IDENTIFICATION OF IRON-OXIDIZING BACTERIA ON STEEL STRUCTURES IN FRESHWATER ENVIRONMENTS

by

Meredith A. Cox

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by

Meredith A. Cox

Greenville, NC

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Approved by:

Dr. Erin K. Field, PhD

Department of Biology, East Carolina University

Biology Honors Thesis

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Meredith A. Cox

May 2023

APPROVED BY:

Director of Thesis: Erin Field
Erin K. Field, PhD

Faculty assessor:
Eric Anderson, PhD

Director of Undergraduate Studies:
Grace Fu-Chun Chen, PhD

On this day, April 11, 2023

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Meredith A. Cox

A thesis submitted to the Department of Biology, East Carolina University, in partial fulfillment of the requirements for Biology Honors Thesis

Advisor: Erin K. Field, Ph.D.
East Carolina University
Department of Biology

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I hereby declare I am the sole author of this thesis. It is the result of my own work in collaboration with Biology master student Maggie Shostak for data collection and analysis, and it has not been submitted elsewhere as coursework for this or another degree.

Signed:

Meredith A. Cox

Date: April 11, 2023

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Meredith A. Cox

Department of Biology, East Carolina University, Greenville N.C. 27858 U.S.A.

Abstract

Microorganisms found on steel structures within aquatic systems impact the integrity of the structures and environmental factors surrounding them. Iron-oxidizing bacteria (FeOB) in particular, can cause damage and corrosion of steel. Understanding how the presence and growth of these microbes in freshwater environments affects local aquatic habitats, as well as the preservation of steel complexes, allows us to identify the best materials for construction, while ensuring that the surrounding environment remains healthy. To begin identifying the species of FeOB present, a freshwater site containing wreckage in Mallows Bay, Maryland, the *Accomac,* was used for sample collection and analysis. I hypothesized that FeOB communities would be found on the wreck and that they would be identified as freshwater species due to the surrounding freshwater conditions. They will play a significant role in the health of the surrounding aquatic environment, and the integrity of the sampled structures. Forty-four biofilm samples from multiple regions of the wreck were collected. Estuary Media, Mallows Bay field site filtered water, and Modified Wolfe's Mineral Media (MWMM) were used in the enrichment cultures to grow FeOB from the samples collected. A total of nineteen biological samples were positive for growth, twelve of these from the starboard, two from the portside, and five from submerged portions of the wreck. Enrichment results suggested that FeOB have grown primarily in MWMM from fully submerged samples and those collected at the waterline on the wreck. I conducted a serial dilution to obtain a pure culture to sequence the 16S rRNA gene to identify the organism and sequence the whole genome for future studies. However, we could only obtain

enrichment cultures with multiple organisms for identification based on the time frame of the study. Data analysis showed that many of the organisms present in the enrichments were known taxa of freshwater FeOB (e.g. Gallionella and Leptothrix) with the largest taxa based on relative abundance falling under classes of Gammaproteobacteria or Alphaproteobacteria. Investigating these microorganisms can further our understanding of present microbial assemblies and the effects they have had on the structures.

Acknowledgments

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Introduction

Microbes and their corresponding communities are often found on steel or metal structures where nutrients are limited, and attachment to such structures, provides ecological benefits. Metal piping and ships are a common source of iron and carbon that advance the concentration of these communities and their nutrients; however, these microbes can cause negative effects on the structures, including deterioration or instability (Emerson, 2018, Vol. 34). Biochemistry plays an important role in conducting and comprehending the research performed on the gathered steel/metal samples, allowing us to ask questions about how to prevent these negative occurrences and salvage the structures that have been affected. This frequently involves performing DNA extraction, data quantification or analysis, and field work, to better understand the influence these communities have on aquatic system structures and habitats. A previous study by Price, et al. (2020), discusses the ways microbial communities assemble on a coastal marine, ferrous-hulled shipwreck and the microenvironments that form.

Microbes can either cause corrosion of wreckage, or aid in its preservation (Videla, et. al. 2005). Multiple industries benefit from understanding the effects caused by present communities, including those related to construction materials, handling, and historical or scientific studies. Participating microorganisms can induce unique qualities on metal surfaces largely including the formation of biofilms. These affect the interactions between the environment and metal surfaces in biodeterioration processes such as microbial influenced corrosion (MIC). Microbes influence corrosion through alteration of electrochemical conditions at a metal-solution intersection, causing different affects based on environmental and microbial surroundings, such as corrosion or corrosion inhibition (Videla, et. al. 2005). Sulfate-reducing bacteria are a commonly studied source of MIC under anoxic aquatic conditions (Enning, et. al. 2014. Vol. 80), whereas iron-

oxidizing bacteria (FeOB) are not yet well-understood or fully identified in these environments. Research regarding FeOB continues to grow; however, with a special interest in the biological, chemical, and geographical cycles within various pH levels and anaerobic conditions, many of these falling into the broad classification of proteobacteria. These can be iron reducers, as well as oxidizers, and are often grouped based on metabolic categories, such as neutrophils and acidophils, where oxygen levels vary. Through isolation of communities in marine environments, several genus' of FeOB have been identified. Iron-oxidizing proteobacteria are commonly found under classes of Gammaproteobacteria, Betaproteobacteria, Zetaproteobacteria and Alphaproteobacteria, with known genera such as *Gallionella, Sideroxydans, Leptothrix, Dechloramonas, Acidovorax, Mariprofundus,* etc. (Hedrich, et. al. 2011, Vol. 157). However, those residing in freshwater, and their impacts on the surrounding habitats, are lesser known (Makita, 2018). The process of iron oxidation is used as a supplemental nutrient source (White, 2016, Ch.3), along with other inorganic compounds and carbon dioxide to perform chemical reactions, hence the classified name they are given of chemolithoautotrophs (Rice, 2005). Chemical oxidation also occurs much faster in an environment with a more neutral pH, rather than those that are very acidic or basic (Emerson, 2005, Vol. 397). Various media types, such as Modified Wolfe's Mineral Media (MWMM), a freshwater media (Emerson, 2005, Vol. 397), and Estuary Media (EM), a brackish water media (Field, et. al., 2016) can be used in enrichment cultures to imitate the environments that these microbes prefer and grow them for analysis or to be cryopreserved for later studies. The composition of media typically consists of common chemical compounds such as NaCl, $MgSO_4$, $CaCl_2$, $NaHCO_3$, $MgCl_2$, $MgSO_4$, etc. depending on the type of media used and what microorganisms are being targeted for growth. Cultured samples can be analyzed using microscopy, where stalks can be distinctly seen to confirm the

presence of FeOB, as they are distinct from chemical iron oxides (Emerson, 2010, Vol. 64). Observation of physical characteristics of FeOB can also be used to identify present microbial growth, including, but not limited to, orange coloring throughout the dish with bright orange fluffy clusters suspended within the media.

Researchers in the Field lab have already begun sampling the corroded metal structures that contain the FeOB and sulfate-reducing bacteria to analyze their abundance, environment, and the affects they have on various types of materials in marine environments. This will provide some insight on ways to maintain the foundation of the structures. Sample collection was completed on the *Accomac*, a steel vessel with the original name of *Virginia Lee* built in 1928 as a convoy vessel during World War II, and later used as a car ferry within the Southern Chesapeake. *Accomac* is now located in Charles County, Maryland within Mallows Bay National Marine Sanctuary, a fresh body of water containing the eminent "Ghost Fleet" which houses over a hundred abandoned steamships from the 20th century used for salvaging scrap metal. Abandoned herself in 1973, *Accomac* is the most recognizable vessels in the sanctuary, as it is one of the only ones remaining that is visible above the waterline (Mariner's Museum). I hypothesize that FeOB communities will be found on the wreck as it is comprised of steel, where they will be cultivated and identified as freshwater species due to the surrounding conditions, allowing us to better understand how their presence affects the integrity of existing steel structures and the environmental factors controlling their lifestyle and habitat.

Methods

Biofilm samples were scraped from corroded portions of the wreck on June 2nd and 3rd, 2022 at levels at and below the waterline from positions at the bow (front), middle, and after-quarters (rear) on both the portside (left side) (Figure 1B) and starboard (right side) (Figure 1A). The ship was originally made of iron, however the source of materials for construction is unknown. It is thought to have been upgraded in some areas with steel, which is a less pure element than iron that has a higher carbon content. Areas where the material was altered tend to be longer, wider, and less thick, as steel is stronger than iron. The *Accomac* has a large bow with plates that look different than the rest of the ship up higher on the hull. These are likely the sections where materials were upgraded, meaning biofilm samples were scraped from lower parts of the ship made of iron. The exact position was measured in meters beginning at 0 meters from the tip of the bow and reached approximately 55.5m at the visible after-quarters of the wreckage with a tape measure spanning the length of the wreck above the waterline. Samples from fully submerged portions of the wreck in the after-quarters sector were collected by trained scuba divers to compare those at various water and air exposure levels. Rudderpost, part of the ship's steering apparatus, and bulkhead, a divider between sections of the ship, biofilm samples were also collected for cultivation. Surrounding environmental samples were collected, such as sediment and water samples, to gather an understanding of the conditions preferred by the microbial communities and to compare the composition of microbes present. Water samples were collected at several different locations around the wreck and five sediment samples were collected beginning at 0m from the rudderpost and continuing in 25m increments to 100m. Water samples were sterilely filtered after collection and placed into falcon tubes for later use in 500 mL increments. Collected samples were kept cool on ice upon collection and for the remainder

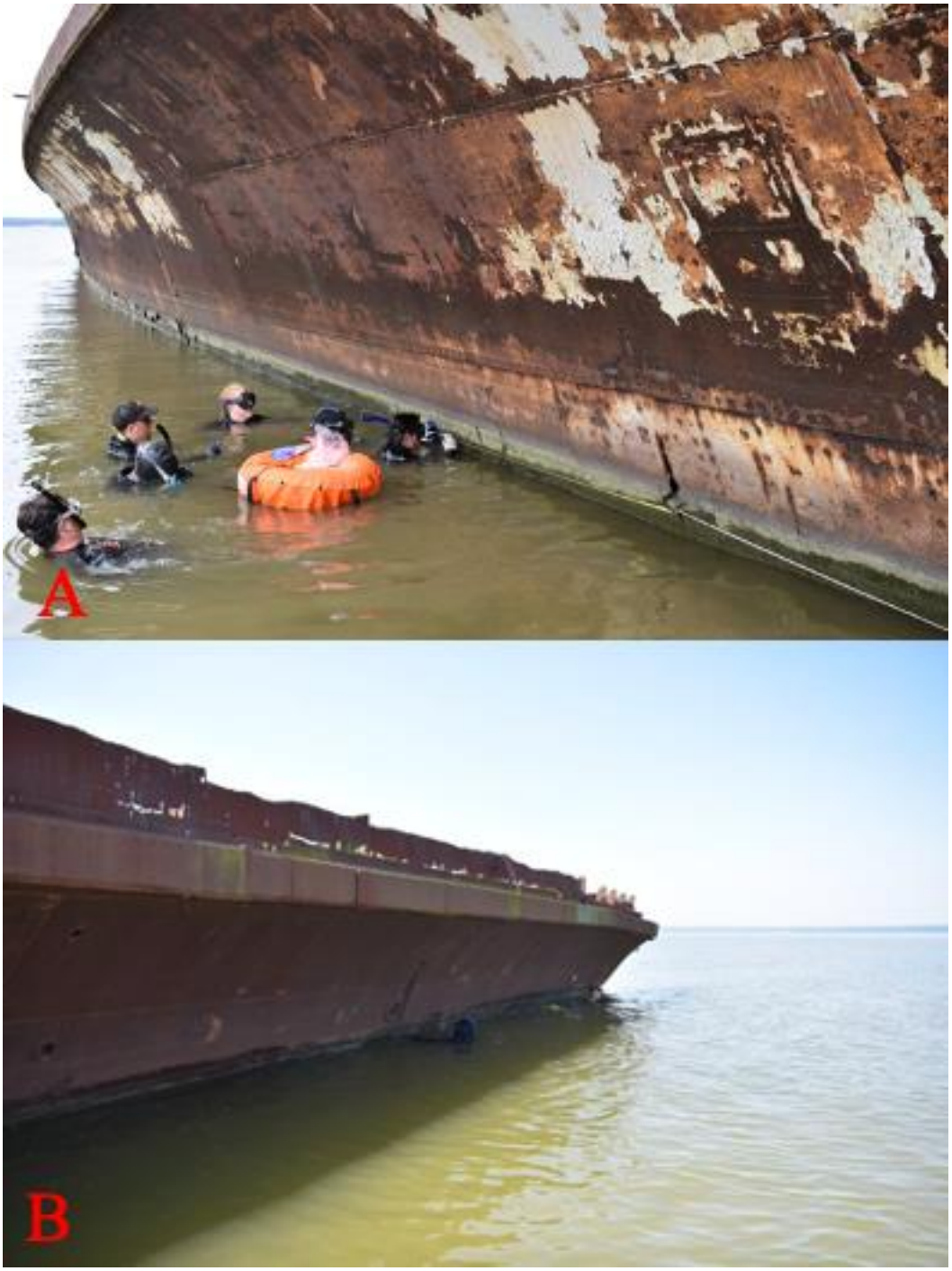

Figure 1: Sampling the SS Accomac Panel A: 18 biofilm samples from the middle of the starboard of the *Accomac* were scraped from levels at and below the waterline to return to the lab for enrichment cultures to cultivate present FeOB.
Panel B: 18 samples from the bow, middle, and aft of the portside were collected at the waterline and below the waterline for later enrichment culturing to cultivate present FeOB. Allyson Ropp, Program in Maritime Studies, East Carolina University.

of the excursion until they were transported back to the lab and refrigerated for immediate preparation for stocks and cultures.

Within the lab, glycerol stocks were made using cryovials and 30% glycerol to ensure samples were not depleted during culturing. Culturing utilized three different media types for enrichments: MWMM (Emerson, 2005, Vol. 397), EM (Field, et. al., 2016), and filtered water (0.22uM PES filter) supplemented with vitamins and minerals from the samples collected at Mallows Bay, where new media was made at least once a month to ensure nutrients within were not reduced and pH was tracked weekly. 20mL of media, 100uL of sample, and sterile iron powder (<0.1 g) were placed into a petri dish and lightly mixed, where the cultures were then incubated at room temperature in a sealed box containing campy-paks, removing some oxygen from the container, to imitate their preferred environment. Control groups, containing 0uL of collected sample, were grown under the same conditions as those with 100uL of sample to isolate and better visualize the growth of FeOB. The incubation period varied depending on the growth observed every few days; however, it was estimated to be around two weeks for a sufficient amount. Record was taken of those that were positive or negative for growth and the media type that they flourished in, identified through both microscopy and visible iron oxide morphology (Figure 2A and 2B). Culturing continued after the first round had grown, creating dilutions and replicates to attempt to obtain a pure culture from those with ample growth. Using microscopy, DNA extraction, and quantification techniques (Qubit), positive enrichment samples with ample growth were analyzed and sent for sequencing to determine community composition and identification of the present taxa of FeOB.

DNA was extracted from the iron samples using a QIAGEN PowerSoil DNA kit and the library prep utilized KAPA Hyper Plus. Samples were plated on a 96-well plate, with 20μL of

sample per well, and sent to the Integrated Microbiome Resource (IMR) at the Centre for Comparative Genomics and Evolutionary Bioinformatics in Nova Scotia, Canada for 16S rRNA bacterial amplicon sequencing. Fragments were amplified by PCR, utilizing Illumina adaptors and recommended IMR primer targets.

To begin data analysis, sequences were loaded into R studio as fastq files and run through the dada2 pipeline R package. DADA2 uses an algorithm that provides high-resolution amplicon sequence variant (ASV) tables instead of the typical operating taxonomic unit (OTU) table (Callahan et al., 2016). Paired reads were trimmed for forward and reverse sequences to remove any bases with low average quality scores. Dereplication removed any sequences that were identical and reduced computational time. Sequences were then merged, those that didn't and any chimeric sequences, were removed. Taxonomy was assigned to all remaining sequence variants. A simper analysis was run to calculate the contribution of each taxon to the dissimilarity between the different biofilm sampling locations. Further literature research on known FeOB classes, their environmental tendencies, and preferences was completed to begin to understand the ways environmental diversity affects microbial assemblage.

Results

To identify microorganisms, present on the *SS Accomac*, enrichment cultures were analyzed using both visual observation and microscopy to determine present microbial communities. An example of visualized growth can be seen in Figure 2A, where the plates contain orange clusters and have small flocks suspended within the media. Microscopic analysis of a sample with visual growth from the middle of the starboard side of the ship (Figure 2B), 27.5m from the tip of the bow at the waterline, displays distinct stalks shown by the measured edges of 100um, where FeOB were identified within the cluster. The recognizable orange color of the FeOB is visible with in the microscopic cluster as well. Of the 54 samples cultivated from the starboard side of the wreck, 12 were positive for growth of FeOB (Table 1), while two from the portside of the wreck were considered positive out of 54 (Table 2A). Of the 12 that were positive on the starboard side, 8 of them were found growing in MWMM, whereas two were in EM, and 1 was in the filtered water samples taken from the sample collection site. From the portside, both cultures that were positive for growth were cultivated in EM. From submerged potions of the wreck, four of the nine samples were positive, those being cultivated in MWMM and EM (Table 2B). One sample from the bulkhead of the wreck was positive and grown in EM. MWMM was consistently found to be the most successful with cultivation of present FeOB. Samples from the starboard of the *Accomac*, as well as the fully submerged portions from the after-quarters sector, yielded the best growth throughout enrichment cultures and were utilized in DNA quantification and sequencing analysis.

Sequencing results represent the relative abundance of present microbial communities within the positive enrichments using taxa classifications. Known iron-oxidizing and reducing genera, such as *Gallionella, Dechloromonas, Acidovorax, Rhodoferax, Sideroxydans and*

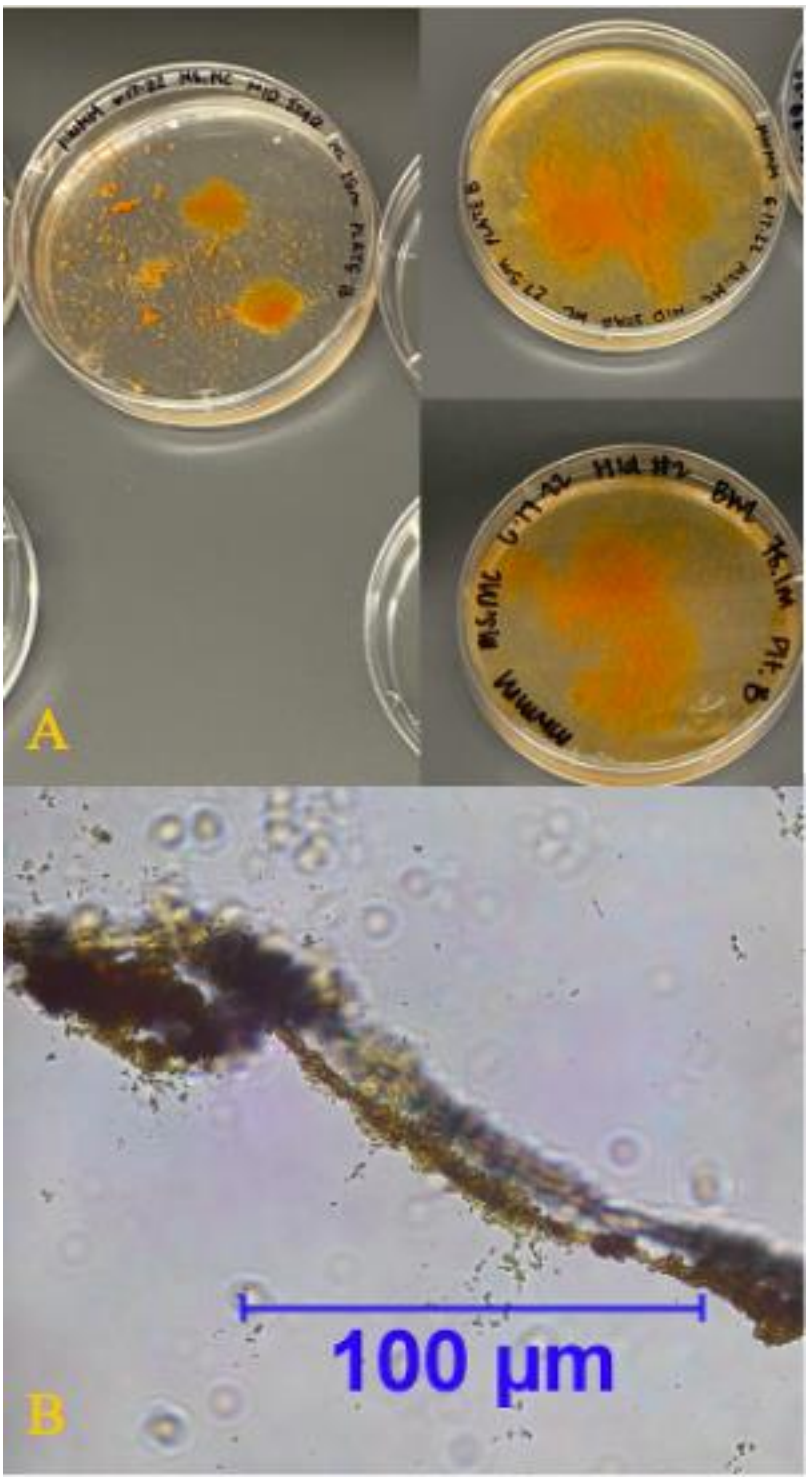

Figure 2: Panel A: Three different examples of observed FeOB growth within enrichment cultures containing MWMM. The orange clumps indicate iron oxidation has occurred. Panel B: Microscopy analysis of a 100 µm FeOB cluster from the middle of the starboard (27.5m) at the waterline.

Table 1: Initial enrichment culture results based on visual observation of growth from the starboard side of the ship. (+) indicates growth of FeOB was observed within the plate. (-) indicates no growth of FeOB was observed within the plate after 14 days.

BOW - STARBOARD			MIDDLE - STARBOARD			AFTERQUARTERS - STARBOARD		
Sample	Media Type	Growth (+/-)	Sample	Media Type	Growth (+/-)	Sample	Media Type	Growth (+/-)
	MWMM	-		MWMM	-		MWMM	+
	EM	+		EM	-		EM	-
BSW 0m	MB	-	MSW 27.5m	MB	-	ASW 53.5m	MB	-
	MWMM	-		MWMM	+		MWMM	-
	EM	-		EM	-		EM	-
BSB 0m	MB	-	MSB 27.5m	MB	-	ASB 53.5m	MB	-
	MWMM	+		MWMM	-		MWMM	-
	EM	-		EM	-		EM	-
BSW 1.5m	MB	-	MSW 28m	MB	+	ASW 54.5m	MB	-
	MWMM	+		MWMM	+		MWMM	+
	EM	-		EM	-		EM	-
BSB 1.5m	MB	-	MSB 28m	MB	-	ASB 54.5m	MB	-
	MWMM	-		MWMM	+		MWMM	-
	EM	-		EM	-		EM	-
BSW 2.5m	MB	-	MSW 28.5m	MB	-	ASW 55.5m	MB	-
	MWMM	+		MWMM	+		MWMM	-
	EM	-		EM	+		EM	-
BSB 2.5m	MB	-	MSB 28.5m	MB	-	ASB 55.5m	MB	-

Table 2: Initial enrichment culture results based on visual observation of growth of FeOB from the Portside of the ship. (+) indicates growth of FeOB was observed within the plate. (-) indicates no growth of FeOB was observed within the plate after 14 days.

BOW - PORTSIDE			MIDDLE - PORTSIDE			AFTERQUARTERS - PORTSIDE		
Sample	Media Type	Growth (+/-)	Sample	Media Type	Growth (+/-)	Sample	Media Type	Growth (+/-)
	MWMM	-		MWMM	-		MWMM	-
	EM	-		EM	-		EM	-
BPW 0m	MB	-	MPW 27.5m	MB	-	APW 53.5m	MB	-
	MWMM	-		MWMM	-		MWMM	-
	EM	-		EM	-		EM	+
BPB 0M	MB	-	MPB 27.5m	MB	-	APB 53.5m	MB	-
	MWMM	-		MWMM	-		MWMM	-
	EM	-		EM	-		EM	+
BPW 1.5m	MB	-	MPW 28m	MB	-	APW 54.5m	MB	-
	MWMM	-		MWMM	-		MWMM	-
	EM	-		EM	-		EM	-
BPB 1.5m	MB	-	MPB 28m	MB	-	APB 54.5m	MB	-
	MWMM	-		MWMM	-		MWMM	-
	EM	-		EM	-		EM	-
BPW 2.5m	MB	-	MPW 28.5m	MB	-	APW 55.5m	MB	-
	MWMM	-		MWMM	-		MWMM	-
	EM	-		EM	-		EM	-
BPB 2.5m	MB	-	MPB 28.5m	MB	-	APB 55.5m	MB	-

Table 3: Initial enrichment culture results based on visual observation of growth of FeOB from submerged portions of the wreck in the after-quarters sector, as well as those taken from the rudderpost and bulkhead. Control group results are also shown. (+) indicates growth of FeOB was observed within the plate. (-) indicates no growth of FeOB was observed within the plate after 14 days.

SUBM. & R.POST SAMPLES			R.POST & BULKHEAD SAMPLES			CONTROL SAMPLES		
Sample	Media Type	Growth (+/-)	Sample	Media Type	Growth (+/-)	Sample	Media Type	Growth (+/-)
	MWMM	+		MWMM	-	Control 1	MWMM	-
	EM	+		EM	-	Control 2	EM	-
Submerged 1	MB	-	RP BWL	MB	-	Control 3	MB	-
	MWMM	+		MWMM	-	Control 4	MWMM	-
	EM	-		EM	-	Control 5	EM	-
Submerged 2	MB	-	BKH WL	MB	-	Control 6	MB	-
	MWMM	+		MWMM	-	Control 7	MWMM	-
	EM	-		EM	-	Control 8	EM	-
Submerged 3	MB	-	BKH BWL	MB	-	Control 9	MB	-
	MWMM	-		MWMM	-	Control 10	MWMM	-
	EM	-		EM	+	Control 11	EM	-
RP WL	MB	-	BKH UPT	MB	-	Control 12	MB	-

Bradyrhizobium, were identified through DNA sequencing, these falling under the two most abundant orders present, Burkholderiales and Rhizobiales. The most abundant families included Azospirillaceae, Chromobacteriaceae, Comamonadaceae, Gallionellaceae, and Rhodocycleaceae (Figure 3, Table 4). Comamonadaceae was the most abundant family in samples FeO2 and FeO7 from the middle of the starboard below the waterline making up 96.4% of the relative abundance for these samples. This family was present in all eight samples and was consistently a large portion of the relative abundance, housing the *Rhodoferax* and *Acidovorax* iron-oxidizing genera. Rhodocycleaceae and Gallionellaceae were also found in each of the eight samples, containing the *Dechloromonas, Gallionella,* and *Sideroxydans* genera. Azospirillaceae made up around 46% of samples FeO3 and FeO8 from the starboard of the after-quarters, as well as 10% of FeO5 from the submerged sample of the after-quarters, however it was not present in great abundance, if at all, in any of the other samples. Chromobacteriaceae was identified in all but two samples, FeO2 and FeO7, in high abundance, other than FeO5 where it made up 5% of enrichment microbes. *Bradyrhizobium* is a member of the Xanthobacteraceae family, and was found in four of the eight samples (FeO3, FeO4, FeO5, FeO8). Neither Azospirillaceae nor Chromobacteriaceae contained known iron-oxidizing taxa.

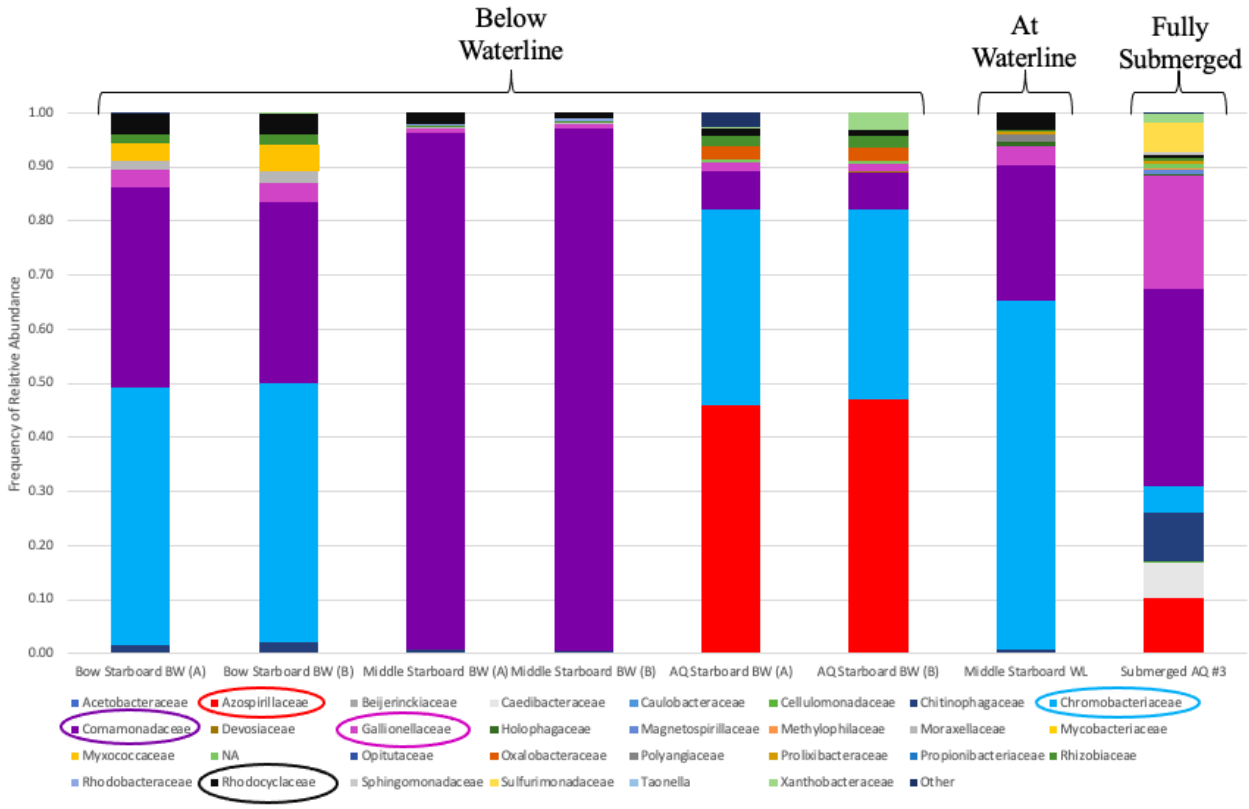

Figure 3: Frequency of relative abundance of present microorganisms in sequenced DNA classified by taxa (family). Samples FeO1 and FeO6 were collected 2.5m into the bow of the starboard at levels below the waterline (BW). FeO2 and FeO7 were collected from 28m into the middle of the starboard below the waterline. FeO3 and FeO8 were collected 54.5m into the after-quarters (AQ) of the starboard side at levels below the waterline. FeO4 was collected 28.5m into the middle of the starboard at the waterline (WL). FeO5 was collected from a fully submerged section of the ship in the after-quarters sector.

Table 4: Figure 3 legend including the location of the samples and the media type in which they were cultivated. Samples FeO6, FeO7, and FeO8 were duplicated from sequenced DNA extractions of FeO1, FeO2, and FeO3.

Sample	Location	Media Type
FeO1	Bow Starboard BW 2.5m (A)	MWMM
FeO2	Middle Starboard BW 28m (A)	MWMM
FeO3	AQ Starboard BW 54.5m (A)	MWMM
FeO4	Middle Starboard WL 28.5m	MWMM
FeO5	Submerged AQ #3	MWMM
FeO6	Bow Starboard BW 2.5m (Duplicate) (B)	MWMM
FeO7	Middle Starboard BW 28m (Duplicate) (B)	MWMM
FeO8	AQ Starboard BW 54.5m (Duplicate) (B)	MWMM

Discussion

DNA sequencing results confirmed the presence of FeOB through identification of known iron-oxidizing taxa, supporting my hypothesis that these microorganisms would be found on the wreck. However, using both microscopic and visual observation techniques, FeOB were confirmed before they were officially identified through sequencing. The variance in the observed orange stalks suspended within enrichment cultures and the microscopic visualization, indicated that several different taxa of FeOB were present, as well as many other microbial communities interacting with the organisms of interest. MWMM is a common freshwater media, its success in growing FeOB suggests that the present microbes on the wreck would generally be found in other freshwater environments. There was little to no salt present in the filtered water samples from Mallows Bay, and only one sample of the 44 taken showed growth in this media. The growth of FeOB in brackish water EM, shown in five cultures, sampled from both sides of the ship, as well as from submerged portions of the wreck, indicates that the presence of salt may make it easier for these microbes to grow. At its current position, the starboard of the Accomac is the side of the ship receiving less wave and wind action, allowing further opportunities for biofilm formation and protection, making it the best place to sample and cultivate enrichments. Samples collected at levels below the waterline, rather than at the waterline, had a higher relative abundance of FeOB. This was also the case with the submerged samples from the after-quarters sector. Being several meters below the surface of the water, wave and wind erosion would not have as great of an impact on the biofilms forming on the submerged structures, allowing for a greater abundance of microorganisms to reside. Based on the identified locations of more prevalent abundance, we can assume that microbial communities present in freshwater systems

will typically be found in areas with less wind and wave exposure where biofilms are not disturbed, and ideal conditions are more consistent.

Sequencing data provided an overview of the composition of microbial communities present on the *Accomac*. Each of the eight samples contained families that house several different types of FeOB. The *Gallionella* genera, which has a tendency to utilize some oxygen and carbon as a source of nutrients within the fibers of its stalks (Hallberg & Ferris, 2004, Vol. 21), was found in every sample, indicating that the use of these compounds for nutrients may be driving iron-oxidation reactions on the wreck. *Dechloromonas* was another iron-oxidizing microorganism identified in all but one sequenced sample (FeO5), that typically performs either iron-reduction or oxidation depending on the available compounds and present strain of genera (Chakraborty & Picardal, 2013, Vol. 29). *Acidovorax* and *Rhodoferax* were found within the most abundant family, Comamonadaceae, which is rather diverse and often utilizes soil or freshwater environments to perform a range of chemical processes, including iron-oxidation and reduction (Willems, et. al., 1991. Vol. 41). Another well-known iron-oxidizing genus, *Leptothrix*, was not identified within the 16s rRNA sequencing, but was found in earlier enrichment cultures taken shortly after collection through visual identification of bacterial sheaths. These microbes are members of the Comamonadaceae family and perform a non-specific iron-oxidizing process in the presence of iron using the formed bacterial sheath surface to bind to reduced iron species (Corstjens, et. al. 1992. Vol. 58). We can assume that the lack of *Leptothrix* in the composition of present communities sequenced here, is due to its difficulty to cultivate in enrichment cultures, where they may grow initially then quickly die out before identification can occur.

Taking into consideration present FeOB and their environmental tendencies, we can begin to predict what factors play into the compositional presence of microorganisms and the chemical mechanisms that they may be using to survive. The significant utilization of inorganic compounds, such as carbon dioxide, iron, oxygen, etc. within all of the present FeOB, suggest that carbon may be the necessary compound for growth and nutrient production; however other organic compounds created from iron-oxidation may also provide crucial components for continuance. FeOB were also identified in each of the eight sequenced samples, leading us to assume that these may contribute to corrosion on the shipwreck.

Coupled with what we already knew about microbial assemblage and biofilms present on steel wreckage in aquatic systems, the new information regarding FeOB communities identified through sequencing allows us to draw conclusions about the management of preservation of similar structures. The location of biofilms and their integrity is dependent upon the environmental factors they are exposed to, providing an idea of where corrosion may be occurring at greater rates than others. Identifying the taxa of the microorganisms present on said structures, can also contribute to better understanding the types of corrosion taking place, such as iron-oxidation, iron-reduction, sulfate-reduction, etc. These factors yield a framework for comprehending how we may be able to preserve steel structures found in freshwater for both the prolonged use of their system and future studies within science and history. Future studies can begin to compare the communities present in freshwater to those in marine environments, emphasizing the ways in which habitat and taxa plays a role in MIC and biofilm formation. Once the environment is understood, we can better comprehend the impact that these processes have on the materials and implement procedures to slow down or inhibit corrosion of steel structures.

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