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Metagenomic analysis of Microbial Communities from a Red Sea Brine Pool

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Christian Jansen, B.Sc.

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Abstract

Microorganisms have been found in essentially every environment on Earth, adapting to a multitude of environmental parameters over a wide range and thriving in some of the most hostile places on earth. Deep-sea brine pools, such as in the Red Sea, are certainly one of these extreme environments in terms of salinity with 250 compared to the usual 35 in the global ocean. In this study we used a metagenomic binning approach to examine the microbial community composition in a Red Sea brine pool in the Thuwal area and assign functions to these microbes using the KEGG database. Water samples were size-fractionated into 0.2-0.8 µm, 0.8-3.0 µm and > 3.0 µm to separate free-living from particle-associated microbes. This approach revealed a clear difference in the community composition between the two larger particle fractions and the free-living community, particularly in the brine pools. Sulfur oxidation plays an important role in the free-living community in combination with inorganic carbon fixation via both the reverse tricarbocylic acid cycle and the Calvin-Benson-Bassham cycle. Our results in combination with previous findings indicate that hydrogen sulfide and other reduced sulfur compounds are limiting resources especially in the free-living community of the brine pool.

Additionally in the brine pool, the particle-associated microbes were dominated by bacteria with carbohydrate fermentation capabilities as well as by organisms using oxygen, nitrate and sulfate as electron acceptors.

This spatial separation of oxidative and reductive pathways in the brine pool, utilizing anaerobic microenvironments on the particles, points towards an interaction between the particle-associated and free-living microorganisms by exchanging oxidized and reduced sulfur compounds.

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Introduction

Microorganisms are the main drivers of biogeochemical cycles on Earth (Madsen 2011; Rousk and Bengtson 2014) and colonized essentially all terrestrial and aquatic environments. Bacteria and Archaea are mediating nitrification, denitrification, sulfur oxidation and reduction and are mainly responsible for the carbon cycling due to their high metabolic activity. Microbes might perform multiple metabolic pathways, such as photo- or chemoautotrophy and heterotrophy using a plethora of electron donors and acceptors.

Chemolithoautotrophy, which is the ability to fix inorganic carbon using energy derived from reduced inorganic compounds, is more widespread in the water column of the global ocean than assumed until a decade ago. The largest group of chemolithoautotrophs in the ocean is the ammonia oxidizing Thaumarchaea (Herndl et al. 2005; Francis et al. 2005; Wuchter et al. 2006), with chemoautotrophic bacteria such as Nitrospinae also playing a major role (Pachiadaki et al. 2017; Zhang et al. 2020). Apart from the oxidation of ammonia and nitrite, sulfur oxidation also serves as an energy source in the oxygenated water column in e.g. SAR324 (Deltaproteobacteria), ARCTIC96BD-19 and Agg47 (Gammaproteobacteria) as well as Oceanospirillales (Swan et al. 2011). In addition, sulfur compounds are oxidized by some microorganisms combined with denitrification in a process called sulfur-driven autotrophic denitrification or anaerobic sulfur oxidation (Shao et al. 2010).

Their enormous diversity and rapid evolutionary adaptation enable microorganisms to thrive also in the most extreme of environments, like hypersaline waters with a salinity of > 270 (Oren 2002) as measured in deep sea brine pools (Anschutz and Blanc 1996). These high salt concentrations are formed by dissolving salt from geological salt deposits in the seafloor occasionally in combination with the release of hydrocarbons and reduced sulfur components (Cita 2006; Antunes et al. 2011). Despite the high salinity, brine pools harbor a diverse microbial community of Bacteria, Archaea and different eukaryotes such as fungi (Wielen et al. 2005; Bernhard et al. 2014). Salt brines occur throughout different depths and have been found in the Mediterranean Sea as well as the Red Sea (Dando et al. 1999; Danovaro et al. 2005; Antunes et al. 2011).

The Red Sea is a peculiar marginal sea, characterized by high solar irradiance, temperature and evaporation. All these factors lead to higher salinity in surface waters than in the global ocean. These environmental parameters make the Red Sea an interesting site for global change studies, with these environmental stressors also becoming increasingly important in other regions of the global ocean for microbial as well as macrobial organisms (Stambler 2005; Edwards 2013). The

large number of brine pools in the Red Sea, however, has been studied only recently (Antunes et al. 2011; Zhang et al. 2014, 2015, 2016a).

One way in which microorganisms cope with the stressors in brine pools is biofilm formation, which has been studied in the Thuwal areas cold seep II in the Red Sea (Wang et al. 2014; Zhang et al. 2014, 2015). It has been shown that a strong selection pressure drives microbial communities into forming biofilms (Zhang et al. 2014). One of the key organisms found there is *Sulfurimonas*, a chemolithoautotrophic Deltaproteobacterium utilizing sulfur components as an electron donor (Zhang et al. 2014). Furthermore, it was found that also nitrite is used as an electron acceptor in brine pools and that this process is coupled with the fermentation of polysaccharides in these anaerobic microenvironments (Zhang et al. 2015).

This finding has been confirmed in a further study using metagenomically assembled genomes (MAG), in which contigous assembled DNA sequences (contigs) are grouped into artificial organisms enabling the allocation of functions to specific organisms. With this approach, novel carbohydrate fermentation genes were found in *Cloacimonetes* and *Marinimicrobia* (Zhang et al. 2016a). Also, evidence has been found of an almost complete acetate fermentation pathway in a sulfate reducing *Bathyarchaeota*, further supporting a link between sulfate and nitrate reduction with polysaccharide fermentation (Zhang et al. 2016a)

The aim of this study was to identify and compare key microorganisms as well as characterize their functional genetic repertoire in particle-associated microbial assemblages and the surrounding free-living community of a Red Sea brine pool. We used a metagenomic binning approach to identify distinct organisms and examine their potential to utilize sulfur compounds as well as the major metabolic pathways.

Material & Methods

Sampling

Sampling was performed during a research cruise in the eastern Red Sea at two sampling stations in the area around the Thuwal cold seeps as well as at a location 550-600km further southeast (Table 1). One sample was taken at the interface between the surrounding deep water and the brine pool (BP) at 853m depth in the Thuwal area (Table 1). Another sample was taken at the southern station at a depth of 209m (Mesopelagic water; MEW) in the vicinity of an oil seep and the third sample from the adjacent deep water of the brine pool at a depth of 930m (ADW) (Table 1). The temperature at all sampling stations ranged between 21.7°C and 22.0°C as commonly measured in the Red Sea below the euphotic layer.

One hundred L of the sampled water of each station, were filtered on board the *RV Thawul 2438*, sequentially through 3.0 µm, 0.8 µm and 0.2 µm polycarbonate filters (142mm filter diameter, Millipore) using a peristaltic pump. Due to a failing filter frame, there was no 3.0 µm microbial community fraction collected from the adjacent deep water (930m). Therefore the 0.8 µm filter contained all the microbes and particles > 0.8 µm. The filters were then stored at -80°C for further analyses in the laboratory.

Due to a calibration error, the oxygen measurements are not available from this research cruise but oxygen data are used from the literature in the further analyses.

Table 1. Metadata of the sampled water masses

	Date	Longitude (°N)	Latitude (°E)	Depth (m)	Temperature (°C)	Salinity
Brine pool	14/04/2017	38.89141	22.28481	853m	21.7	59-63
Sub-euphotic water	16/04/2017	41.43315	17.64909	209m	22.0	37-40
Adjacent deep water	19/04/2017	38.79145	22.23845	930m	21.7	40

The sequence files for the metagenomic analysis were provided by the Red Sea Research Center of the King Abdulah University of Science and Technology (KAUST; Saudi Arabia). The extraction was done with the phenol-chloroform method, modified for filters (Sambrook and Russell 2006). The resulting nucleic acid was then purified using a column-based method and the library preparation was done using NuGen-Ovation (Head et al. 2018). The actual sequencing was done using paired-end Illumina sequencing on an Illumina 2500. The base calling and pre-processing of the resulting reads were performed by KAUST and the data provided in FASTQ format. For the genomic analysis, the quality of the reads was checked using the program fastqc and no trimming had to be applied.

Diversity estimates

To estimate the diversity of the individual samples, we used the indexed non-redundant (nr) database (mOTU.v2b.nr.padded) provided by the tool mOTU. This database contains a collection of household genes whose diversity in a sample, according to previous research, closely reflects the diversity of the community composition (Sunagawa et al. 2013; Milanese et al. 2019).

The reads of each individual sample were aligned to the database using the bwa mem algorithm (Li 2013). The resulting coverage was then converted to reads per kilobase million (RPKM) using the pileup.sh script from the bbmap tool set (Bushnell 2014).

The results were then combined into one matrix with the abundances of all mOTU units and using the libraries rtk and vegan of the programming language R. The Shannon diversity index, richness and evenness of the individual samples were calculated.

Binning workflow

The reads from the size-fractions were concatenated and co-assembled for each individual station. The assembly was done with the program MEGAHIT (1.1.2) using its default parameters (Li et al. 2015, 2016).

To determine the coverage of the assembly, the reads were aligned with the contigs using the bbwrap.sh script from the BBmap (37.61) tool set (Bushnell 2014), which provided an alignment in a gzip compressed sam-format.

The alignment was then analyzed using the pileup.sh script from the same tool set, revealing the coverage as well as the hits per contig in a normalized format. The normalization was done by the program using the following formula to calculate the fragments per kilobase million (FPKM).

$$FPKM = \frac{FragmentsMapped}{\frac{ContigLength}{1000} * \frac{AllFragments}{1{,}000{,}000}}$$

The fragments presented here are the combined sequences from the forward and reverse reads. The FPKM represents the amount of fragments mapped to a contig for each 1000 bp of the contigs length and for each 1,000,000 fragments sequenced in total.

To group the contigs into bins, MaxBin (2.2.4) and MetaBat (2.12.1) were used and the results were combined with the metawrap pipeline (1.0.5) (Wu et al. 2014, 2016; Kang et al. 2015; Uritskiy et al. 2018).

Maxbin requires abundance estimates of all contigs since it uses differential coverage in the binning process. These abundances were extracted from the output of pileup using awk and saved in an individual file for each size fraction. These files together with the contigs of the metagenome

were transferred to the runMaxBin.pl script, which grouped the contigs into bins using otherwise default parameters.

Metabat also uses differential coverage but requires a different input format. To create the required depth summary, the alignment was converted to bam-format and sorted using the program samtools. The sorted alignment files were then analyzed using jgi with the *--outputDepth* parameter to obtain the sequencing depth in a text file. Using the calculated contig depth as well as the contigs, metabat was then used to group the contigs into bins.

To combine the results of the two binning approaches we used the bin_refinement module from the metawrap pipeline (1.0.5) (Uritskiy et al. 2018). The thresholds of the program were set to > 50% completion and < 10% contamination of the genome and the binning results of both metabat and maxbin were provided to the program in FASTA-format.

Taxonomic classification

The resulting bins were then classified taxonomically using the program gtdbtk, which aligns the individual genomes against a reference tree constructed from the genome taxonomy database (gtdb). The program can thereby assign the taxonomy of Bacteria as well as Archaea using a total of 120 marker genes. For the classification, we used the classify_wf workflow with otherwise default parameters (Parks et al. 2018).

Functional annotation

For the functional annotation, the bins were further improved in quality by reassembling them from their reads using the reassemble_bins module of the metawrap pipeline. The parameters of the module were set in a way that all bins with a completeness of more than 50% and a contamination below 10% were included along with the minimum contig length in the reassembled bins of 500 basepairs. The program was then provided with the refined bins as well as one read file of all size fractions concatenated together for forward and reverse reads.

The resulting re-assembled bins were then analyzed in terms of quality and all bins with a completeness of < 70% as well as a contamination of > 5% were excluded from further analysis to obtain reliable, more conservative results.

To assign a functional repertoire to the individual bins, predicted genes were annotated by the KEGG and EggNOG database. For the gene prediction, the program prodigal (Hyatt et al. 2010) was used which was provided with the bins selected in the previous step and returned the nucleotide and protein sequences of the predicted genes in fasta-files, as well as the gene positions in a gff-file.

The predicted genes of the individual bins were searched against the eggNOG protein database in a diamond (dmnd) format using the emapper.py script with the *-m* Flag set to diamond.

Using the same tool, the resulting output file was parsed using the *--annotate_hits_table* Flag which resulted in an output file with all the accession numbers from the KEGG database (KO-numbers) which were found in each individual bin (Huerta-Cepas et al. 2017).

Abundance analysis

To compare the abundance of all bins across all samples, the bins were clustered together using the dereplicate_wf workflow of the program dRep with its parameters left at default to remove redundancy (Olm et al. 2017).

For exact identification, the bins were given a unique identifier. This consisted of an abbreviation of the environment where they were originally recovered from (BP: brine pool; MEW: mesopelagic water; ADW: adjacent deep water) followed by a unique index for that environment (e.g. BP_22).

The resulting dereplicated bins were then analyzed in terms of their abundance using the metawrap quant_bins module provided with the dereplicated bins as well as a folder with all reads across all stations. The output of the module provides the average contig abundance of each bin per sample, weighted by the length of the contigs.

$$abundance_{Bin} = \frac{\sum_{all\,contigs} \frac{abundance}{length}}{number\ of\ contigs}$$

The results from gtdbtk, emapper.py and metawrap bin_abundance were then imported into R, merged, analyzed and visualized using the tidyverse packages as well as pheatmap and ggplot2.

Results

Physical and chemical conditions

Among the environmental parameters of the water masses (Table 1) salinity was varying most among the stations. The salinity varied between 37 and 40 in the waters outside the brine pool while the brine pool exhibited a salinity of 59-63. The brine pool and adjacent deep water samples were taken at similar depths of 853 and 930 m respectively, while the mesopelagic water sample was taken at 209 m depth just below the euphotic zone. The water temperature of all the stations was essentially constant varying only between 21.7°C and 22.0°C.

Due to a malfunctioning oxygen sensor, oxygen measurements are not available. Measurements from a previous study at the same brine pool (Wang et al. 2014) were used to interpret the analyses described below.

Comparison of the microbial diversity among the sampling sites

The diversity estimates based on mOTUs showed a fairly consistent pattern across the stations, with Shannon diversity indices ranging from 8.65 to 10.51 (Table 2). The rarefaction curve of the analysis shows that all samples reached a plateau when rarefying them to the sample with the lowest number of reads (9.8 million) (Fig. 1), which means that all of the diversity estimates can be compared between the different size fractions and stations.

The lowest Shannon diversity index was found in the particle-associated size fraction > 3.0 µm in the brine pool and the medium size fraction 0.8 µm - 3.0 µm exhibited the highest diversity of all samples. While the Shannon diversity indices of the microbial community in the brine pool varied between the different size fractions, the Shannon diversity indices of the mesopelagic waters and the adjacent deep water varied only marginally (Table 2).

Table 2. mOTU based alpha-diversity estimation

mOTU based alpha-diversity estimation of each size fraction from the brine pool (BP), mesopelagic water (MEW) and adjacent deep water (ADW)

	BP 0.2µm	BP 0.8µm	BP 3.0µm	MEW 0.2µm	MEW 0.8µm	MEW 3.0µm	ADW 0.2µm	ADW 0.8µm
Shannon diversity	9,9402	10,2382	8,6524	10,5083	9,8486	10,2941	9,2430	9,9203
Richness	94516,2	95108,0	95135,8	95097,1	94834,4	95184,8	94920,0	94884,0
Eveness	0,8676	0,8932	0,7548	0,9167	0,8594	0,8980	0,8065	0,8656

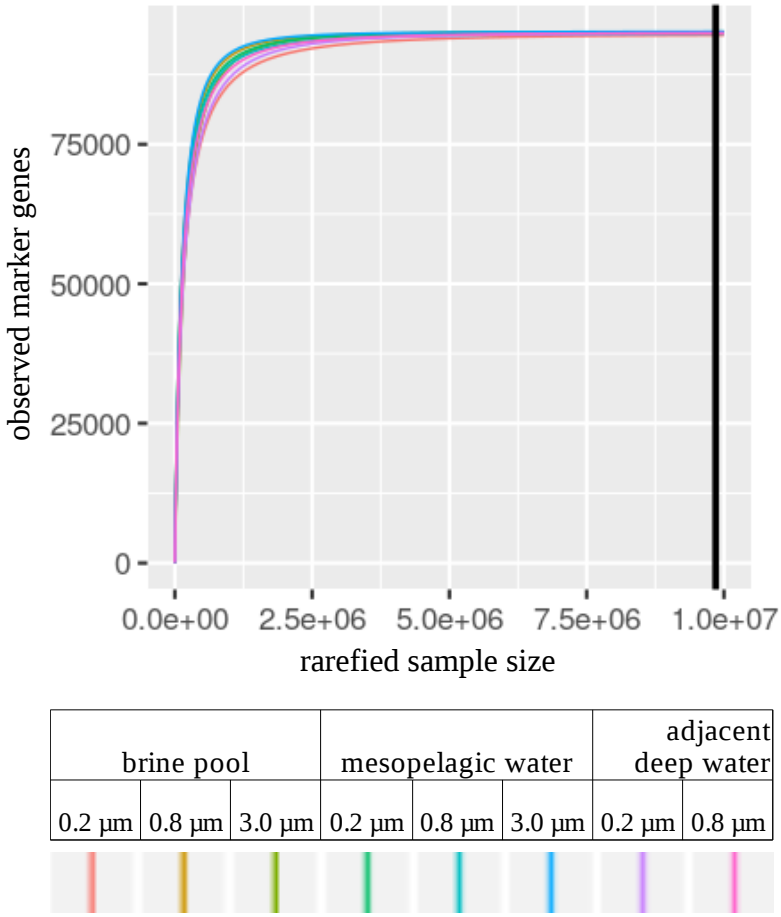

Figure 1 Rarefaction curve of the mOTU diversity estimate with the number of observed marker genes on the y-axis and the rarefied sample size (in reads) on the x-axis. The black line marks the sample size (number of reads) of the smallest sample.

Abundant microbes in the brine pool

Our binning approach resulted in a total of 84 bins (Table 3) with a completeness > 70% and a contamination < 10% (only 3 bins had a contamination of > 5%). All these 84 bins (3 Archaea, 81 Bacteria) grouped into 18 phyla, which consisted of 26 classes, 38 orders and 44 families.

The relative abundance (reads per million, RPM) of the bins at the phylum level distinguished the dominant phyla of the different environments (Fig. 2). The Gammaproteobacteria were the most dominant in all the samples except in the particle-associated microbial community (> 3.0 µm) and the medium size fraction in the brine pool, where Cloacimonadota and Desulfobacterota were the most abundant phyla. Other phyla like the Marinisomatota, Bacteroidota and Fermentibacterota showed higher abundances in the brine pool than at the outside brine pool sites. Additionally, the brine pool microbiota showed a distinct distribution pattern across the different size fractions. Gammaproteobacteria, Alphaproteobacteria and Campylobacterota were more abundant in the free-

living fraction than in the particle-associated fractions. Free-living Alphaproteobacteria and Campylobacterota were also more abundant in the brine pool than outside of the brine pool sites.

In the mesopelagic waters, highest abundances were recorded for the Gammaproteobacteria followed by Crenarchaeota, Actinobacteria, Marinisomatota and SAR324 in the 0.2 µm – 0.8 µm size fraction and Planctomycetota, Myxococcota, Crenarchaeota and Actinobacteriota for the 0.8 µm – 3.0 µm and > 3.0 µm size fraction.

In the adjacent deep-water sample, the most abundant phyla were Gammaproteobacteria, Actinobacteriota and Marinisomatota from the 0.2 µm – 0.8 µm size fraction and Gammaproteobacteria and Alphaproteobacteria for the > 0.8 µm size fraction.

Figure 2. Sum of the abundances of the bins from all recovered phyla in reads per million base pairs, displayed across all size fractions from the brine pool (BP), mesopelagic water (MEW) and adjacent deep water (ADW)

Table 3. Completeness, contamination and taxonomy of the filtered bins

Bin name	Compl. (%)	cont. (%)	Kingdom	Phylum	Class	Order	Family	Genus	Species
MEW_25	86,49	1,94	Archaea	Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrosopumilaceae	Nitrosopelagicus	unknown
BP__68	94,17	3,88	Archaea	Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrosopumilaceae	Nitrosopumilus	GCF_001541925.1
ADW_21	86,80	1,60	Archaea	Thermoplasmatota	MGII	MGIII	CG-Epi1	CG-Epi1	unknown
BP__32	94,99	0,00	Bacteria	AABM5-125-24	AABM5-125-24	AABM5-125-24	AABM5-125-24	unknown	unknown
ADW_26	94,01	4,27	Bacteria	Actinobacteriota	Acidimicrobiia	Microtrichales	MedAcidi-G1	S20-B6	unknown
BP__16	93,16	1,28	Bacteria	Actinobacteriota	Acidimicrobiia	Microtrichales	MedAcidi-G1	UBA3125	GCA_002687745.1
MEW_20	90,33	3,85	Bacteria	Actinobacteriota	Acidimicrobiia	Microtrichales	MedAcidi-G1	UBA9410	unknown
MEW_35	77,37	0,43	Bacteria	Actinobacteriota	Acidimicrobiia	Microtrichales	MedAcidi-G1	UBA9410	GCA_002717365.1
MEW_33	92,92	2,99	Bacteria	Actinobacteriota	Acidimicrobiia	Microtrichales	TK06	MedAcidi-G3	GCA_002721305.1
BP__25	94,01	0,43	Bacteria	Actinobacteriota	Acidimicrobiia	UBA5794	UBA5794	UBA5794	unknown
MEW_06	96,68	3,04	Bacteria	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	Cutibacterium acnes
BP__67	90,86	3,38	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	4484-276	unknown	unknown
BP__31	96,23	2,15	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	ML635J-15	unknown	unknown
BP__22	95,93	1,22	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Arcobacteraceae	unknown	unknown
BP__27	85,97	0,81	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Arcobacteraceae	unknown	unknown
BP__05	80,87	5,74	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Sulfurovaceae	Sulfurovum	unknown
MEW_13	90,54	2,19	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Sulfurovaceae	Sulfurovum	unknown
BP__10	100,00	1,43	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	unknown
BP__36	81,07	2,73	Bacteria	Chloroflexota	Anaerolineae	Anaerolineales	envOPS12	unknown	unknown
ADW_18	85,08	2,97	Bacteria	Chloroflexota	Dehalococcoidia	SAR202	UBA11138	UBA1123	unknown
ADW_07	87,78	0,99	Bacteria	Chloroflexota	Dehalococcoidia	UBA3495	UBA3495	UBA1141	unknown
BP__45	91,20	1,10	Bacteria	Cloacimonadota	Cloacimonadia	Cloacimonadales	TCS61	unknown	unknown
BP__01	92,24	1,65	Bacteria	Cloacimonadota	Cloacimonadia	Cloacimonadales	TCS61	SDB	unknown
BP__37	83,87	0,10	Bacteria	Cloacimonadota	Cloacimonadia	Cloacimonadales	TCS61	TCS61	unknown
BP__43	70,21	0,10	Bacteria	Cloacimonadota	Cloacimonadia	Cloacimonadales	TCS61	TCS61	TCS61 sp1
BP__12	98,90	0,00	Bacteria	Cloacimonadota	Cloacimonadia	JGIOTU-2	unknown	unknown	unknown
BP__04	78,57	0,00	Bacteria	Cloacimonadota	Cloacimonadia	JGIOTU-2	JGIOTU-2	JGIOTU-2	unknown
BP__30	96,88	0,00	Bacteria	Delongbacteria	UBA4055	unknown	unknown	unknown	unknown
BP__61	91,61	2,58	Bacteria	Desulfobacterota	Desulfobacteria	Desulfatiglanales	NaphS2	unknown	unknown
BP__72	77,67	1,29	Bacteria	Desulfobacterota	Desulfobacteria	Desulfatiglanales	NaphS2	NaphS2	unknown
BP__23	87,61	1,00	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacula	unknown
BP__66	96,42	2,38	Bacteria	Desulfobacterota	Desulfobulbia	Desulfobulbales	Desulfocapsaceae	unknown	unknown
BP__69	100,00	1,47	Bacteria	Desulfobacterota	Desulfobulbia	Desulfobulbales	Desulfocapsaceae	Desulfocapsa	unknown
BP__51	90,29	1,10	Bacteria	Fermentibacterota	Fermentibacteria	Fermentibacterales	Fermentibacteraceae	unknown	unknown
BP__65	84,45	1,85	Bacteria	Fermentibacterota	Fermentibacteria	Fermentibacterales	Fermentibacteraceae	unknown	unknown
ADW_34	84,53	2,26	Bacteria	Gemmatimonadota	Gemmatimonadetes	SG8-23	UBA6960	UBA887	UBA887 sp2
BP__11	91,70	1,20	Bacteria	Marinisomatota	AB16	AB16	TCS52	TCS52	GCA_001577215.1
BP__42	79,31	0,00	Bacteria	Marinisomatota	Marinisomatia	unknown	unknown	unknown	unknown
MEW_07	82,41	0,10	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	Marinisomataceae	Marinisoma	unknown
MEW_49	84,06	3,85	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	S15-B10	S15-B10	S15-B10 sp1
MEW_24	85,52	1,65	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	TCS55	unknown	unknown
MEW_26	85,71	0,00	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	TCS55	UBA2126	unknown
BP__33	92,84	0,55	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	UBA8229	unknown	unknown
BP__41	89,49	2,26	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	UBA8229	unknown	unknown
ADW_25	95,60	1,10	Bacteria	Marinisomatota	Marinisomatia	Marinisomatales	UBA8229	AB-629-J13	unknown
BP__59	90,10	0,06	Bacteria	Marinisomatota	Marinisomatia	SCGC-AAA003-L08	SCGC-AAA003-L08	unknown	unknown
ADW_09	79,82	2,29	Bacteria	Myxococcota	UBA4248	UBA4248	unknown	unknown	unknown
MEW_04	91,93	2,26	Bacteria	Myxococcota	UBA9160	UBA9160	unknown	unknown	unknown
MEW_27	90,64	1,97	Bacteria	Myxococcota	UBA9160	UBA9160	unknown	unknown	unknown
MEW_01	78,81	2,84	Bacteria	Myxococcota	UBA9160	UBA9160	UBA4427	unknown	unknown
MEW_03	91,23	1,51	Bacteria	Myxococcota	UBA9160	UBA9160	UBA9160	unknown	unknown
MEW_14	94,44	0,00	Bacteria	Planctomycetota	Planctomycetes	Planctomycetales	Planctomycetaceae	unknown	unknown
MEW_46	91,28	1,08	Bacteria	Planctomycetota	UBA1135	UBA1135	GCA-002686595	GCA-2720975	unknown
MEW_09	93,54	1,08	Bacteria	Planctomycetota	UBA8108	UBA1146	UBA1146	unknown	unknown
MEW_23	92,88	1,08	Bacteria	Planctomycetota	UBA8108	UBA1146	UBA1146	UBA1146	unknown
MEW_30	97,78	4,30	Bacteria	Planctomycetota	UBA8108	UBA8108	UBA8108	unknown	unknown
BP__17	77,20	2,58	Bacteria	Proteobacteria	Alphaproteobacteria	Oceanibaculales	unknown	unknown	unknown
BP__09	93,10	2,67	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unknown	unknown
BP__58	98,48	0,77	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unknown	unknown
BP__26	76,17	4,31	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Shimia	unknown
ADW_15	99,54	0,60	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Thiobacimonas	Thiobacimonas profunda
ADW_06	84,21	1,73	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Erythrobacter_A	unknown
ADW_04	97,60	0,59	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	Alteromonas_A	GCA_002335925.1
BP__74	80,15	7,29	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	Alteromonas	Alteromonas macleodii
ADW_53	86,71	6,11	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	Alteromonas	Alteromonas macleodii
BP__35	76,11	2,44	Bacteria	Proteobacteria	Gammaproteobacteria	GCA-2400775	unknown	unknown	unknown
BP__64	94,63	0,57	Bacteria	Proteobacteria	Gammaproteobacteria	Halothiobacillales	Halothiobacillaceae	WRN-7	unknown
ADW_14	95,77	0,18	Bacteria	Proteobacteria	Gammaproteobacteria	Nitrosococcales	Methylophagaceae	Methylophaga	unknown
ADW_23	79,65	0,34	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Hahellaceae	Marinobacter	unknown
MEW_12	75,86	0,00	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Hahellaceae	Marinobacter	Marinobacter lutaoensis
MEW_37	92,79	1,02	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Nitrincolaceae	Neptuniibacter	unknown
MEW_39	81,03	8,62	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Nitrincolaceae	Neptuniibacter	Neptuniibacter caesariensis
MEW_29	90,89	4,86	Bacteria	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	unknown	unknown	unknown
BP__03	93,29	2,44	Bacteria	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Hydrogenovibrio	unknown
BP__19	92,48	0,71	Bacteria	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrorhabdus	unknown
BP__44	95,77	0,10	Bacteria	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrorhabdus	unknown
BP__60	94,24	0,51	Bacteria	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrorhabdus	unknown
ADW_30	89,63	3,05	Bacteria	Proteobacteria	Gammaproteobacteria	UBA10353	LS-SOB	REDSEA-S09-B13	REDSEA-S09-B13 sp1
BP__56	85,97	3,20	Bacteria	Proteobacteria	Gammaproteobacteria	UBA10353	LS-SOB	UBA5682	unknown
ADW_03	91,76	3,42	Bacteria	Proteobacteria	Gammaproteobacteria	UBA10353	LS-SOB	UBA5682	unknown
BP__40	94,11	0,00	Bacteria	Proteobacteria	Magnetococcia	unknown	unknown	unknown	unknown
BP__54	86,49	0,17	Bacteria	SAR324	SAR324	SAR324	NAC60-12	unknown	unknown
BP__55	88,74	0,00	Bacteria	SAR324	SAR324	SAR324	NAC60-12	Arctic96AD-7	Arctic96AD-7 sp3
BP__34	85,35	2,25	Bacteria	Wallbacteria	unknown	unknown	unknown	unknown	unknown

We focused more closely on the dominant microbial groups which differentiated the brine pool microbiota. The bins from the Bacteroidota, Campylobacterota, Cloacimonadota, Delongbacteria, Desulfobacterota, Fermentibacterota and Marinisomatota, Alphaproteobacteria and Gammaproteobacteria were selected for the following functional analysis.

Microbial Carbon fixation

Key genes for the reverse tricarboxylic acid cycle (rTCA) are ATP-citrate lyase (*aclA*, *aclB*) and the oxalate oxidoreductase (*oorA*), while the key genes for the Calvin-Benson-Bassham cycle (CBB) are the large and small subunit of ribulose-1-5-bisphosphate-carboxylase/Rubisco (*cbbL*, *cbbM*). The presence and absence of these genes in each of the individual bins according to the KEGG annotation were used to determine their potential for microbial carbon fixation (Fig. 3).

Four out of five Campylobacterota bins (BP_22, BP_27, BP_05, MEW_13) had at least two of the three rTCA marker genes and no cbbL/M present, with the remaining one (BP_10) harbored both cbbL and cbbM but no aclA/B. Other organisms with at least two of the three rTCA marker genes but no cbbM/L genes were affiliated with the Bacteroidota (BP_31), Cloacimonadota (BP_01), Desulfobacterota (BP_72) and Marinisomatota (ADW_15).

Beside the one Campylobacterota bin, there were four Alphaproteobacteria (BP_17, BP_09, BP_58, ADW_15) and six Gammaproteobacteria (BP_35, BP_64, MEW_29, BP_19, BP_56, ADW_03) which had at least one subunit of Rubisco in their genome.

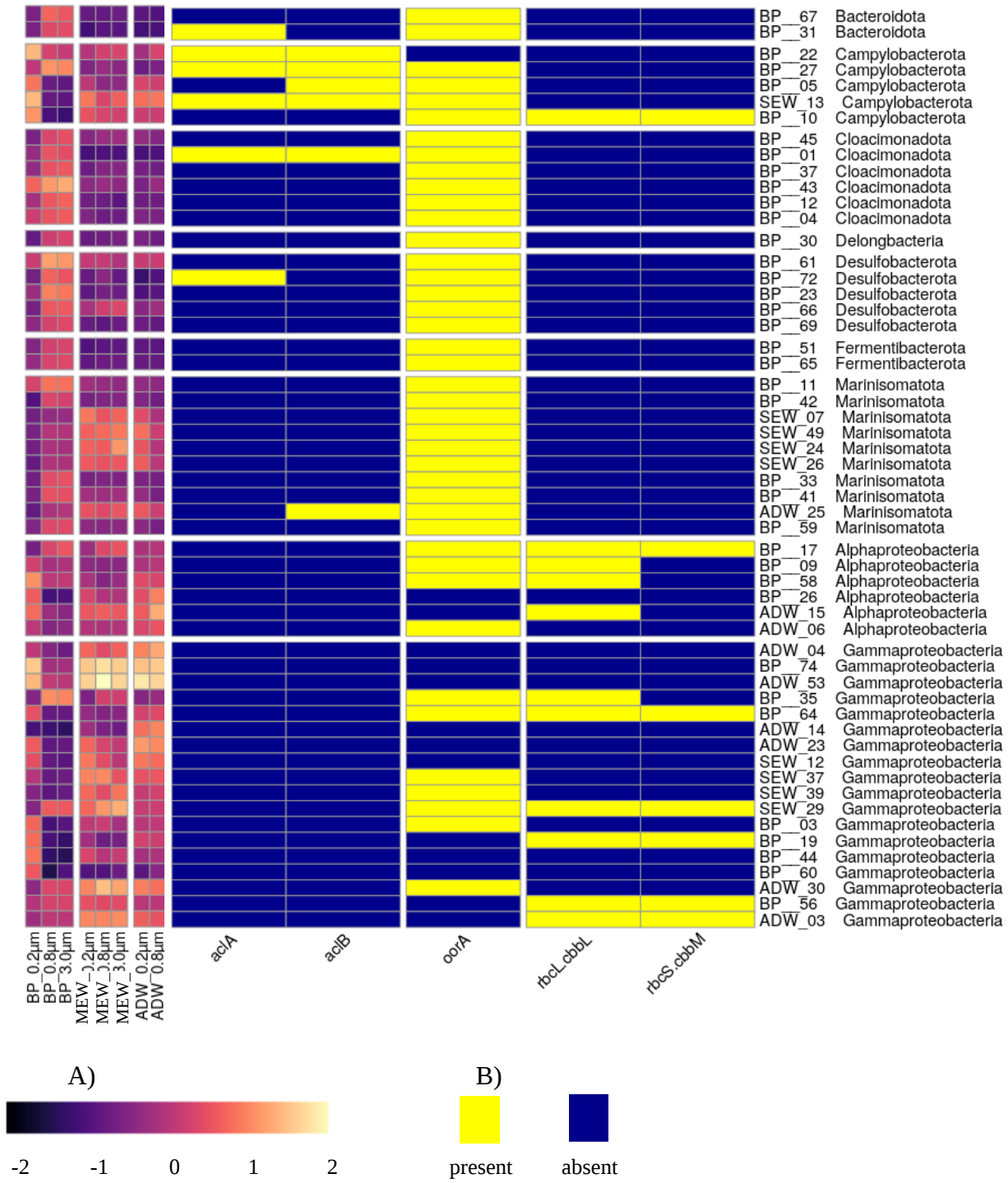

Figure 3. (A) Small heatmap of bin abundance normalized per row using the z-score and (B) large heatmap showing the presence and absence of microbial carbon fixation related genes in the respective bins

Carbohydrate fermentation

To assess the potential of the bins for carbohydrate utilization, the genes of the different categories of the carbohydrate active enzymes (CAZymes) in each bin were normalized to the total predicted number of functional genes in the respective bin (Fig. 4).

The enzymes grouped into six categories. The first group was glycosyltransferases (GT) which form glycosidic bonds and are therefore used in the formation of carbohydrates. The next are glycoside hydrolases (GH) hydrolyzing glycosidic bonds, polysaccharide lyases (PL) for non-hydrolytic cleavage of glycosidic bonds and carbohydrate esterases (CE) for cleavage of ester bonds. These three categories are all used for cleavage and are therefore important for the degradation of carbohydrate compounds. The last two categories are auxiliary activities (AA), which often work together with other CAZymes as well as carbohydrate binding modules (CBM) promoting the binding of carbohydrates to enzyme complexes and enable degradation as well as assemblage of carbohydrates.

Generally, only a low number of CAZymes across all CAZyme categories were found in the Campylobacterota bins, which are the most dominant phylum in the free-living microbial fraction of the brine pool community. In the Cloacimonadota and Desulfobacterota bins, which were very dominant in the two particle-associated size fractions of the brine pool, an opposite trend was observed, with the Cloacimonadota having a high number of glycoside hydrolases, polysaccharide lyases and carbohydrate binding modules and a low number of genes assigned to carbohydrate esterases, glycosyltransferases and auxiliary activities. Contrary to that, the Desulfobacterota exhibited a low number of polysaccharide lyases, carbohydrate binding modules and glycoside hydrolases category genes and a high number of carbohydrate esterases and glycosyltransferases related genes (Fig. 4).

The Fermentibacterota had average numbers of CAZyme genes, except for the glycosyltransferases category where a higher than average number of genes was detected in both of the two bins from this phylum.

The phylum Marinisomatota exhibited a variable number of CAZyme genes in the respective bins and categories with high relative numbers of genes in the categories polysaccharide lyases, carbohydrate binding modules, glycoside hydrolases and carbohydrate esterases and low to average relative numbers in the categories glycosyltransferases and auxiliary activities (Fig. 4).

The Alphaproteobacteria had average to high relative numbers of glycosyltransferases and auxiliary activities and low to average in the other categories.

Gammaproteobacteria also displayed a large variation in the relative numbers of CAZyme genes. There was a negative relationship between the number of genes from carbohydrate binding modules and glycoside hydrolases detected in a bin and the bin abundance in the brine pool particle-associated microbes. Bins obtained from the free-living bacterial fraction, like BP_35, MEW_29, ADW_29, BP_56 and ADW_03 exhibited low numbers of carbohydrate binding modules and glycoside hydrolase genes compared to the other gammaproteobacterial bins.

Figure 4. Heatmap of bin abundance normalized per row using the z-score (A) and (B) heatmap showing the number of genes of a category divided by the total number of predicted genes in a bin and normalized per column using the z-score. The visualized categories are the polysaccharide lyases (PL), carbohydrate binding modules (CBM), glycoside hydrolases (GH), carbohydrate esterases (CE), glycosyltransferases (GT) and auxiliary activities (AA).

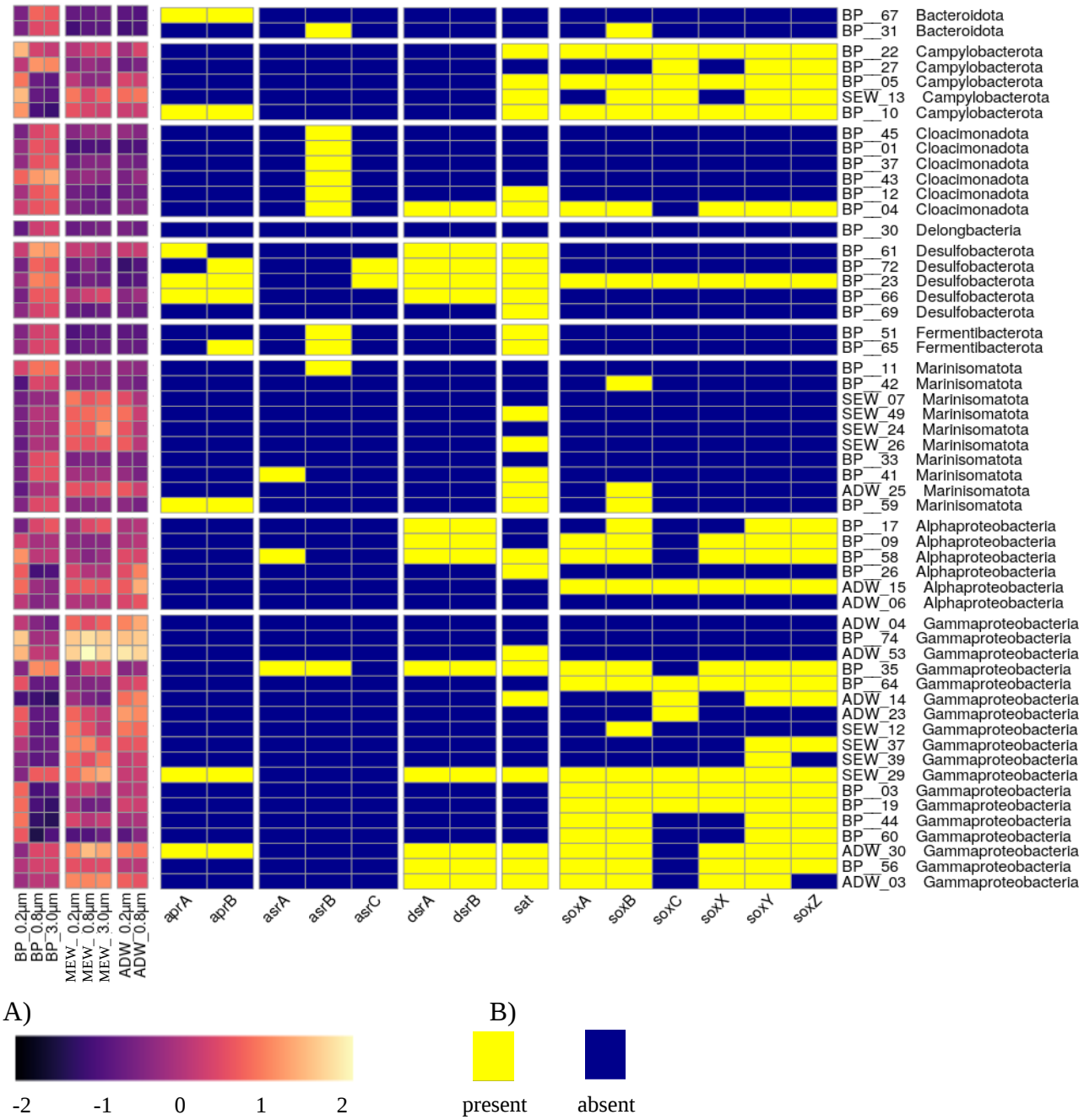

Figure 5. Heatmap of bin abundance normalized per row using the z-score (A) and (B) heatmap showing the presence and absence of sulfur metabolism-related genes in the respective bins

Sulfur oxidation

The sulfur oxidation related genes (Fig. 5), L-cysteine S-thiosulfotransferase (*sox*A, *sox*X), S-sulfosulfanyl-L-cysteine sulfohydrolase (*sox*B), sulfane dehydrogenase (*sox*C) and two other sulfur-oxidizing genes (*sox*Y, *sox*Z) were found mostly in three phyla, Campylobacterota, Alphaproteobacteria and Gammaproteobacteria. Additionally, there was one bin in the Desulfobacterota (BP_23) and one in the Cloacimonadota (BP_04), which contained all or most of the *sox* genes.

Sulfate reduction

The reductive sulfur metabolism of the microbial communities was examined by analyzing the genes encoding adenylylsulfate reductase (*apr*), anaerobic sulfate reductase (*asr*), dissimilatory sulfate reductase (*dsr*) and sulfate adenylyltransferase (*sat*), which are all involved in the reduction of sulfate and other sulfur compounds (Fig. 5).

The key genes for sulfate reduction, *dsr*A and *dsr*B were present in the Desulfobacterota, as well as the Alpha- and Gammaproteobacteria and additionally, in one bin of the phylum Coloacimonadota (BP_04). *Dsr* genes were detected in four out of the five Desulfobacterota bins and all of these four bins (BP_61, BP_72, BP_23, BP_66) also had at least one of the *apr*A and *apr*B genes as well as *sat*, while Desulfobacterota BP_69 only had *sat*. The only other organisms with all these three genes were two gammaproteobacterial bins (MEW_29, ADW_30) while no other gammaproteobacterial bins with both *dsr*A/*dsr*B and *sat* had *apr*A or *apr*B. In terms of abundance, gammaproteobacterial bins which contained *dsr*A, *dsr*B and *sat* genes were relatively more abundant in the brine pool than in the other environments.

Alphaproteobacteria had only one bin containing *dsr*A, *dsr*B and *sat* while *apr*A and *apr*B were not detected in this class.

The *asr* genes, *asr*A, *asr*B and *asr*C, were not observed together in any single analyzed organism, with only one gammaproteobacterial bin containing two of the sub units (BP_35; *asr*A, *asr*B). Additionally, the *asr*B gene was detected in all of the Cloacimonadota.

Denitrification

To assess the potential for sulfur driven denitrification as well as heterotrophic denitrification, the genes for periplasmatic nitrate reductase (napA, napD, napE) and (ferredoxin-) nitrate reductase (narB, narG/Z, narH/Y, narI/V, narJ/W) were analyzed (Fig. 6).

The two phyla Campylobacterota and Desulfobacterota contained the two genes napA and narB, except for the Desulfobacterota BP_23 lacking the *nap*A gene. Apart from that there was only one bin from the Marinisomatota, one from the Alphaproteobacteria and three from the Gammaproteobacteria which contained both, the *nap*A and *nar*B genes.

Nitrogen fixation

The genes for the nitrogenase molybdenum-iron protein (nifD, nifH, nifK) used for nitrogen fixation was only detected in the Campylobacterota BP_05 (Fig. 6), where all three genes of the different sub units of the enzyme were present.

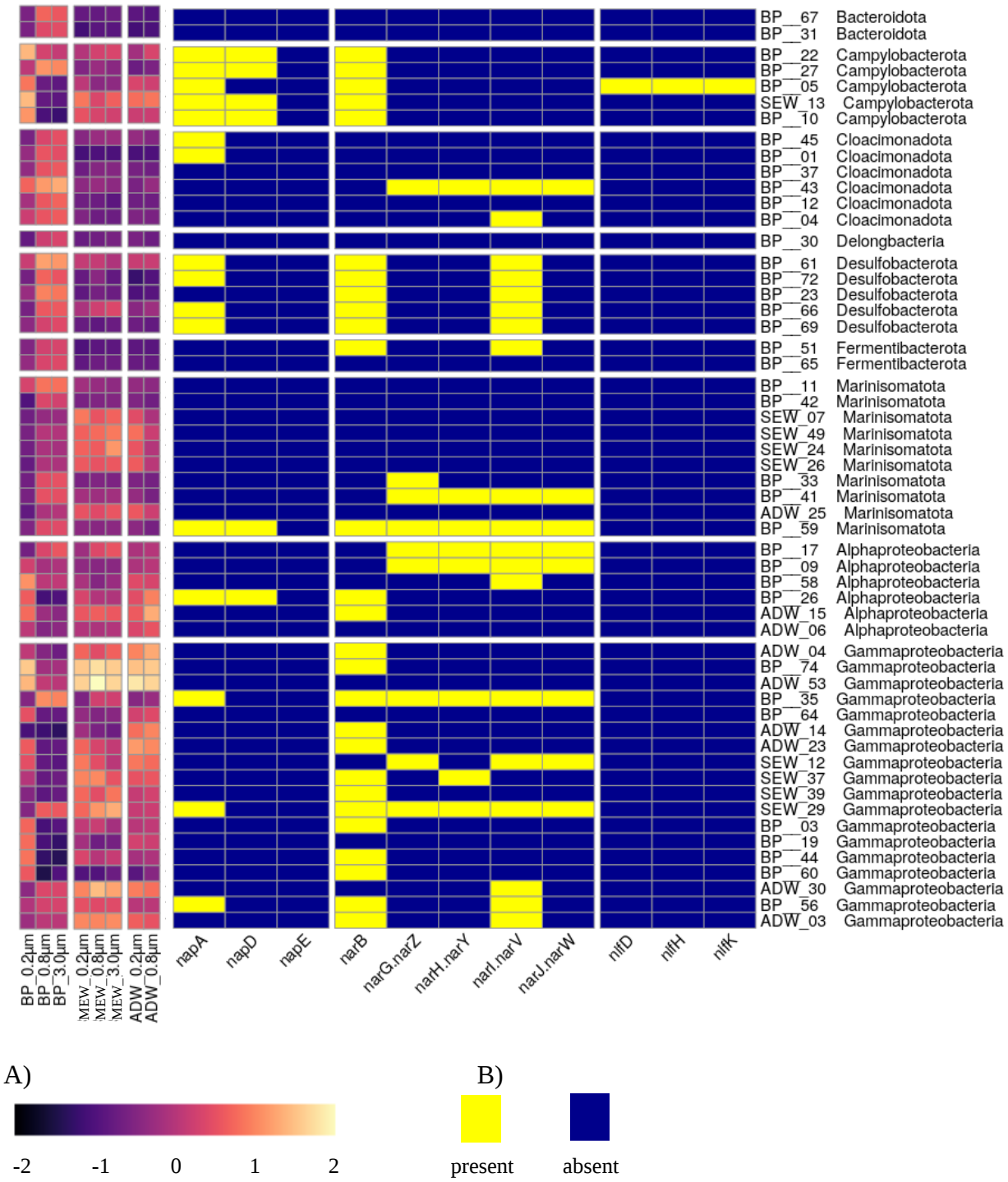

Figure 6. (A) Heatmap of bin abundance normalized per row using the z-score and (B) heatmap showing the presence and absence of nitrogen metabolism related genes in the respective bins

Discussion

Our results indicate that there are distinct differences in the community composition between the stations as well as different size fractions of the microbial community, thus between the free-living and particle associated microbes.

The observed differences in the prokaryotic community composition can be partially explained by environmental parameters. The higher abundance of Planctomycetes in the mesopelagic waters in the size fraction 0.8 µm – 3.0 µm and > 3.0 µm size fraction is likely caused by the differences in the depth where the samples have been taken. Planctomycetes are known to be preferentially particle-associated (DeLong et al. 1993). Due to the shallower sampling depth at the mesopleagic sampling site, larger particles with likely higher nutritional values are present as the microorganisms had less time to degrade the sinking particulate organic matter at this comparatively shallow depth compared to the other stations (McCave 1975; Burd and Jackson 2009).

The differences in the community composition of the different size fractionated microbial communities of each station were particularly pronounced in the brine pool. Such differences in the microbial community composition between the particle associated and the free-living microbial community have been reported also in previous studies (Acinas et al. 1999; Zhang et al. 2016).

Microbial inorganic carbon fixation

To explore the driving factors behind the community composition of the brine pool, we analyzed the metabolic pathways of these organisms.

Overall, a large fraction of the microorganisms inhabiting the brine pools had the potential to fix inorganic carbon (Fig. 3). The phylum Campylobacterota, which showed genetic potential for inorganic carbon fixation, was highly abundant in the free-living size fraction of the brine pool but much lower in abundance at all other locations. This indicates that the free-living community of the brine pool is a hot spot for inorganic carbon fixation, mostly driven by the Campylobacterota utilizing the rTCA cycle. This agrees with the findings of studies from deep-sea hydrothermal vents where Campylobacterota, formerly coined Epsilonproteobacteria, have been found to be an important carbon-fixing organism also utilizing the rTCA cycle (Campbell and Cary 2004; Hügler et al. 2010).

One of the prominent sources of energy for microbial inorganic carbon fixation is sulfur oxidation. According to our findings, the potential for sulfur oxidation as indicated by the presence of the *sox* genes can be found in all Campylobacterota bins giving them the genetic potential to fix inorganic carbon in combination with sulfur oxidation.

Alphaproteobacteria also exhibited a high abundance in the free-living size fraction in the brine pool. A large fraction of the Alphaproteobacteria had the genetic potential for sulfur oxidation and inorganic carbon fixation. In contrast to Campylobacterota, however, Alphaproteobacteria harbored the necessary genetic potential for the CBB cycle and not for the rTCA pathway. As reduced sulfur components have been previously reported to be depleted in these environments, there seems to be a notable competition among the Alphaproteobacteria and Campylobacterota for the available resources (Zhang et al. 2016a).

The fairly large number of representatives of the phylum Gammaproteobacteria also harbored the genetic potential for sulfur oxidation. In this phylum, however, sulfur oxidation genes were not as often associated with genes for inorganic carbon fixation as compared to the Alphaproteobacteria and thus, seem to use sulfur as a potential additional energy source only.

In addition to hydrogen sulfide, oxygen is required for sulfur oxidation. A previous study found oxygen concentrations of 14.75 µmol l^{-1} in the boundary layer of this brine pool (Wang et al. 2014), which albeit low in concentration is sufficient to allow sulfur oxidation.

The CAZyme categories also reflect the potential for inorganic carbon fixation in the Alphaproteobacteria bins as elevated numbers of glycosyltransferases were detected in most of the members of this phylum. These enzymes are involved in sugar bond formation facilitating in microbial carbon fixation (Fig. 4) (Lombard et al. 2014). The glycosyltransferases were, however, not enriched in the Campylobacterota, possibly due to the carbon fixation pathways differing from the other recovered phyla capable of inorganic carbon fixation.

Carbohydrate fermentation

To decipher the heterotrophic utilization of the carbon compounds in these environments by the microorganisms, the CAZY database categories were used.

Apart from the glycosyltransferases and some auxiliary activity genes detected in the Alphaproteobacteria the microorganisms characteristic for the free-living size fraction in the brine pool did not show high numbers of genes associated with carbohydrate metabolism.

In contrast, Desulfobacterota and Cloacimonadota, the two major phyla of the size fraction 0.8 µm – 3.0 µm and > 3.0 µm in the brine pool, harbored a much higher number of CAZyme genes from multiple categories as the similarly abundant Gammaproteobacteria from the same environment (Fig. 4). Additionally the CAZyme categories of these two phyla showed a contrasting pattern (see Fig. 4), indicating that these organisms utilize different types of carbohydrates and thereby occupy different ecological niches than Gammaproteobacteria. Cloacimonadota had more gene copies encoding polysaccharide lyases and glycoside hydrolases compared to the

Desulfobacterota, indicating that they may use polysaccharides as carbon source from the particles they inhabit. In contrast, Desulfobacterota exhibited high numbers of carbohydrate esterases (Fig. 4). This pattern coincides with the finding of a previous study, which also found Desulfobacterota (former Deltaproteobacteria) lacking polysaccharide lyases although the lack of carbohydrate esterases was less pronounced in previous studies (Zhang et al. 2016a). In our analysis, Marinisomatota showed a more diverse set of CAZyme genes giving them the potential to compete with both, Desulfobacterota and Cloacimonadota for the available carbohydrates.

As Gammaproteobacteria were by far the largest group of bins it is not surprising that they host a very diverse set of CAZyme genes from all categories depending on the individual bin. One clear trend was a negative relation between the abundance of the Gammaproteobacteria in the brine pool in the size fractions 0.8 µm – 3.0 µm and > 3.0 µm and the number of CAZyme genes of the carbohydrate binding modules and glycoside hydrolases categories. This trend indicates that the respective Gammaproteobacteria are not able to compete for the required carbohydrates with other microbes such as Cloacimonadota or Marinisomatota, which are the other phyla with the necessary genetic potential present in high numbers.

Since oxygen is generally available in the brine pool, it is the obvious electron acceptor for the carbohydrate utilizing microbes. This seems to hold true for both the Cloacimonadota and Marinisomatota as they lack the necessary genes to utilize sulfate reduction or denitrification as an electron sink, apart from a few individual bins.

In contrast, Desulfobacterota (former Deltaproteobacteria) harbored both the *dsr* and the *sat* gene giving them the potential to utilize sulfate, supporting previous reports on their metabolism (Miyatake et al. 2009; Bergmann et al. 2011). As dissimilatory sulfate reduction is an anaerobic process, this raises the question how the Desulfobacterota are able to thrive in an oxygenated water mass (Zhang et al. 2015). This might be explained by the high concentration of POM as described in previous studies (Zhang et al. 2014, 2015). Anaerobic microenvironments can be created on such particles providing anaerobic microorganisms the required ecological niches (Alldredge and Cohen 1987).

To exclude the possibility of a reverse usage of the *dsr* gene to oxidize reduced sulfur compounds, additional genes were taken into account in the analyses. According to previous findings, detecting the combination of *apr*A and *apr*B as well as *dsr*A and *dsr*B genes in a member of the Deltaproteobacteria means that the specific organism utilizes the dissimilatory sulfate reductase in a reductive manner (Anantharaman et al. 2018). At least one of the two *apr* subunit genes was detected in each of the Desulfobacterota thought to utilize sulfate reduction. This points to a reductive usage of the *dsr* genes. Other organisms containing *dsr*A/B were the

Alphaproteobacteria, in which no *apr*A/B genes were detected. Therefore, a reverse usage of *dsr*A/B cannot be ruled out indicating that these organisms could potentially use the reverse dissimilatory sulfate reductase pathway as an alternative way to oxidize sulfur compounds besides the above mentioned *sox* genes.

Denitrification can be an alternative electron sink for sulfate and other oxidized sulfur compounds as they have been reported to be the preferred electron acceptors if both resources are present (Lam and Kuypers 2011). In our study, both the Campylobacterota, which have been found to be involved in denitrification in similar environments (Zhang et al. 2015), and the Desulfobacterota harbored the two marker genes, *napA* and *narB*, indicating the genetic potential for denitrification. This, in combination with the above described anaerobic microenvironments and previously reported high concentrations of nitrate in these environments (Zhang et al. 2014, 2016a), shows that denitrification should be a viable electron sink for the described Desulfobacterota in combination with carbohydrate fermentation for the particle-associated size fraction where they were primarily detected. The Campylobacterota, however, as they were mainly detected in the oxygenated waters of the free-living size fraction, do not have the necessary anaerobic conditions to perform denitrification. When oxygen concentration decreases, the genetic potential of these organisms would enable them to utilize sulfur driven autotrophic denitrification which is the process of oxidizing sulfur compounds coupled with denitrification as an electron sink (Shao et al. 2010). This means that they might be resistant to oxygen fluctuations in the brine pool as they can switch between oxygen and nitrite as electron acceptors, if necessary.

Nitrogen fixation did not seem to play a significant role in the brine pool as the necessary *nif* genes were only detected in one Campylobacterota, which was most abundant in the oxygenated free-living size fraction of the brine pool. The total supply of nitrogen compounds for nitrification and denitrification is therefore dependent on external input into the environment as well as internal recycling.

Overall our findings indicate a spatial separation of the reductive and oxidative pathways in the brine pool. We found both, the possibility for sulfur oxidation as well as sulfate reduction in the community, providing the opportunity for an exchange of oxidized and reduced sulfur compounds. The extend of sulfate reduction in the brine pool is, however, also dependent on the availability of nitrate and nitrite as the dominant organisms capable of sulfate reduction also had the capability for denitrification which is the preferred pathway (Lam and Kuypers 2011).

Conclusion

Our findings show that the brine pool environment harbors highly adapted communities in both its free-living as well as its particle-associated fraction of the microbial community.

In the free-living community, sulfur oxidation via the *sox* genes and in case of the Alphaproteobacteria potentially via the r*dsr* pathway, in combination with the potential for microbial inorganic carbon fixation are widespread genetic features in two most dominant phyla (Campylobacterota, Alphaproteobacteria). Additionally, the Campylobacterota have the potential to fix inorganic carbon and oxidize sulfur via sulfur driven denitrification, if anaerobic conditions should arise in the brine pool.

In the particle-associated community of the brine pool all major phyla were enriched in one or several CAZyme categories with the abundance patterns of these categories indicating niche specialization among the Desulfobacterota and Cloacimonadota as well as a systematically lower abundance in some Gammaproteobacteria due to competition with other phyla for parts of the carbohydrate pool. To decompose these organic particles, oxygen is utilized as an electron acceptor as well as nitrate and sulfate in anaerobic microenvironments on particles by Desulfobacterota.

Our findings provide new insights into the interactions of microorganisms in brine pools and show both, interactions in between the particle-associated and free-living communities. The spatial separation seems to be an important factor in these interactions as it enables the microbial community of the brine pool to utilize metabolic pathways under different environmental conditions.

Zusammenfassung

Mikroorganismen sind die am weitesten verbreiteten und häufigsten Lebewesen der Erde und haben sich an einige der extremsten Umgebungen unseres Planeten angepasst. Salzseen in der Tiefsee des Roten Meeres mit Salinitätswerten von rund 250 verglichen mit 35 im globalen Ozean sind sicherlich eine der extremsten Umgebungen.

In dieser Studie haben wir metagenomische Binning Analysen verwendet, um die mikrobielle Zusammensetzung eines Salzsees in der Tiefsee der Thula Region des Roten Meeres zu charakterisieren. Des Weiteren nutzten wir die KEGG Datenbank um den gefundenen Organismen metabolische Funktionen zu zuordnen.

Die vorgenommene Größenfraktionierung zeigte deutliche Unterschiede zwischen den freilebenden Mikroorganismen der kleinsten Fraktion (0,2 µm – 0,8 µm) und den Organismen der größeren Fraktionen (0,8 µm – 3,0 µm, > 3,0 µm) welche eher mit Partikeln assoziiert leben. Schwefeloxidation schien eine wichtige Rolle für die Mikroben der freilebende Fraktion des Salzsees zu spielen, häufig kombiniert mit Genen assoziiert mit mikrobieller anorganischer Kohlenstoff-Fixierung durch den reversen Tricarboxyl- sowie den Calvin-Benson-Bassham Zyklus. Unsere Ergebnisse in Kombination mit früheren Studien deuten auf eine Limitierung durch die reduzierten Schwefelkomponenten hin, insbesondere in der freilebenden Größenfraktion (0,2 µm – 0,8 µm).

Zusätzlich konnten wir in den beiden größeren Fraktionen des Salzsees eine Anreicherung an Mikroben feststellen, welche vermehrt Gene aufwiesen die im Zusammenhang mit mikrobieller Kohlenhydratfermentierung stehen. Gleichzeitig wiesen die Organismen in dieser Umgebung vermehrt das genetische Potential für die Nutzung verschiedener Elektronenakzeptoren wie Sauerstoff, Nitrat und Sulfat auf.

Diese räumliche Trennung der oxidativen und reduktiven Stoffwechselwege zwischen den verschiedenen Größenfraktionen, in Kombination mit der möglichen Nutzung von anaeroben Mikronischen auf den Partikeln im Wasser des Salzsees, deutet auf eine ökologische Nischenaufteilung der Organismen und einen möglichen Austausch der entstehenden Stoffwechselprodukte zwischen den Organismen auf den Partikeln und denen im umgebenden Wasser hin.

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