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Metagenomic Insights Into Ecosystem Function in the Microbial Mats of a Large Hypersaline Coastal Lagoon System

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The hypersaline lagoon system of Araruama (HLSA) is one of the largest in the world and one of the most important sources of evaporative salt in Brazil. The biogeochemical characteristics of this lagoon system led it to be considered a Precambrian relic. The HLSA also harbors extensive microbial mats, but the taxonomic and metabolic attributes of these mats are poorly understood. Our high-throughput metagenomics analyses demonstrated that the HLSA microbial mats are dominated by Proteobacteria, Cyanobacteria, and Bacteroidetes. Among Proteobacteria, Deltaproteobacteria comprises approximately 40% of the total population and it includes sulfate-reducing bacteria such as Desulfobacterales, Desulfuromonadales, and Desulfovibrionales. Differing in composition and function of their reaction centers, other phylogenetic diverse anoxygenic phototrophic bacteria were detected in the HLSA microbial mats metagenomes. The presence of photolithoautotrophs, sulfate reducers, sulfide oxidizers, and aerobic heterotrophs suggests the existence of numerous cooperative niches that are coupled and regulated by microbial interactions. We suggest that the HLSA microbial mats hold microorganisms and the necessary machinery (genomic repertoire to sustain metabolic pathways) to promote favorable conditions (i.e., create an alkaline pH microenvironment) for microbially mediated calcium carbonate precipitation process. Metagenome-assembled genomes (*Ca. Thiohalocapsa araruaensis* HLSAbin6 sp. nov. and *Ca. Araruabacter turfae* HLSAbin9 gen. nov. sp. nov.) obtained support the relevance of Sulfur metabolism and they are enriched with genes involved in the osmoadaptive networks, hinting at possible strategies to withstand osmotic stress. Metabolically versatile bacteria populations, able to use multiple nutrient sources and osmolytes, seem to be a relevant attribute to survive under such stressful conditions.

Keywords: biofilms, microbiome, metagenome, metagenome-assembled genomes, sulfate-reducing bacteria, carbonatogenesis, calcium carbonate, compatible solutes

INTRODUCTION

Microbial mats are one of the oldest known ecosystems on Earth. They support complex consortia of many interdependent species belonging to different functional groups (van Gemerden, 1993; Bolhuis et al., 2014). Fossil records (dated > 3.5 Ga) and modern microbial mats have been investigated extensively from geological, biochemical, and microbiological perspectives (Walter et al., 1980; Vasconcelos et al., 2006; Nutman et al., 2016), raising interesting questions about interactions of microorganisms and its environment. Although modern microbial mats hold taxa that likely arose relatively recently, most metabolic pathways processed by them emerged early in Earth's history and are likely retained at the community level (Bolhuis et al., 2014; Louca et al., 2018). Physicochemical microgradients and taxonomic stratification (Harris et al., 2013) are thus generated to accommodate the coexistence of a wide range of complementary metabolic strategies such as photosynthesis, chemosynthesis, and heterotrophy (Fullmer et al., 2015). Exopolymeric substances (EPSs) excreted by these complex microbial communities protect them against environmental stressors such as desiccation and excessive light. These substances also represent an important source of Organic Carbon under oligotrophic conditions and can serve as nucleation centers for carbonate precipitation processes (Rossi and De Philippis, 2015; Cangemi et al., 2016). These characteristics allow microbial mats to thrive in a variety of harsh environments around the world including hypersaline ecosystems, where intense evaporation and low levels of freshwater input lead to high salt concentrations in the water.

In aquatic ecosystems, the total concentration of inorganic ions such as NaCl (i.e., salinity) is a key environmental factor affecting the distribution of microbial communities (Lozupone and Knight, 2007; Schapira et al., 2009; Dupont et al., 2014). Hypersaline microbial mats are usually composed of extremophile bacteria and archaea that actively regulate cytoplasmic osmotic pressure, thereby maintaining protein integrity under hyperosmotic stress (Oren, 1994; Das et al., 2015). Under high salinity (>10% NaCl), cells tend to lose water to the environment through osmosis, causing dehydration and ultimately cell death. To survive and maintain cell turgor, acclimation processes are needed, including compatible solute accumulation and the expression of channel proteins and osmosensitive enzymes (Das et al., 2015).

The hypersaline lagoon system of Araruama (HLSA) is the largest complex of coastal hypersaline lagoons and salty ponds in Brazil, and one of the largest and commercially most important hypersaline sources of evaporative salt in the world (Kjerfve et al., 1996; Clementino et al., 2008; Laut et al., 2017). HLSA is a rare biogeochemical system, representing an analog for Precambrian environments (Vasconcelos et al., 2006). An excess of evaporation over precipitation maintains the lagoons hypersaline (approximately 52 g/L^{-1} salinity), although some annual unbalance might happen (Moreira-Turcq, 2000). High salinity, together with strong daily fluctuations of temperature, light intensity, UV radiation, and desiccation make this shallow system a harsh environment for any organism. These shifts impose important challenges for the ecosystem function, such as how microbial communities adapt to stay active, while maintaining the characteristic structure of vertically stratified groups of microorganisms. Anthropogenic activities also impose pressure on water quality in these lagoons. Understanding how the HLSA microbial mats are characterized is crucial to provide insights of the system and allow to monitor changes of microbial diversity in these unique ecosystems.

The set of hypersaline lagoons of Araruama has been a subject of study for over 30 years, and the examination of microbial communities inhabiting such environment has been performed through classical cultivation methods and 16S rRNA sequencing (Baeta Neves, 1983; Clementino et al., 2008; Ramos et al., 2017). Clementino et al. (2008) detected a high number of novel prokaryotic phylotypes in the HSLA water column, whereas a better understanding of the cyanobacterial composition in the HLSA microbial mats was given by Ramos et al. (2017). However, little is known about the metabolic diversity of the taxa composing the microbial mats of this lagoon system. In addition, a microbial-induced carbonate precipitation model has been described for the microbial mats of Lagoa Vermelha (L. Vermelha) in Araruama (Vasconcelos et al., 2006, 1995); however, it is not entirely clear what species of bacteria are involved in the carbonate formation in the HLSA mats. The main microbial diversity studies of HLSA lack information about the microorganisms involved in the Sulfur cycling, a key metabolism connected to the calcium carbonate precipitation and dissolution in microbial mats. The aim of the present study was to analyze the taxonomic and metabolic potential of the HLSA microbial mats using shotgun metagenomic sequencing, to avoid the taxonomic primer bias of the 16S rRNA sequencing approach (Jovel et al., 2016). We also sought to investigate the metabolic pathways enabling these microbes to thrive in such a unique environment, by shedding light on the genomic repertoire related to osmoadaptation.

MATERIALS AND METHODS

Study Site and Sample Collection

Microbial mats were sampled in eight salty ponds across the HLSA (16°40′, 19°40′S–39°10′, 37°20′W) (**Figures 1A,B**). This shallow hypersaline lagoon system covers an area of approximately 300 km^2 and is located on the coast 150 km east of Rio de Janeiro where it is subject to a semi-arid climate, an upwelling zone (Kjerfve et al., 1996; Spadafora et al., 2010), and northeast trade winds that promote strong daily fluctuations of temperature, light intensity, and desiccation (Vasconcelos et al., 2006). The low rainfall (annual evaporation rate of 1,390 mm) (Kjerfve et al., 1996) and high evaporation rates in this region result in high salt content in the lagoons (>5.2% total salts) (Kjerfve et al., 1996; Clementino et al., 2008).

The abundant microbial mats are small and organized in stratified (stacked) layers (**Figure 1C**). To obtain a broad representation of the microbial taxonomic composition and metabolic potential, mat samples were collected in two seasons: summer (January 2013) at Brejo do Espinho (Br. Espinho), Monte

FIGURE 1 | (A) Map of the study area. **(B)** Overview of the hypersaline lagoons. **(C)** An excised fraction of the microbial mat ecosystem from the hypersaline lagoon system of Araruama. A stratified structure is observed.

Alto (M. Alto), Mossoró, Queira, Sal Cisne (S. Cisne), and Silva; and winter (June 2013) at BR, Br. Espinho, L. Vermelha, M. Alto, Mossoró, S. Cisne, and Silva (**Figure 1A**). The microbial mats were taken at different stages of maturity or stratification. Samples were collected with a small shovel and sterile metal spatulas, which were sterilized with ethanol and flame between samples. Approximately 75 g samples were collected, transferred to polypropylene tubes in the field and stored in liquid nitrogen. Samples comprised a mixture of the different layers.

DNA Extraction and Sequencing

The samples were separately ground in liquid nitrogen using ceramic mortars and pestles that were washed with SDS detergent, soaked in 10% bleach for 30 min, and autoclaved between samples. Approximately 200 mg of each sample were used for DNA extraction with the DNeasy PowerSoil Kit (Qiagen, Germantown, MD, United States). DNA integrity was evaluated by 1% agarose gel electrophoresis (GelRed^{TM}, Biotium, Inc., Hayward, CA, United States), and DNA purity was assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, United States). The DNA was quantified with a Qubit® 3.0 Fluorometer (Life Technologies-Invitrogen, Carlsbad, CA, United States). Metagenomic libraries were prepared with the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA, United States). Library size distribution was evaluated with a 2100 Bioanalyzer (Agilent, Santa Clara, CA, United States), and library quantification was carried out with a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, United States) and KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, MA, United States). Paired-end sequencing (2 × 300 bp) was performed on a MiSeq System (Illumina).

Bioinformatics and Statistical Analysis

The fastq files generated by Illumina sequencing were qualitatively evaluated with FASTQC v.0.11.2 (Andrews, 2010). The sequences were preprocessed with PRINSEQ v0.20.4 (Schmieder and Edwards, 2011) to remove low-quality DNA sequences (Phred score < 20), duplicates, and short sequences (<35 bp). The resulting sequences were assembled using MIRA

software (Chevreux et al., 1999) with default parameters. The assembled sequences (contigs) were then annotated *via* Metagenome Rapid Annotation using the Subsystem Technology (MG-RAST) (Meyer et al., 2008) with the following cut-off parameters: *e*-value $\leq 1e^{-5}$, 60% minimum sequence identity, and alignment length $\geq$ 15 bp. Taxonomic annotation was performed using the GenBank database (Benson et al., 2008), the largest (Strasser, 2008; Porter and Hajibabaei, 2018) and reliable (Leray et al., 2019) repository of genetic data for biodiversity; whereas the functional annotation was performed using the SEED Subsystems database (Overbeek et al., 2005), an accurate collection of functionally related protein families.

We compared the HLSA microbial mat metagenomic datasets from the present study ($n = 13$) with microbial mat metagenomic datasets from the following sources: Diamond Fork, Utah, United States (hot spring, $n = 2$) (Gomez-Alvarez et al., 2012), Yellowstone National Park, United States (Mushroom Springs, $n = 4$; Octopus Springs, $n = 2$) (Bhaya et al., 2007; Bolhuis et al., 2014), Guerrero Negro, Mexico ($n = 10$) (Kunin et al., 2008; Harris et al., 2013), Shark Bay, Australia ($n = 6$) (Ruvindy et al., 2016; Wong et al., 2018), Lake Meyghan, Iran ($n = 3$) (Naghoni et al., 2017), Clinton Creek, Canada ($n = 2$) (Unpublished, McCormick, M.)[1], Schiermonnikoog, Netherlands (tidal and intermediate zone mats, $n = 2$) (Bolhuis and Stal, 2011), Abrolhos Bank, Brazil ($n = 19$) (Walter et al., 2016), Neutral Zone, Norway ($n = 1$) (Stokke et al., 2015), Cuatro Cienegas, Mexico (lithifying and non-lithifying microbialites, $n = 2$) (Breitbart et al., 2009; Peimbert et al., 2012), and Highbourne Cay, The Bahamas (stromatolites, $n = 1$) (Khodadad and Foster, 2012). All metagenomes were annotated using the same pipelines and settings, and they are publicly available on the MG-RAST website under the ID provided in **Supplementary Table 1**.

Statistical analyses were performed with R version 3.0.3 (R Core Team, 2011) with the vegan package (Oksanen et al., 2012). One-Way Analysis of Similarities (ANOSIM) were used to test differences between sampling locations at genera, phyla, and SEED Level 1 levels using Bray–Curtis distances and 999 permutations. Non-metric multidimensional scaling (nMDS) analyses were used to display the sampling locations based on Bray–Curtis dissimilarity matrices. The hierarchical clusters were built using Euclidian distances and Ward's clustering method. The relative abundance of microbial taxa and the nMDS results were plotted with the ggplot2 (Wickham, 2009) and reshape (Wickham, 2007) packages.

Metagenome-Assembled Genomes

Cross-assembly of reads from all metagenomes was performed by metaSPAdes v.3.6.2 (Nurk et al., 2017), using the default parameters. Protein sequences were predicted from assembled scaffolds with Prodigal (Hyatt et al., 2010). The predicted protein sequences were searched against the NCBI nr database for functional and taxonomic annotation with DIAMOND (Buchfink et al., 2015) setting an *e*-value cut-off of 10^{-5}. The assembled contigs were binned together using the *super-specific* configuration of MetaBAT (Kang et al., 2015) to obtain partial or complete microbial genomes. Genome quality was assessed by CheckM (Parks et al., 2015). The cross-assembly of reads between metagenomes approach is a central feature in most automated binning algorithms and often implemented in different studies (Sharon et al., 2013; Parks et al., 2017; Stewart et al., 2018). The cross-assembly of reads among the HLSA metagenomes aimed to increase the chances of full-length recovery of genomes from the metagenomes as long as the taxonomic profiles of the individual metagenomes seem to be similar. These data have been deposited in GenBank, https://www.ncbi.nlm.nih.gov, under the BioProject accession number PRJNA675017: BioSample SAMN16710161 and SAMN16710317.

Phylogenomic Analysis of the Reconstructed MAGs

Average amino acid identity (AAI), average nucleotide identity (ANI), and genome-to-genome distance (GGD) were used for genomic taxonomy (species cutoff of 95% AAI/ANI and 70% GGD) (Konstantinidis and Tiedje, 2005). Phylogenomic trees were generated for the two metagenome-assembled genomes (MAGs). Clustal Omega (Sievers and Higgins, 2014) was used to align the 43 phylogenetic markers used by CheckM (Parks et al., 2015) and that were identified in the bins 6 and 9, and in a set of bacterial genomes publicly available in the RefSeq database (O'Leary et al., 2016). These alignments were concatenated and used as input for phylogenomic reconstruction with FastTree 2.0 using default parameters (Price et al., 2010). One thousand bootstrap replications were calculated to evaluate the relative support of the branches.

RESULTS

Overview of the Metagenomic Sequencing Dataset

We sequenced a total of 13 microbial mat samples (corresponding to 12.69 million reads) from eight different HLSA locations in summer ($n = 6$) and winter ($n = 7$) (**Table 1**). After quality control, the number of metagenome sequences pairs per sample ranged from 153,231 to 1,856,780, and the total number of contigs ranged from 36,860 to 1,140,943 (**Table 1**).

Taxonomic Composition of HLSA Microbial Mats

The HLSA microbial mats sustain a taxonomically diverse assemblage of microorganisms (**Figures 2A,B**). A total of 32 phyla and 806 different genera were detected belonging to the prokaryotic fraction in the metagenomes of the HLSA microbial mats. The sequences were predominantly bacterial (95.9% on average) with a relatively minor proportion of Archaea (2.2% on average) (**Supplementary Figure 1**). The most abundant phylum was Proteobacteria (30.9–53.6%), followed by Cyanobacteria (9.7–33.0%), and Bacteroidetes (8.8–22.6%) (**Figure 2A**).

[1]McCormick, M. *Shotgun Metagenome of Clinton Creek Biofilm, Canada*. Clinton, NY: Hamilton College. Available online at: https://www.mg-rast.org/linkin.cgi?project=mgp15973

TABLE 1 | Summary statistics of quality filtering and metagenomic assembly for the hypersaline lagoon system of Araruama.

Metagenome	Number of sequences (pairs)	Number of sequences after quality control (pairs)	Total number of contigs	Largest contig size	Median contigs size	Number of bacterial contigs	Number of archaeal contigs	Number of eukaryotic contigs	Number of viral contigs
Br. Espinho | s	653,686	578,239	192,530	7,782	141	56,964	1,727	1,137	49
Br. Espinho | w	1,176,295	945,015	377,975	2,548	144	122,781	9,068	1,723	94
M. Alto | su	1,396,511	1,204,019	636,979	7,666	154	236,053	11,643	3,307	108
M. Alto | w	1,231,528	1,110,004	380,659	4,922	167	164,706	3,775	1,980	119
Mossoró | s	317,239	300,523	167,792	1,467	120	53,368	980	539	9
Mossoró | w	892,599	797,471	418,036	11,457	187	197,178	5,203	2,301	184
S. Cisne | s	1,961,966	1,856,780	1,140,943	30,227	153	650,386	4,846	7,577	172
S. Cisne | w	155,276	153,231	36,860	914	100	9,205	148	130	5
Silva | s	678,359	644,104	289,430	6,914	148	128,834	1,883	1,482	56
Silva | w	1,054,328	957,287	399,736	13,619	153	134,732	3,801	1,553	83
Queira | s	1,404,748	1,284,214	486,043	34,253	155	260,988	2,879	3,168	127
BR | w	1,281,029	1,231,445	649,467	37,879	162	261,791	7,599	3,005	136
L. Vermelha | w	487,728	424,905	223,730	11,865	126	77,480	1,480	680	28

s, summer; w, winter.

Among Proteobacteria, the most abundant groups included the orders Desulfobacterales (1.3–4.6%), Desulfovibrionales (1.5–4.1%), and Desulfuromonadales (1.0–2.8%) and the genera *Rhodobacter* (0.4–1.8%), *Rhodopseudomonas* (0.3–0.8%), and *Nitrosococcus* (0.4–1.0%) (**Figure 2B**), likely because of the importance of their metabolic roles. Among Cyanobacteria, the difference in the profile abundance was attributed to an increase in reads associated with the orders Chroococcales (4.2–10.4%) and Oscillatoriales (2.1–14.6%). The cyanobacterium genus *Coleofasciculus* (formerly *Microcoleus*) was the most abundant genus in most metagenomes (0.6–11.6%), and the species *Coleofasciculus chthonoplastes* alone represented 23.6% of the total abundance of Cyanobacteria (ranging from 6.4 to 39.9%; $n = 95{,}995$). Reads related to *Cyanothece* sp. PCC 7425 (2.0% in Br. Espinho winter to 5.8% in L. Vermelha winter) were detected in all HLSA metagenomes. Most of the recovered archaeal sequences were assigned to the phylum Euryarchaeota (ranging from 0.7% in S. Cisne summer to 6.4% in Br. Espinho winter), with high relative abundances of the genera Halobacterium (0.1% in S. Cisne summer to 4.1% in Br. Espinho winter) and Methanomicrobia (0.3% in S. Cisne summer to 1.1% in BR winter).

Metabolic Potential of HLSA Microbial Mats

The metagenomic sequences were classified into 28 SEED subsystems (**Supplementary Figure 2**), a wide range of metabolic pathways which allows microbes to detect changes in the environment conditions to survive. Metabolisms related to Carbohydrates, Protein, and Amino Acids and Derivatives subsystems accounted for 35% of all identified sequences. Cyanobacteria were found to be a key component of this system as the main group responsible for Photosynthesis (50.9–82.4%), and the order Chroococcales alone was the main contributor of genes related to Nitrogen Fixation (6.1–27.3%) and Ammonia Assimilation (5.5–36.4%) metabolisms. Proteobacteria was the main contributor of genes related to Respiration (45.3–64.4%) and Fermentation (32.4–50.0%) subsystems. Genes related to Sulfur metabolism (0.5–0.8%) were attributed mainly to Proteobacteria, whereas genes related to Methanogenesis metabolism were attributed mainly to the bacterial phyla Actinobacteria (0.0–61.54%) and Proteobacteria (7.69–100%), and also to the archaeal orders Methanosarcinales (0.0–30.0%) and Methanopyrales (0.0–18.18%).

When examining the taxonomic profile of the HLSA metagenomes, we see that five out of the six most abundant phyla (Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, and Chloroflexi) (**Figure 2A**) contain members that are capable of oxygenic and anoxygenic reaction center-based phototrophy. Metabolic versatility of specific taxa present in the HLSA metagenomes is discussed below. Furthermore, genes related to Osmotic Stress were detected in all HLSA microbial mat metagenomes, following: L-ectoine synthesis (0.01–0.05%), L-proline transport, glycine betaine (0.1–0.2%), hyperosmotic potassium uptake (0.02–0.1%), glutathione-regulated potassium-efflux system (0.05–0.2%), and voltage-gated potassium efflux systems (0.01–0.1%).

Taxonomic and Metabolic Profiles Across Microbial Mats Metagenomic Samples

We compared the HLSA microbial mat metagenomes ($n = 13$) with metagenomes from 11 other microbial communities ($n = 55$). Notwithstanding the similar nature of the structures used for comparison, general bacterial composition differed by sample origin (ANOSIM: $R = 0.706$, $P = 0.001$; **Figure 3**). Cyanobacteria diversity and abundance accounted for much of the variation between samples. The HLSA metagenomes clustered together, without distinction between seasons or samples (**Figures 3**, **4**). The HLSA metagenomes were more closely related to the hypersaline microbial mat metagenomes from Mexico (Guerrero Negro, in Baja

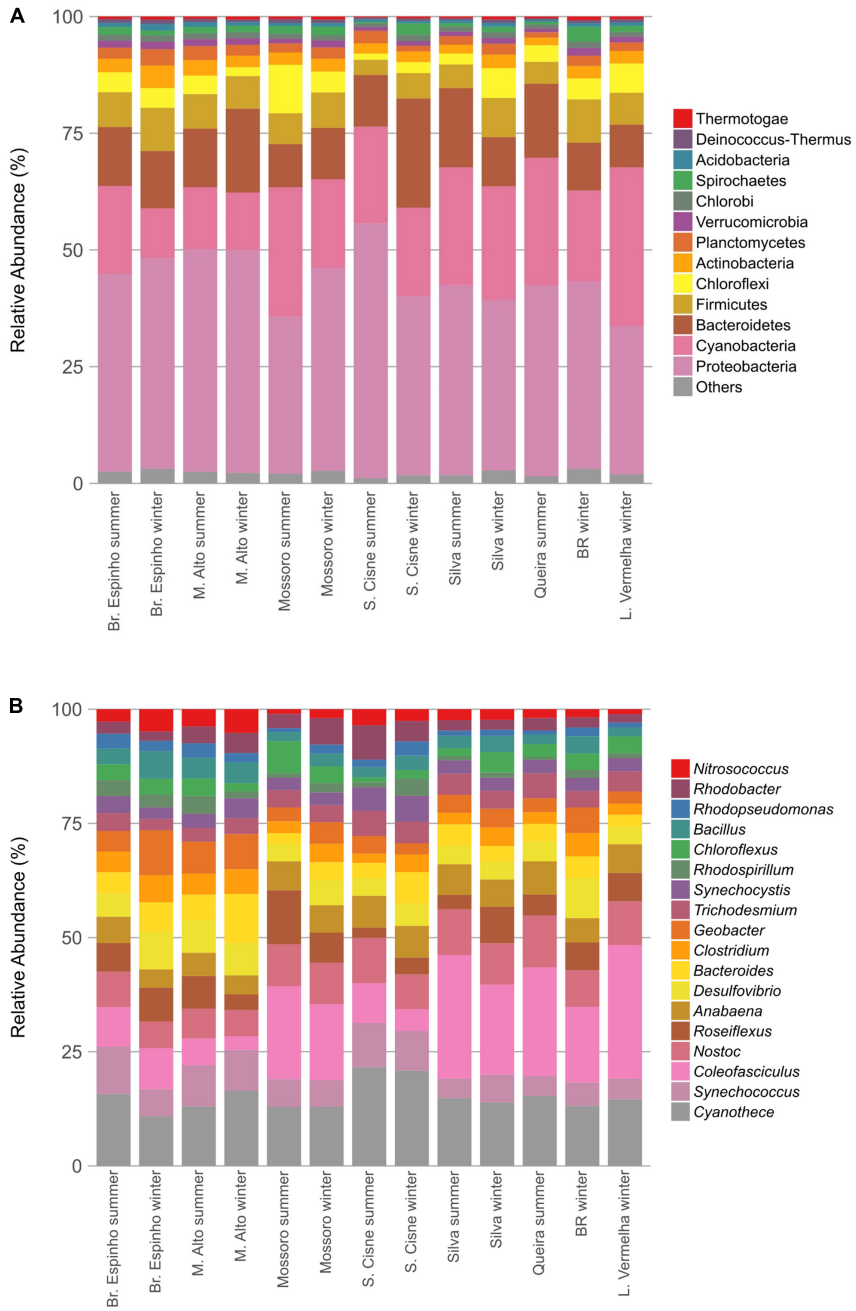

FIGURE 2 | Taxonomic composition of the microbial mats from the hypersaline lagoon system of Araruama. Bar plots depict the relative abundances of bacterial phyla **(A)** and genera **(B)** detected in the metagenomes normalized to 100%. Only the most abundant taxa are shown.

California Sur, and Cuatro Cienegas green mat) (**Figure 4** and **Supplementary Figure 3**).

Comparative Genomics and Functional Complexity of Recovered MAGs

To provide insights into the genomic context of microorganisms interacting within the HLSA microbial mats, we recovered genomes from the metagenomes and explored their taxonomic and functional diversity. Bacterial genomes with completeness >87% and presenting genome sizes of approximately 4 Mbp were obtained from the HLSA metagenomic dataset (**Supplementary Table 2**). Because of the relatively low sequencing depths obtained for the HLSA metagenomes, only the most abundant sequences were binned into individual genomes. Here, we highlighted the annotated taxonomic and functional genes of two reconstructed genomes. Following the standards suggested by Bowers et al. (2017), Bin6 is referred as a high-quality

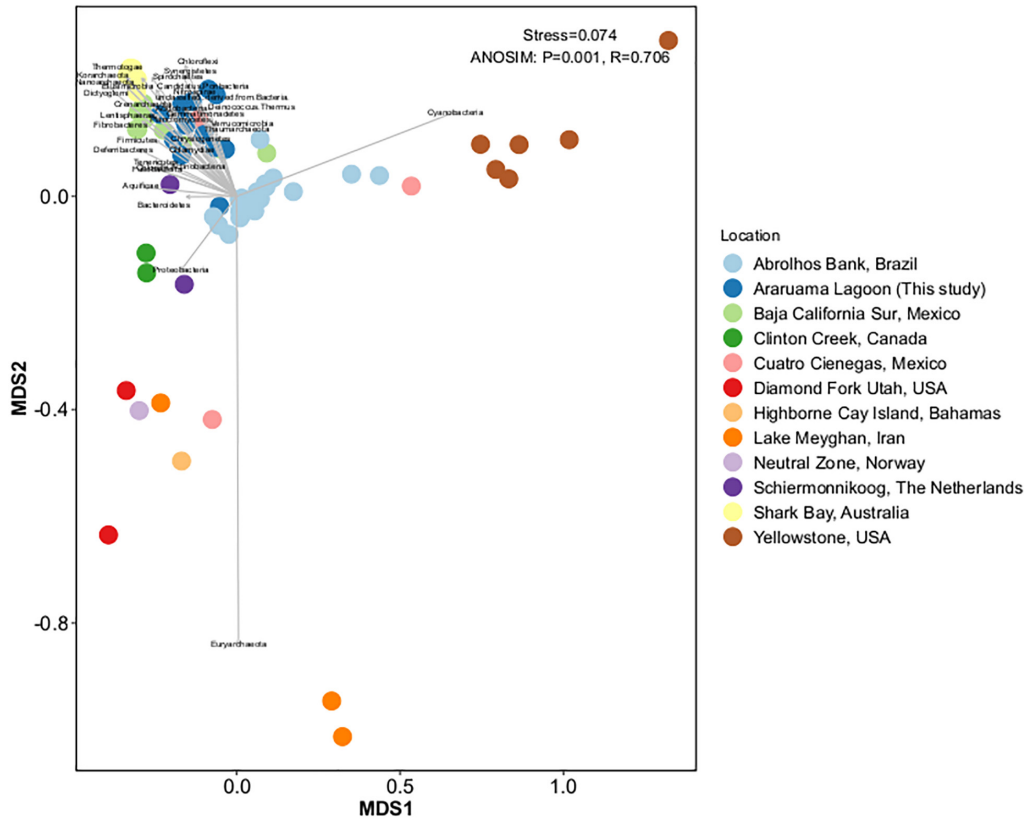

FIGURE 3 | Non-metric multidimensional scaling (nMDS) plot representing the Bray–Curtis similarity of general microbial community structure composition of 68 metagenomes of microbial mats from different habitats. Each dot represents a sample, color represents the sampling location, and the arrows represent the nMDS phyla scores.

draft (>90% complete, <5% contamination), while Bin9 is a medium-quality draft (>50% complete, <10% contamination). A comparison of both reconstructed genomes with their most closely related reference genomes showed that AAI, ANI, and GGD values were much lower than the species cutoff, indicating the novelty of these microorganisms (**Table 2**). Bin6 represents a new species of *Thiohalocapsa* that is closely related to *Thiohalocapsa* sp. ML1 (70.2% AAI), a Gammaproteobacteria belonging to the order Chromatiales (**Table 2** and **Supplementary Figure 4**). Bin9 represents a new genus, and the closest reference genome belongs to a member of Bacteroidetes, *Phaeodactylibacter xiamenensis* (47.6% AAI) (**Table 2** and **Supplementary Figure 5**). To further identify these two reconstructed genomes, phylogenomic analysis were performed. Whereas Bin6 was placed closely with *Thiohalocapsa* sp. ML1 (**Supplementary Figure 4**), the phylogenomic placement of Bin9 shown a relatively distant evolutionary relationship with *P. xiamenensis* (**Supplementary Figure 5**). The novel species were named *Ca. Thiohalocapsa araruaensis* HLSAbin6 sp. nov. (Bin6), and *Ca. Araruabacter turfae* HLSAbin9 gen. nov. sp. nov. (Bin9).

The closest relatives of these two recovered genomes are salt tolerant; therefore, we tested for the presence of genes related to osmoregulation. We identified genes encoding glycine betaine/proline transport systems (ABC transport systems, e.g., *pro*V, *pro*W, and *pro*X), high-affinity choline uptake protein (*bet*T), carnitine/choline transporter (*opu*CB), betaine uptake/biosynthesis systems, genes involved in glucan synthesis, an aquaporin Z, and another outer membrane protein (*omp*A) (**Table 3**).

Key genes related to Carbon, Nitrogen, and Sulfur biogeochemical cycling were compiled and allowed delineation of the functional role of the taxa associated to the bins (**Table 4**). For instance, both bins encode complete and partial sulfate-reduction pathways (**Table 4**), potentially indicating the importance of Sulfur cycling in the HLSA microbial mats. Partial recovery of a determined pathway might be a result of lack of coverage in both not complete MAGs. Annotations for major metabolisms such as Sulfur (*dsr*, *apr* genes, and the *sox*ABHWYZ complex), Nitrogen (*nif* and *nar* genes), and bacteriochlorophyll-based Photoautotrophy (e.g., *bch*, *psb*, *puf*, *rbc*, *ccm*, *apcc*, *coo*) are found in Bin6, which is associated with purple sulfur bacteria.

DISCUSSION

This study aimed at gaining insights into the diversity of microorganisms in the HLSA microbial mats, expanding

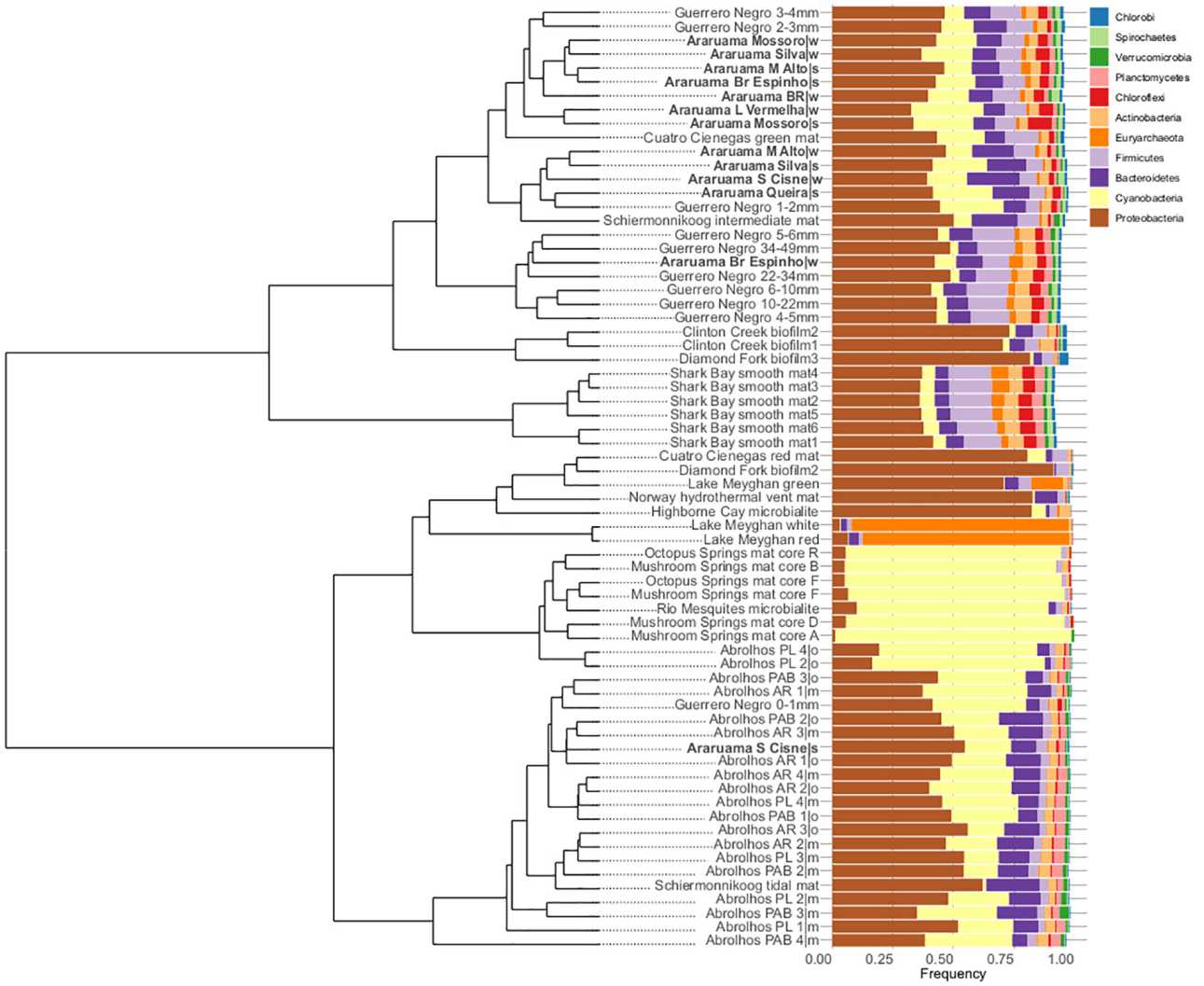

FIGURE 4 | Hierarchical clustering and bar plots of relative abundances of the major microbial phyla found within 68 metagenomes of microbial mats from different habitats. Clustering was based on Euclidian distances and Ward's clustering method.

the knowledge generated by previous studies that employed operational taxonomic unit-based approach to focus on the cyanobacterial populations of these microbial mats (Ramos et al., 2017), and the prokaryotic diversity of the HLSA water column (Clementino et al., 2008). The use of shotgun metagenomics allowed us to delineate a broader characterization of the microbial mats coping with extreme environmental conditions in the coastal Araruama lagoon system. This approach circumvents the use of culture-dependent methodology as part of the polyphasic strategy employed previously (Clementino et al., 2008; Ramos et al., 2017). Although bacterial isolation technique has yielded valuable biodiversity information in the past, currently it provides little information which limits substantial characterization. For the first time in the HLSA ecosystem, shotgun metagenomics was generated, and near-complete genome bins were retrieved from the metagenomic data.

On the southeastern Brazilian continental margin, structures that dominate the HLSA have been extensively studied with focus on the mineralogical and biogeochemical features (Vasconcelos and McKenzie, 1997; van Lith et al., 2002; Delfino et al., 2012; Bahniuk et al., 2015), giving insights into stromatolite genesis. These laminated structures produced by the successive deposition of layers of microbial mat are found in proximity with their modern counterparts. Comprehensive fossil record found worldwide indicates that ancient microbial mat structures are the oldest biological communities known, dating back to 3.7 billion years for structures found in Greenland (Nutman et al., 2016) and 3.5 billion years for Australian stromatolites (Walter et al., 1980; Allwood et al., 2006).

Evaporation, flooding, and salinity fluctuations processes contribute to the dynamic of the naturally occurring shallow HLSA ecosystem. Modern microbial mats descended from stromatolites and are likely to harbor microorganisms adapted

TABLE 2 | Genetic relatedness between the metagenome-assembled genomes and the most closely related reference genomes based on average amino acid identity (AAI), average nucleotide identity (ANI), and genome-to-genome distance (GGD).

	AAI (%)	ANI (%)	GGD (%)
	Bin6		
Thiohalocapsa sp. ML1	70.2	81.0	23.5
Uncultured *Thiohalocapsa* sp. PB-PSB1	55.5	75.6	20.9
Allochromatium vinosum DSM 180^T	51.3	76.6	19.1
Allochromatium warmingii DSM 173^T	47.3	73.8	17.6
Thioflavicoccus mobilis 8321	50.9	76.5	19.9
	Bin9		
Phaeodactylibacter xiamenensis KD52^T	47.6	74.0	19.1
Lewinella nigricans DSM 23189^T	44.1	73.1	18.4
Lewinella agarilytica	39.2	71.3	12.5
Lewinellaceae bacterium SD302^T	39.4	71.8	17.4
Lewinella persica DSM 23188^T	39.0	71.2	16.4

to such stressful conditions. The new sequences generated in the present study substantially increase the representation of all phyla described previously for the HLSA ecosystem (e.g., Proteobacteria and Cyanobacteria) (Clementino et al., 2008; Ramos et al., 2017). The HLSA microbial mats sustain taxonomically diverse assemblage of microorganisms, which exhibit high metabolic diversity.

In contrast to microbial mats in other extreme environments such as hot springs (e.g., Mushroom Springs and Octopus Spring, Yellowstone samples), which are dominated by Cyanobacteria (Bhaya et al., 2007; Bolhuis et al., 2014), the HLSA microbial mats are dominated by Proteobacteria. The prevalence and relative abundances of the three prevailing phyla in the HLSA microbial mats metagenomes were similar to other hypersaline microbial mats from Mexico, such as Guerrero Negro (Kunin et al., 2008; Harris et al., 2013), and Cuatro Cienegas green mat (Breitbart et al., 2009; Peimbert et al., 2012). The dominant proteobacterial groups in the HLSA microbial mats are similar to those in the Mexican microbial mats. Besides, the taxonomic similarity between both locations has been observed previously for the cyanobacterial community (Ramos et al., 2017). Taken together, a taxonomic signature for hypersaline environments may exists. Cyanobacteria were found to be a key component of this system as the main group responsible for photosynthesis and nitrogen fixation. Moreover, the relatively high abundance of the cyanobacterial orders Chroococcales and Oscillatoriales is in agreement with a previous study combining morphology and molecular-based tools to characterize the diversity of Cyanobacteria in the HLSA (Ramos et al., 2017). The abundance of the cyanobacterial genus *Coleofasciculus* in the HLSA microbial mats may be explained by its tolerance to high saline levels and its metabolic flexibility (i.e., ability to perform both photosynthesis and anoxic fermentation) (Burow et al., 2013). OTUs related to this halophilic Cyanobacteria was reported previously in the HLSA ecosystem (Clementino et al., 2008; Ramos et al., 2017), whereas microbial mats dominated by *Coleofasciculus* are found in hypersaline ponds of Guerrero Negro, Mexico (Garcia-Pichel et al., 1996; Marais, 2010; Harris et al., 2013). Acting as the primary producer in the mat, this microorganism maintains high numbers of metabolically active heterotrophs which hold catabolic and transport capabilities, for instance.

When examining the taxonomic profile of the HLSA metagenomes, we see that five out of the six most abundant phyla contain members that are capable of oxygenic and anoxygenic reaction center-based phototrophy. High taxa heterogeneity and metabolic versatility occurs in the HLSA mats, particularly considering the diverse taxa of anoxygenic phototrophic bacteria and oxygenic cyanobacterial communities driving the energetic flow. The utilization of different electron donors is well represented by the photoheterotrophic purple non-sulfur *Rhodobacter* spp., capable of anoxygenic photosynthesis, as well as aerobic and anaerobic respiration (Pérez et al., 2017). Another abundant genus, *Rhodopseudomonas*, is capable to switch among photoautotrophic, photoheterotrophic, chemoautotrophic, and chemoheterotrophic metabolisms (Larimer et al., 2004). *Nitrosococcus*, the most abundant genus of Chromatiales found in the HLSA, is a widespread chemolithoautotrophic ammonia-oxidizing bacterium that possesses monovalent cation transporters that confer salt tolerance (Klotz et al., 2006). Notably, Deltaproteobacteria make up to 37.5% of the Proteobacteria population and include sulfate-reducing bacteria such as Desulfobacterales, Desulfuromonadales, and Desulfovibrionales that obtain energy reducing sulfates to sulfides (Wasmund et al., 2017). Also, very abundant (up to 40.5%) and more diverse is Gammaproteobacteria, which contain anoxygenic phototrophic sulfide-oxidizing members that provide the heterotrophic sulfate reducers with some Organic Carbon, hence closing the Sulfur cycle within the HLSA mats. In correspondence to that, several metabolically versatile microorganisms were identified in the HLSA mats, including a high-quality reconstructed genome related to *Thiohalocapsa* sp. (Bin6), a purple bacterium, which indicates its involvement in the Sulfur metabolism. Bin6 contains annotations for both Sulfur (*dsr*, *apr* genes, and the *sox*ABHWYZ complex) and Nitrogen (*nif* and *nar* genes) metabolisms. These, together with the annotations for bacteriochlorophyll-based Photoautotrophy (e.g., *bch*, *psb*, *puf*, *rbc*, *ccm*, *apcc*, *coo*) suggest a dynamic role of Bin6 in the HLSA microbial mats. This purple sulfur bacteria contain a *puf* operon encoding a type-2 photochemical reaction center (subunits PufL, PufM, and PufH) for aerobic anoxygenic metabolism. Another indication of its anoxygenic metabolism is the presence of BchF, which is exclusively found in those groups of bacteria that can synthetize bacteriochlorophyll *a* (Bryant et al., 2012). Observations of active Sulfur and Nitrogen metabolisms in other purple sulfur bacteria have been shown elsewhere (Bebout et al., 1993; Yurkov et al., 1994).

Another genome recovered from the metagenomes, Bin9, is related to Bacteroidetes. Members of this phylum act as specialists for the degradation of high molecular weight organic matter and complex polysaccharides (Fernandez-Gomez et al., 2013). They have been detected in high abundance and diversity in several hypersaline microbial mats (e.g., Guerrero Negro, Shark

TABLE 3 | Osmoprotectant and osmoregulation profile in the two reconstructed genomes.

Annotation	Gene	Bin6	Bin9
Trehalose synthase (EC 5.4.99.16)	*treS*		
Trehalose-6-phosphate phosphatase (EC 3.1.3.12)	*ostB*		
Trehalose phosphorylase (EC 2.4.1.64)	*treP*		
Malto-oligosyltrehalose trehalohydrolase (EC 3.2.1.141)	*mth*		
Alpha-trehalose-phosphate synthase (EC 2.4.1.15)	*otsA*		
Alpha-amylase (EC 3.2.1.1)	*amyA*		
1,4-alpha-glucan (EC 2.4.1.18)	*glg*		
Glycogen debranching enzyme (EC 3.2.1.-)	*treX*		
Glucoamylase (EC 3.2.1.3)	*ssg*		
Beta-phosphoglucomutase (EC 5.4.2.6)	*pgm*		
YggS, proline synthase	*yggS*		
Gamma-glutamyl phosphate reductase (EC 1.2.1.41)	*gpr*		
Pyrroline-5-carboxylate reductase (EC 1.5.1.2)	*proC*		
Glutamate 5-kinase (EC 2.7.2.11)	*proB*		
RNA-binding C-terminal domain PUA	*pua*		
NADP-specific glutamate dehydrogenase (EC 1.4.1.4)	*gdhA*		
Glycine betaine/L-proline ABC transporter, ATP-binding protein ProV (TC 3.A.1.12.1)	*proV*		
Glycine betaine/L-proline ABC transporter, periplasmic binding protein ProW (TC 3.A.1.12.1)	*proW*		
Glycine betaine/L-proline ABC transporter, protein ProX (TC 3.A.1.12.1)	*proX*		
High-affinity choline uptake protein BetT	*betT*		
Choline-sulfatase (EC 3.1.6.6)	*betC*		
Choline dehydrogenase (EC 1.1.99.1)	*betA*		
Sarcosine *N*-methyltransferase	*bsmA*		
Glycine *N*-methyltransferase (EC 2.1.1.20)	*bsm*		
Dimethylglycine *N*-methyltransferase	*bsmB*		
Aquaporin Z	*aqpZ*		
Outer membrane protein A precursor	*ompA*		
Glucans biosynthesis glucosyltransferase H (EC 2.4.1.-)	*opgH*		
Proline iminopeptidase (EC 3.4.11.5)	*pipX*		
Proline-rich protein/signal peptide	*prb*		
Transporter linked to choline/ethanolamine kinase and OMR	*pnuC*		
Potassium uptake protein TrkA	*trkA*		
Potassium uptake protein TrkH	*trk1*		
Potassium channel protein	*kch*		
Osmosensitive K+ channel histidine kinase KdpD (EC 2.7.3.-)	*kdpD*		
Potassium voltage-gated channel subfamily KQT	*kcn*		
Potassium efflux system KefA protein	*kefA*		
Glutathione-regulated potassium-efflux system protein KefB	*kefB*		
Glutathione-regulated potassium-efflux system protein KefC	*kefC*		
Glutathione-regulated potassium-efflux system ancillary protein KefG	*kefG*		
Glutathione-regulated potassium-efflux system ATP-binding protein	yhe		

Key genes related to halotolerance recovered in genomes obtained from the HLSA metagenomes.
The heatmap displays the presence or absence of key genes related to halotolerance.

Bay), and the occurrence of specialists have been hypothesized. A strain specialized on the scavenging of Cyanobacteria was found in a hypersaline microbial mat (Hania et al., 2017). Complex cyanobacterial exudates become available to the general microbial community thought those bacteria. Therefore, it is likely that Bacteroidetes play a key role in the degradation and cycling of mat compounds.

Altogether, they indicate the importance of the energy flow (e.g., Carbon and Sulfur) (Canfield and Marais, 1993; Baumgartner et al., 2006) in the HLSA microbial mats. The major role of sulfur-bacteria to calcium mineralization has been demonstrated (Visscher et al., 1998; Braissant et al., 2007; Dupraz et al., 2009; Saghai et al., 2015). Previous Oxygen and Sulfur profiles taken at L. Vermelha demonstrated oxygen

TABLE 4 | Functional genetic diversity of biogeochemical cycling (C, N, S) in the two reconstructed genomes.

Cycling	Annotation	Gene	Bin6	Bin9
Assimilatory sulfate reduction	Adenylylsulfate kinase/reductase (EC 2.7.1.25)	*apsK*		
	Adenylylsulfate reductase alpha-subunit	*aprA*		
	Adenylylsulfate reductase beta-subunit	*aprB*		
	Arylsulfatase (EC 3.1.6.1)			
	Dihydrofolate reductase (EC 1.5.1.3)	*lapr*		
Dissimilatory sulfate reduction	Dissimilatory sulfite reductase, gamma subunit	*dsr*		
	DsrE oxidoreductase	*dsrE*		
	Sulfite reduction-associated complex DsrMKJOP multiheme protein DsrJ (=HmeF)	*dsrJ*		
	Sulfite reduction-associated complex DsrMKJOP protein DsrK (=HmeD)	*dsrK*		
	Sulfite reduction-associated complex DsrMKJOP protein DsrM (=HmeC)	*dsrM*		
	Sulfite reduction-associated complex DsrMKJOP iron–sulfur protein DsrO (=HmeA)	*dsrO*		
	Sulfite reduction-associated complex DsrMKJOP protein DsrP (=HmeB)	*dsrP*		
	IscA-like protein, DsrR	*dsrR*		
	DsrS	*dsrS*		
	Sulfate adenylyltransferase, dissimilatory-type (EC 2.7.7.4)	*sat*		
	Sulfite oxidase SoxA protein	*soxA*		
	Sulfite oxidase SoxB protein	*soxB*		
	Sulfite oxidase SoxH protein	*soxH*		
	Sulfite oxidase SoxW protein	*soxW*		
	Sulfite oxidase SoxY protein	*soxY*		
	Sulfite oxidase SoxZ protein	*soxZ*		
Nitrogen	Nitrogenase protein NifA	*nifA*		
	Nitrogenase protein NifB	*nifB*		
	Nitrogenase protein NifE	*nifE*		
	Nitrogenase protein NifN	*nifN*		
	Nitrogenase protein NifO	*nifO*		
	Nitrogenase protein NifQ	*nifQ*		
	Nitrogenase protein NifX	*nifX*		
	Nitrogenase cofactor carrier protein NafY	*nafY*		
Anoxygenic photosynthesis	Light-harvesting LHII, beta subunit	*pufB*		
	Light-harvesting LHI, alpha subunit	*pufA*		
	Photosynthetic reaction center H subunit	*pufH*		
	Photosynthetic reaction center L subunit	*pufL*		
	Photosynthetic reaction center M subunit	*pufM*		
	Photosynthetic reaction center cytochrome *c* subunit	*pufC*		
	Photosystem II proteins	*psb*		
	Putative photosynthetic complex assembly protein			
	2-vinyl bacteriochlorophyllide hydratase	*bchF*		
	Bacteriochlorophyll *c* synthase	*bchC*		
Heterotrophy (fermentation)	Cytochrome *c* oxidase subunit CcoG	*ccoG*		
	Cytochrome *c* oxidase subunit CcoN	*ccoN*		
	Cytochrome *c* oxidase subunit CcoO	*ccoO*		
	Cytochrome *c* oxidase subunit CcoP	*ccoP*		
	2-oxoglutarate oxidoreductase	*kor*		
	2-oxoglutarate dehydrogenase complex (EC 2.3.1.61)	*odh*		
	6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44)	*pgd*		
	Phosphoglucomutase (EC 5.4.2.2)	*pgm*		
	Phosphoglucosamine mutase (EC 5.4.2.10)	*glmM*		
	Enolase	*ens*		
	Pyruvate kinase	*pyk*		
Carbon fixation	Ribulose bisphosphate carboxylase small chain (EC 4.1.1.39)	*rbc*		
	Ribulose bisphosphate carboxylase large chain (EC 4.1.1.39)	*rbcA*		
	Carbonic anhydrase (EC 4.2.1.1)	*cah*		
	Carboxysome	*ccm*		
	Acetyl-CoA carboxylase	*apcc*		
	Propionyl-CoA carboxylase			
	Carbon monoxide dehydrogenase	*coo*		
	Putative sodium-dependent bicarbonate transporter			

Heatmap displays the presence or absence of a selected subset of genes previously associated with pathways involved in nutrient utilization and energy metabolism.

TABLE 5 | Key genes related to microbial-induced calcium carbonate precipitation.

	BR | w	Br. Espinho | s	Br. Espinho | w	S. Cisne | s	S. Cisne | w	L. Vermelha | w	M. Alto | s	M. Alto | w	Mossoro | s	Mossoro | w	Queira | s	Silva | s	Silva | w	Bin6	Bin9
Carboxysome protein CcmL																
Carboxysome protein CcmM																
Carboxysome protein CcmN																
Carboxysome shell protein CsoS1																
Carboxysome shell protein CsoS2																
Carboxysome shell protein CsoS3																
Putative carboxysome peptide A																
Putative carboxysome peptide B																
Carbonic anhydrase (EC 4.2.1.1)																
Carbonic anhydrase, gamma class (EC 4.2.1.1)																
Carbonic anhydrases/ acetyltransferases																

Heatmap displays the presence or absence of a selected subset of genes previously associated with the mineralization of calcium carbonate in both metagenomes and recovered genomes from the HLSA metagenomes.

peak (oxygen-producing Cyanobacteria) and decrease (oxygen consuming heterotrophs), followed by sulfide consumption (anaerobic sulfide-oxidizing purple bacteria), and sulfide increase (sulfide producing sulfate- and sulfur reducers) (Warthmann et al., 2011). Notably, high sulfate reduction rates coincided with zones of carbonate precipitation in oxygenated zones of another hypersaline microbial mats (Highborne Cay, Bahamas) (Visscher et al., 2000; Dupraz et al., 2004). Different sulfur-reducing bacteria display different tolerances for oxygen exposure and, hence, may present a broad distribution in the microbial mat. Like in the hypersaline mats from Guerrero Negro (Minz et al., 1999), different genera of sulfur-reducing bacteria most likely populate different depths within the HLSA mats, and the oxic zone near the mat surface may present the highest rates of sulfate reduction (Canfield and Marais, 1991). Key genomic repertoire related to calcium carbonate was identified in the HLSA microbial mats metagenomes (**Table 5**). Microbial mats found across the HLSA seem to present thin and discontinuous calcium carbonate deposition, and two lagoons (L. Vermelha and Br. do Espinho) are well-known for containing Ca–Mg carbonate formations alternating with non-lithified organic layers (Vasconcelos et al., 2006; Nascimento et al., 2019). In addition to the cyanobacterial contribution to the precipitation of calcium carbonate, *Cyanothece* sp. PCC 7425 and *Thermosynechococcus elongatus* BP-1 strains are known to accumulate calcium carbonate inclusions in their cytoplasm (Benzerara et al., 2014), and both were abundantly present in the HLSA metagenomes.

Interestingly, the final net production of carbonates depends on the balance of different microbial metabolisms. Metabolisms such as oxygenic and anoxygenic photosynthesis (Dupraz and Visscher, 2005; Bundeleva et al., 2012), sulfate reduction (Visscher et al., 2000; Gallagher et al., 2014), and anaerobic methane oxidation coupled to sulfate reduction (Michaelis et al., 2002) contribute to a state of carbonate saturation, in an alkaline pH, promoted by a matrix of EPS that leads to calcium ions to precipitate as calcium carbonate (Baumgartner et al., 2006; Zhu and Dittrich, 2016). On the other hand, aerobic respiration, sulfide oxidation, and fermentation (Dupraz and Visscher, 2005) tend to promote dissolution by acidification.

Microorganisms adapted to saline and hypersaline environments display different strategies to cope with high osmotic pressure. These microorganisms may use two main strategies to maintain osmotic balance: (1) accumulate (biosynthesize and/or import) organic compatible solutes (osmoprotectants) that do not interfere with enzymatic activity (e.g., L-ectoine, L-proline, sucrose, trehalose, glucosylglycerol, and glycine betaine) and (2) control ion flow across cellular membranes through regulated potassium uptake and efflux pumps (Martinac et al., 1987). Efflux pumps are not sufficient to cope with high osmolarity (Roberts, 2005), because microorganisms may only transiently accumulate potassium ions. Thus most halotolerant organisms use multiple osmolyte strategies to cope with hypersaline environments (Yaakop et al., 2016). The two recovered genomes contain salt tolerant genomic repertoire. Bin6, associated with purple sulfur bacteria, has genes that encode aquaporin Z water channels that may enhance the flux of water across the cellular membrane in response to abrupt changes in osmotic pressure (Calamita, 2000). This species may also achieve osmotolerance by importing proline, glycine, and betaine through the proU operon (*pro*V, *pro*W, and *pro*X). Whereas Bin9, related to the family Saprospiraceae, also possesses osmoregulation genes encoding proteins involved in the biosynthesis of trehalose and proline and in the uptake and biosynthesis of choline and betaine (Chen et al., 2014). In addition, this species has a gene that encodes the OmpA outer membrane protein, which has multiple functions, including osmoprotection (Hong et al., 2006). Osmoprotectant compounds can be used as Carbon and Nitrogen sources and for energy storage (Welsh, 2000), which may help microorganisms, including the novel candidate species identified in this study, to survive under stressful conditions, such as a sudden temperature increase, desiccation, and UV radiation. Indeed, the new candidate species exemplify different strategies for halotolerance as mentioned before.

Although detected in all HLSA metagenomes, the reconstruction of a particular bin associated with a Cyanobacteria representative (the most closely related reference genome was *C. chthonoplastes*) did not pass the binning thresholds (Bowers et al., 2017) due to high level of sequences contamination. Despite that, annotation of the cyanobacterial bin could provide some interesting information (results not shown). This bin contains a genetic repertoire for compatible solute metabolism (e.g., trehalose biosynthesis and glycine betaine uptake and biosynthesis). Interestingly, most mat-forming filamentous Cyanobacteria accumulate trehalose, and the combination of EPS with trehalose protects against desiccation (Potts, 1994). Another compound that may be used by *Coleofasciculus* as an osmoprotectant is carnitine, which can protect against fluctuations in salinity, water content, and temperature (Meadows and Wargo, 2015). Also, we identified sequences related to the permease proteins involved in carnitine transport. Although many bacteria can generate carnitine from direct precursors, these metabolic pathways are not completely understood. *Coleofasciculus* sp. may also use Na^+/H^+ antiporters for ion exclusion (e.g., Na^+) under hypersaline conditions, as described for other Cyanobacteria (Waditee et al., 2002). Na^+ is the main inorganic cation in saline environments and thus, active sodium ion export mechanisms exist in these cells. Clusters of genes encoding the Mrp operon system were also found in all HLSA metagenomes. The Mrp cluster is a monovalent cation/proton antiporter system also involved in Na^+ extrusion (Hagemann, 2011). MAGs allow to disentangle the drivers of functional complexity in other microbial mats (Saghai et al., 2015; Wong et al., 2020), where the genomic repertoire of such candidate microbial taxa was investigated. The genomes recovered from the HLSA metagenomes support the environmental relevance of the microorganisms represented by the assemblies described in this study.

CONCLUSION

Hypersaline lagoon system of Araruama microbial mats have evolved to encompass high taxonomic and metabolic diversity,

illustrated by the autotrophic and heterotrophic guilds found in their metagenomes. The similarity between HLSA, Cuatro Cienegas and Guerrero Negro hint to possible adaptative mechanisms to thrive in hypersaline environments. High metabolic flexibility and the production of osmoprotectant compounds appear to be important for survival in the HLSA microbial mats. Halotolerance, phototrophy, and chemosynthesis pathways by bacterial representatives in both the HLSA microbial mats metagenomes and the recovered genomes are indicative of a diverse metabolic repertoire needed to sustain life in the HLSA. A high proportion of sulfur bacteria is remarkable. Deltaproteobacteria, which includes sulfate-reducing bacteria such as Desulfobacterales, Desulfuromonadales, and Desulfovibrionales, comprise approximately 40% of the Proteobacteria population, the most abundant phylum in the HLSA microbial mat metagenomes. This result supports the relevance of sulfate-reducing bacteria in the hypersaline microbial mats of HLSA, where versatile populations in synergy with other taxa cover most of the metabolic activities within the mat, including the precipitation of calcium carbonate in these unique microbial structures.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SD, RC, FT, CT, MN, DB, and AC conceived the study and designed the experiments. LO and DB processed the samples and performed DNA sequencing. JW, LO, DT, and PM analyzed the data. JW, LO, and FT wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.715335/full#supplementary-material

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