across stations and media types. This was done using the CFU counts calculated in Table 4. The phenotypes described in Table 2.

Discussion:

Overall, my results corroborate my hypothesis: there was some variation in phenotype and in growth across the different metabolite mediums. This indicates that the ability of certain bacteria to metabolize metabolites varies amongst these communities, and allows for different bacteria to operate in specialized ecological niches. The most diverse growth occurred on the ½ YTSS plates, which is a more complete medium than the other substrates in the sense that it is designed to provide all nutrients necessary for bacterial growth, and was thus anticipated to have more phenotypic diversity. Some of the media types contained bacteria that were specific to that media across all sampling stations; specifically, Glycine Betaine (GBT) showed unique bacterial phenotypes compared to other media types, particularly the type-N and type-L phenotype. In this case, they were pinpoint colonies that were almost “splotchy” in appearance and a pale white (type-N) or bluish white (type-L). There is potential that both are the same type, but from an inspection of phenotype pictures taken after the 5-day incubation, they seemed to be slightly different due to a perceived difference in pigmentation.

There were two to three phenotypes which seemed to thrive across all mediums and thus may be more prevalent or adaptable than the rest. In particular, the presence of type-A, type-B, and to a lesser extent type-C phenotypes were seen across nearly every medium and location. This may suggest that there are a few species that can be found regardless of condition or nutrient types. These were also the smallest phenotypes in terms of size, which begs the question if size is a significant control of whether or not a bacteria can thrive in nutrient-replete conditions. There has been limited research on some common marine heterotrophic bacteria, such as the SAR11 clade, which suggests they have minimized their size/complexity to increase their efficiency in extremely oligotrophic environments via a phenomenon known as

"streamlining" (Giovannoni, 2017). The SAR11 clade also has been found to either require or utilize unique nutrients that are phytoplankton metabolites, such as DMSP and GBT, which could indicate that they rely on interactions with phytoplankton for survival. While SAR11 is extremely difficult to culture using traditional methods and thus likely not represented in my cultures, this relationship could hold true for other small, well-distributed marine heterotrophic bacteria that are within my cultures. However, a smaller colony size does not necessarily correlate with smaller cell size, and this would require further analysis of my samples using microscopy and other methods. Overall, it does appear that there are a few bacterial phenotypes that seem to be well-distributed across all locations of the equatorial region that can subsist on multiple phytoplankton metabolites as a source for carbon and potentially other key nutrients such as nitrogen and sulfur.

Furthermore, the bacterial community appears to be increasingly diverse as one heads north from the equator. This may be due to increasingly stormy conditions north of the equator during the course of our cruise. These conditions could have resulted in increased mixing which may have created a more diverse bacterial community. This would be due to increases in surface mixing, which then results in nutrient levels increasing and phytoplankton blooms occurring (Whitt et al., 2019). This could then affect the metabolites present and the heterotrophic bacterial community. This also could have been caused by variation in currents and upwelling in regions north of the equator due to equatorial mixing, but this requires further research and corroboration with physical/chemical oceanographic data. Specifically, I would need to determine if a shift in currents or upwelling did occur, and if an associated shift in local nutrient conditions caused these regions of diversity.

There are many limitations to this experiment that could be improved or remedied in future iterations. This study took samples from 3 locations at a uniform depth over a period of 2 weeks; there may be relationships or factors that rely on depth or seasonality that would require a series of experiments with much broader scope. My samples were taken during an El Nino year, which could also have impacted the bacterial community composition due to altered nutrient and phytoplankton conditions. There is also the potential that some of these colonies came from contamination. This could be a cause of some of the "outlier" phenotypes found in isolated bacterial plates or at a single station. There is the possibility of the same species presenting as different phenotypes on different media, or conversely different species presenting as the same phenotype on the same or different media. Additionally, some colonies could be aggregates of multiple bacterial species. These differences can only be determined by additional analysis of samples such as 16S rRNA gene sequencing, which is currently being processed and the natural next step in this study. The visual identification of the bacterial phenotypes was particularly difficult. There is always the potential for human error, and in many cases I found it difficult to discern between two phenotypes accurately. It is possible that some of the phenotypes were the same, but I perceived a difference in their phenotype, or they were in different stages of growth; this was accounted for by using set incubation times and using phenotypes for reference, but the possibility is still there. The use of machine-learning methods could help remove the human error element of this identification process and increase the speed/efficiency of the identification process, but this software is limited and difficult to use.

It should be noted that my samples were limited to the equatorial Pacific, and thus whether the results can be extrapolated to other environments like those in more extreme latitudes remains unknown. Finally, there is also the "unculturable dogma" found within marine

microbiology, which asserts that around 1% or less of marine bacteria can be cultured via traditional microbiological methods (Rodrigues & Carvalho, 2022). While this is largely true, there is evidence that the success rate of culturing heterotrophic bacteria via plating on marine agar is far more successful with results approaching 50% in terms of cultivability. However, this is still not ideal, and thus my results likely captured on a glimpse of the diversity when it comes the heterotrophic marine bacterial community in this region. I would advise future projects to identify bacteria to the best of their ability while at sea and utilize scanning microscopes if they are available to better identify different colony phenotypes using finer detail. Also, I would recommend adding some element of genomic analysis if permissible, whether it be 16S rRNA, complete genome sequencing, and/or metagenomic analysis of bacteria in the water column. This would allow future researchers to gather more insights on the bacterial species, metabolisms, and community composition.

Conclusion:

Heterotrophic bacteria serve important roles in biogeochemical cycling and the recycling of organic carbon and DOM (Dissolved Organic Matter) in marine ecosystems. Within these ecosystems, phytoplankton are equally important due to the roles they serve as primary producers. There have been studies on how these two communities interact, but research on how this relationship plays out in remote regions of the ocean like the equatorial Pacific is limited. My study has been valuable in highlighting these potential relationships. I found that there is a promising relationship between bacteria and phytoplankton metabolites in this region, with certain bacterial phenotypes readily utilizing these metabolites. This would suggest that these bacteria are users of these metabolites, and even reliant on these metabolites for certain nutrients. The metabolites released can vary based on the phytoplankton species and/or physical conditions. This study and future iterations could inform us on how phytoplankton populations influence the local bacterial community and vice versa. With advancement of this knowledge, our understanding of how microbes and phytoplankton influence biogeochemical cycling can be greatly improved. A natural progression of this study would be identification of bacterial species using 16S rRNA analysis, metagenomic analysis to get the full bacterial community composition, sampling during different seasons and during La Nina conditions for comparison, and integration of nutrient and physical oceanographical data to form a more complete picture of this region.

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