

1 **SUPPLEMENTAL RESULTS AND DISCUSSION**

2 **Supp. Table 1.** Predicted diversity coverage and inverse Simpson indices of 16S rRNA gene sequences
3 recovered from microbialites-associated mat communities in seven locations from the SA of GSL.

| Location | Average Coverage | Average Inverse Simpson |
|-----------------|-------------------------|--------------------------------|
| BB | 0.90 | 32.4 |
| BP | 0.90 | 46.4 |
| R | 0.90 | 34.2 |
| SI | 0.89 | 29.6 |
| SP | 0.89 | 30.8 |
| WE | 0.91 | 35.4 |
| WW | 0.87 | 39.9 |

4 Abbreviations: BB, Bridger Bay; BP, Bison Point; R, Ridges; SI, Stansbury Island; SP, Stansbury
5 Polygons; WE, Windward East; WW, Windward West.

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24 **Supp. Table 2.** Bray-Curtis dissimilarity indices of community compositions recovered from seven sites
 25 in the SA of GSL. Bray-Curtis distances were calculated using the relative abundances of SSU rRNA
 26 gene OTUs as defined at 97% sequence similarity.

| | BB | BP | R | SI | SP | WE | WW |
|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|
| BB | 1.00 | 0.77 | 0.85 | 0.84 | 0.85 | 0.89 | 0.87 |
| BP | | 1.00 | 0.71 | 0.70 | 0.70 | 0.79 | 0.77 |
| R | | | 1.00 | 0.82 | 0.91 | 0.80 | 0.80 |
| SI | | | | 1.00 | 0.83 | 0.79 | 0.86 |
| SP | | | | | 1.00 | 0.78 | 0.81 |
| WE | | | | | | 1.00 | 0.84 |
| WW | | | | | | | 1.00 |

27 Abbreviations: BB, Bridger Bay; BP, Bison Point; R, Ridges; SI, Stansbury Island; SP, Stansbury
 28 Polygons; WE, Windward East; WW, Windward West.

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45 **Supp. Table 3.** Bray Curtis dissimilarity of the taxonomic composition of seven Great Salt Lake (GSL)
 46 microbialite communities and the average (Avg) GSL microbialite community calculated after organizing
 47 16S rRNA gene sequences at the order level of taxonomic classification.

| | Avg | BB | BP | R | SI | SP | WE | WW |
|------------|------------|-----------|-----------|----------|-----------|-----------|-----------|-----------|
| Avg | 1.00 | 0.92 | 0.87 | 0.90 | 0.92 | 0.92 | 0.91 | 0.91 |
| BB | | 1.00 | 0.84 | 0.88 | 0.87 | 0.87 | 0.93 | 0.88 |
| BP | | | 1.00 | 0.80 | 0.82 | 0.82 | 0.84 | 0.82 |
| R | | | | 1.00 | 0.87 | 0.92 | 0.86 | 0.84 |
| SI | | | | | 1.00 | 0.89 | 0.85 | 0.90 |
| SP | | | | | | 1.00 | 0.84 | 0.86 |
| WE | | | | | | | 1.00 | 0.87 |
| WW | | | | | | | | 1.00 |

48 Abbreviations: BB, Bridger Bay; BP, Bison Point; R, Ridges; SI, Stansbury Island; SP, Stansbury
 49 Polygons; WE, Windward East; WW, Windward West.

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Supp. Table 4. Sequencing statistics associated with the Bridger Bay, Great Salt Lake, Utah, microbialite mat metagenome and its assembly.

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| Read Lengths | 2x125 |
| # Reads (Post Quality Filter) | 79,692,256 |
| Total Sequencing (bp) | 10,026,675,156 |
| # Contigs | 212,139 |
| Contig no. > 1 kbp | 71,830 |
| Longest Contig | 233,003 |
| Total Assembly Size | 354,039,636 |
| Assembly size > 1 kbp | 284,850,691 |
| N50 | 5,244 |

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66 **SUPPLEMENTAL RESULTS AND DISCUSSION**

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68 **Bin 1: *Euhalothece* (Cyanobacteria; Chroococcales)**

69 Estimated genome size = 4.82 Mb; completeness = 98.3%; contamination = 0.0%; abundance
70 within community = 12.2%.

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72 The beta subunit of the DNA directed RNA polymerase (RpoB) in the most abundant
73 MAG from the microbialite metagenome (**Fig. 3A**) shared 97% sequence identity to that of
74 *Euhalothece* sp. PCC 7418. The genus *Euhalothece* comprises thermo- and halotolerant
75 members within the order Chroococcales (1). Consistent with the dominance of this MAG in the
76 hypersaline GSL metagenome, *Euhalothece* spp. were also shown to be dominant members of
77 microbial communities in several hypersaline environments including estuaries in South Africa
78 (1) and salt lakes in India (2), among others. The *Euhalothece* GSL MAG encodes a complete
79 Calvin cycle, both photosystems I and II, and pathways for fermentation of pyruvate into
80 formate, lactate, and acetate. Further, *Euhalothece* encodes a molybdenum (Mo)-dependent
81 nitrogenase (NifHDKENB) (**Supp. Dataset 2**). The presence of a molybdate importer and
82 machinery for synthesizing the active site iron-molybdenum cofactor of Mo-nitrogenase is
83 consistent with this population being capable of nitrogen fixation. This indicates that
84 *Euhalothece* likely supplies both fixed carbon and nitrogen for secondary consumers in GSL, a
85 finding that supports previous suggestions that this taxon is a keystone species in the GSL
86 ecosystem (3). The GSL *Euhalothece* MAG also encodes a bidirectional type 3d [NiFe]-
87 hydrogenase (HoxUFE), indicating a general ability to metabolize H₂ (4). The ability of this
88 population to catalyze reversible H₂ oxidation via [NiFe]-hydrogenase is supported by the

89 detection of homologs of proteins involved in the synthesis of the [NiFe] active site as well as
90 nickel import proteins. Hydrogenase may allow *Euhalothece* to re-capture H₂ generated as a
91 byproduct of nitrogen fixation activity, or may function to regenerate oxidized pyridine
92 nucleotides that accumulate when these cells ferment organic carbon reserves at night (5). The
93 NifD protein from the GSL *Euhalothece* MAG clusters phylogenetically with NifD genes from
94 canonical nitrogen fixing cyanobacteria including *Trichodesmium erythraeum* (data not shown),
95 which temporally segregates the O₂ sensitive process of N₂ fixation from oxygenic
96 photosynthesis by only fixing N₂ at night (6). This suggests that the GSL *Euhalothece* population
97 may also temporally segregate N₂ fixation. Further, the GSL *Euhalothece* MAG encodes CikA,
98 Pex, KaiA, KaiB, KaiC, and SASa, genes required for circadian timing (7). In addition, this
99 MAG encodes xanthorhodopsin, a retinal-based green-light absorbing proton pump (8), and for
100 apocarotenoid-15,15' oxygenase, a protein that synthesizes retinal (9). This suggests this to be an
101 additional mechanism allowing it to generate an electrochemical gradient across the membranes
102 of these cells (8). Other pathways encoded by this MAG include two-component signaling,
103 quorum sensing, and carbohydrate secretory systems, the latter of which may function to
104 facilitate cross-feeding of secondary consumers in the community and thus contribute to
105 microbialite community structure and function.

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107 **Bin 2: *Fabibacter* (Bacteroidetes; Bacteroidetes Order II. Incertae sedis)**

108 Estimated genome size = 3.34 Mb; completeness = 94.8%; contamination = 1.7%; abundance
109 within community = 9.3%.

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111 The second most abundant MAG (**Fig. 3A**; 9.3% of total reads) in the GSL microbialite
112 metagenome is distantly related (60% RpoB identity) to the genus *Fabibacter* within the
113 Bacteroidetes Order II. Incertae sedis. The *Fabibacter*-affiliated MAG encodes complete
114 glycolytic, pentose phosphate, and TCA cycle pathways (**Supp. Dataset 2**). The closest cultured
115 representative, *F. pacificus*, was shown to be obligately aerobic (10). Consistent with this, the
116 GSL MAG encodes homologs for cytochrome *c* oxidase (complex IV), indicating it too may
117 respire O₂. This MAG can likely use starch as a carbon source and electron donor based on the
118 presence of homologs of isoamylase that hydrolyze starch into maltodextrin. Maltodextrin can
119 then be hydrolyzed to maltose and glucose by alpha amylase. Maltodextrin can also be
120 hydrolyzed to yield glucose polymers by maltodextrin glucosidase. This organism may also
121 utilize glycerolipids and fatty acids as sources of carbon and electrons as evidenced by the MAG
122 encoding multiple lipases, including triacylglycerol lipase, and fatty acid degradation pathways.
123 The GSL MAG does not encode glycerate 2-kinase for entry of glycerate from glycerol
124 metabolism into glycolysis but it does encode other enzymes that allow for processing glycerol
125 into glycerate. Thus, it is unclear if this MAG can utilize glycerol as a carbon and/or energy
126 source, like has been demonstrated for *F. pacificus* (10). The GSL MAG encodes pathways for
127 the degradation of leucine and isoleucine as other potential reduced carbon sources accessible to
128 this population. In addition, homologs for sodium ion-transporting rhodopsin (11) and
129 halorhodopsin, a retinal-based green/yellow light-absorbing unidirectional chloride pump (12),
130 were identified. This suggesting the organism can use light to maintain its cytoplasmic osmotic
131 balance. Collectively, these observations point to the *Fabibacter*-affiliated GSL population as
132 being an aerobic photoheterotroph that is cross-fed reduced carbon compounds that serve as
133 sources of carbon and reductant.

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Bin 3: *Longibacter* (Bacteroidetes; Bacteroidetes Order II, *Incertae sedis*)

Estimated genome size = 4.05 Mb; completeness = 88.8%; contamination = 1.4%; abundance within community = 5.8%.

A MAG was recovered from the GSL microbial mat that was most closely affiliated (97% RpoB identity) with *L. salinarum* and this MAG comprised 5.8% of the total reads (**Fig. 3A**). The detection of sequences affiliated with *L. salinarum* in hypersaline GSL is consistent with the environment where the type strain was isolated: a marine solar saltern in China (13). Moreover, the type strain was shown to grow over a salinity range of 5-20% (14), which is similar to the salinity of GSL waters (14.5 to 17.0%) when mat samples for the metagenome were collected. The MAG encoded nearly complete glycolysis, pentose phosphate, and starch/sucrose degradation pathways but did not encode any known autotrophic pathways (**Supp. Dataset 2**). The MAG also encodes for xanthorhodopsin, a retinal-based green-light absorbing proton pump (8), and for β -carotene 15,15'-dioxygenase, a protein involved in the synthesis of the carotenoid salinixanthin (15). This suggests a possible role in supplementing the generation of electrochemical gradients across the membrane of the cell. The organism also encoded cytochrome *c* oxidase (complex IV) but did not encode other protein complexes (e.g., nitrate reductase) allowing for anaerobic respiration. Together, these data suggest that the organism is an aerobic photoheterotroph. To this end, the predicted metabolism of the GSL *Longibacter*-affiliated population is consistent with previous studies of the type strain that showed that aerobic growth of the organism was supported by oxidation of glycerol, sucrose, starch, and mannitol (13), however, it differs in the suggested ability to use light.

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Bin 4: *Pelagibaca* (Proteobacteria; Rhodobacterales)

Estimated genome size = 3.00 Mb; completeness = 86.2%; contamination = 0.0%; abundance within community = 3.7%.

A MAG closely affiliated with *P. abyssi* (94% 50S ribosomal protein L2 identity) and that represented 5.8% of the total reads was identified in the GSL microbial mat metagenome (**Fig. 3A**). Consistent with its detection within GSL mats, the type strain of *P. abyssi* was isolated from a marine habitat and exhibited growth across a range of salinities (5 to 13%) (16). The type strain was reported as an aerobic heterotroph. Consistent with this report, the GSL *P. abyssi* MAG encodes cytochrome *c* oxidase (complex IV) and lacked homologs of other identifiable protein complexes that would allow for respiration of additional oxidants such as nitrate or sulfate (**Supp. Dataset 2**). Homologs for nearly complete TCA, glycolytic, and pentose phosphate pathways were also identified in the GSL MAG. Like the genome of *P. abyssi*, the GSL MAG encodes a wide range of carbohydrate, oligopeptide, and amino acid transporters, suggesting it can import and degrade organic carbon substrates (17). Specific carbohydrate transporters identified in the MAG include those for maltose/maltodextrin, raffinose/stachyose/melibiose, and glucose/mannose. Maltose is possibly hydrolyzed to glucose by alpha-glucosidase and raffinose/stachyose/melibiose could be hydrolyzed to glucose by alpha-galactosidase; glucose released from these hydrolysis reactions could then enter glycolysis. Alternatively, homologs of an ABC transporter for glucose/mannose could allow glucose to enter glycolysis in the absence of a phosphotransferase system for this sugar. Additional ABC transporters were identified for alpha-glucoside, ribose/xylose, sn-glycerol-3-phosphate and

180 phospholipids. In addition, two of the three genes for transport of urea were identified. The type
181 strain was shown to encode the Calvin cycle allowing for CO₂ fixation, with electrons likely
182 coming from thiosulfate oxidation via the Sox system (17). However, while the GSL MAG
183 encodes for the Sox system, it does not encode for the Calvin Cycle or any other autotrophic
184 pathways. This suggests an ability to oxidize thiosulfate to potentially supplement heterotrophic
185 growth in what has been described as chemolithoheterotrophy (18). Intriguingly, the GSL MAG
186 encodes an antenna complex, bacteriochlorophyll biosynthesis genes, and photosynthetic
187 reaction center L, M and H, which could also supplement heterotrophic growth through what has
188 been described as aerobic anoxygenic photoheterotrophy.

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190 **Bin 5: *Wenzhouxiangella* (Proteobacteria; Chromatiales)**

191 Estimated genome size = 2.94 Mb; completeness = 82.8%; contamination = 1.7%; abundance
192 within community = 3.1%.

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194 A MAG closely affiliated to *Wenzhouxiangella sediminis* (96% RpoB identity) that
195 accounts for 3.1% of the total reads was identified in the GSL microbialite mat metagenome
196 (**Fig. 3A**). The MAG encodes cytochrome *c* oxidase (complex IV), which when combined with
197 the absence of other canonical protein complexes that allow for anaerobic respiration (e.g.,
198 nitrate reductases; **Supp. Dataset 2**), suggests the population to be aerobic. This agrees with
199 previous characterizations of closely related strains that were also shown to be aerobes . The
200 MAG encodes an incomplete glycolytic pathway and lacks homologs of glucose-6-phosphate
201 isomerase, phosphofructokinase, diphosphate--fructose-6-phosphate 1-phosphotransferase, and
202 aldose reductase, suggesting a limited ability to metabolize sugars. However, the MAG encodes

203 a complete TCA cycle suggesting that the organism may be acquiring acetyl-CoA via pathways
204 other than glycolysis. Perhaps consistent with this hypothesis, the GSL MAG encodes fructose
205 1,6-bisphosphatase, an enzyme that functions in gluconeogenesis. The MAG has all the genes
206 required for the transport of oligopeptides and encodes valine, leucine, and isoleucine
207 degradation pathways. When taken together, these data suggest that the bacterium may acquire
208 acetyl-CoA for biosynthesis via amino acid degradation. The presence of phospholipid transport
209 genes also points toward the possibility of lipid degradation as a source of carbon and reductant.
210 Consistent with this hypothesis, fatty acid degradation pathways are encoded by the MAG.
211 Considering that an endpoint of fatty acid degradation is acetyl-CoA, the genome data provide a
212 possible path for how this organism may be acquiring acetyl-CoA.

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214 Interestingly, the MAG encodes several proteins that form photosystem II, reaction center
215 proteins PufM, PufL, PufC, PucC, PuhE, PuhA and alpha/beta subunits of an antenna complex. It
216 also encodes most of the proteins required for the biosynthesis of the carotenoid, spheroidene,
217 which forms a complex with the reaction center in *Rhodobacter sphaeroides* (19). It is unlikely
218 that the organism can synthesize bacteriochlorophyll, however, since the MAG lacks most of the
219 proteins required including dark-operative protochlorophyllide oxidoreductase (DPOR;
220 BchLNB) and chlorophyllide *a* oxidoreductase (COR; BchXYZ) (20). Intriguing, the MAG
221 encodes the large subunit of RuBisCO but not the small subunit. When taken together, the
222 genome data suggests this organism is a facultative anaerobe and heterotroph that relies on
223 amino acid and fatty acid degradation to generate ATP and acquire carbon. The incomplete
224 glycolytic pathway might indicate that this aerobic organism specializes in utilizing select amino
225 and fatty acids to minimize niche overlap with co-inhabiting heterotrophs that utilize sugars to

226 support their energy metabolisms. The presence of reaction center proteins and an antenna
227 complex but no Calvin Cycle suggest that the organism may also be capable of anoxygenic
228 photoheterotrophic carbon assimilation.

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230 **Bin 6: *Arboriscoccus* (Proteobacteria; Geminococcales)**

231 Estimated genome size = 3.36 Mb; completeness = 99.7%; contamination = 3.6%; abundance
232 within community = 2.9%.

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234 A MAG distantly affiliated (80% RpoB identity) with *Arboriscoccus pini* (21) was
235 identified in the GSL metagenome and it comprised 2.9% of the total reads (**Fig. 3A**). The MAG
236 encodes nearly complete glycolytic and pentose phosphate pathways, as well as the TCA cycle
237 (**Supp. Dataset 2**). The MAG also encodes transporters for glycerol, lipoproteins, and
238 lipopolysaccharides and encodes pathways allowing for starch metabolism, a hallmark of other
239 closely related alphaproteobacterial (e.g., *A. pini* and free-living *Geminicoccus roseus* and
240 *Alysiosphaera europaea*) (21-23). The MAG also encodes for a bidirectional [NiFe]-
241 hydrogenase, suggesting an ability to reversibly oxidize H₂ (4). The MAG also encodes for
242 cytochrome *c* oxidase (complex IV) and the supporting proteins necessary for oxidative
243 phosphorylation. The MAG encodes a homolog of xanthorhodopsin, a retinal-based green-light
244 absorbing proton pump (8). To this end, the MAG appears to be an aerobic photoheterotroph
245 with the capacity to use oxygen as its terminal electron acceptor to metabolize sugars and starch.

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247 **Bin 7: *Roseibaca* (Proteobacteria; Rhodobacteriales)**

248 Estimated genome size = 2.58 Mb; completeness = 82.8%; contamination = 2.6%; abundance
249 within community = 2.2%.

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251 A MAG closely affiliated with *Roseibaca calidilacus* (93% 50S rRNA sequence
252 identities) within the proteobacterial order Rhodobacterales was identified in the metagenome,
253 representing 2.2% of the total reads (**Fig. 3A**). These findings are consistent with previous
254 studies that identified *Roseibaca* to be abundant components of waters and phototroph mats from
255 hypersaline, alkaline habitats [e.g., (24)]. The GSL *Roseibaca* MAG encodes full glycolytic,
256 TCA, and pentose phosphate (PP) pathways, as well as transporter for xylose that can be input
257 into the PP pathway (**Supp. Dataset 2**). Other homologs of transport systems identified in the
258 MAG include those for urea, glucose, glycerol, lipoproteins, and lipopolysaccharides. Based on
259 these identified protein homologs and the absence of homologs of autotrophic pathways, the
260 bacterium is hypothesized to be a heterotroph. Likewise, the presence of several homologs of
261 cytochrome *c* oxidase (complex IV) and the absence of identifiable pathways for anaerobic
262 respiration, suggests that the GSL *Roseibaca*-affiliated bacterium is aerobic. Intriguingly, this
263 MAG encodes homologs for genes involved in bacteriochlorophyll synthesis but only encodes
264 one reaction center (PufH) homolog. This might be due to the MAG incompleteness (82.8%) and
265 suggests this organism is a photoheterotroph.

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267 **Bin 8: *Longibacter* (Bacteroidetes; Bacteroidetes Order II. Incertae sedis)**

268 Estimated genome size = 4.27 Mb; completeness = 87.9%; contamination = 5.9%; abundance
269 within community = 1.9%.

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271 A MAG closely related to *Longibacter salinarum* (84% RpoB identity) was detected in
272 the GSL mat metagenome at an abundance of 1.9% of total reads (**Fig. 3A**). The type strain of *L.*
273 *salinarum* was isolated in a marine system in China (13) and has been shown to grow over a
274 salinity range of 5-20% (14), which is comparable to conditions within GSL (14-17%, **Table 1**).
275 The MAG encoded a nearly complete glycolytic pathway (exceptions being genes coding for
276 phosphofructokinase and hexokinase), a complete TCA cycle, and a complete pentose phosphate
277 pathway (**Supp. Dataset 2**). Homologs of enzymes involved in autotrophic pathways were not
278 detected. The MAG encodes cytochrome *c* oxidase (complex IV) suggesting an ability to respire
279 O₂ but it does not encode homologs of proteins that would allow it to respire other oxidants.
280 These findings suggest that this MAG is from an aerobic heterotroph. This is consistent with a
281 physiological characterization of the type strain that showed the organism could grow aerobically
282 using glycerol and starch, among other carbon sources (13). In addition, this MAG encodes the
283 enzyme lactate dehydrogenase, which can convert lactate to pyruvate and pyruvate to lactate.
284 This MAG also contains the sequences for lactate utilization suggesting that the bacterium could
285 potentially use lactate as a carbon source (25). Some of the transporters encoded in the MAG
286 include a sodium/glucose co-transporter in addition to amino acid and metal transporters. This
287 MAG does not encode for reaction centers, bacteriochlorophyll biosynthesis genes, or
288 rhodopsins.

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290 **Bin 9: *Coralimargarita* (Verrucomicrobia; Puniceicoccaceae)**

291 Estimated genome size = 4.30 Mb; completeness = 92.2%; contamination = 7.1%; abundance
292 within community = 1.8%.

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294 A MAG distantly related to *Coralimargarita akajimensis* (78% RpoB identity), a
295 member of the bacterial phylum Verrucomicrobia, order Puniceococcales, was detected in the
296 GSL at a relative abundance of ~1.8% total reads (**Fig. 3A**). This strain is an obligate aerobe
297 isolated from seawater (26). The GSL MAG encodes complete glycolytic, TCA, and PP
298 pathways. In addition, this MAG encodes cytochrome *c* oxidase (complex IV), suggesting an
299 ability to respire O₂ (**Supp. Dataset 2**). This MAG does not encode homologs for proteins
300 involved in autotrophy. Homologs for transporters for the following reduced organic carbon
301 compounds are encoded in this MAG: polysaccharides, lipopolysaccharides, lipoproteins,
302 glycine betaine/proline, oligopeptides, and a sodium/glucose cotransporter. This MAG may
303 utilize fructose by first converting it to glucose using the encoded xylose isomerase. Glycogen
304 can be phosphorylated to 1P-glucose by the encoded glycogen phosphorylase or converted to
305 maltose and dextrin by the encoded alpha-amylases, and sucrose can be converted to fructose by
306 alpha-glucosidase. The potential for fermentation is suggested by the presence of alcohol
307 dehydrogenase and the ability to convert acetate to acetyl-CoA. Moreover, a suite of enzymes
308 allowing for the conversion of propanoate to propanoyl-CoA, which can be converted to succinyl
309 CoA or succinate to feed other metabolic pathways, are also encoded by the GSL MAG. It may
310 also be possible for this population to reduce nitrous oxide, as a homolog for nitrous oxide
311 reductase was encoded in this MAG; however, no other denitrification genes were identified. In
312 addition, this MAG encodes for β -carotene 15,15'-dioxygenase, a protein involved in the
313 synthesis of the carotenoid salinixanthin (15), and for two proteorhodopsins: one is a homolog
314 of xanthorhodopsin, a retinal-based green-light absorbing proton pump (8), and the other is a
315 homolog of the partially characterized blue-light absorbing rhodopsin (27). Either of these likely
316 allow the organism to support the generation of an electrochemical gradient across its membrane

317 using light (8). Altogether, these observations suggest that this population is a facultative
318 anaerobic photoheterotroph.

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320 **Bin 10: *Psychroflexus* (Bacteroidota; Flavobacteriales)**

321 Estimated genome size = 2.58 Mb; completeness = 98.3%; contamination = 1.7%; abundance
322 within community = 1.8%.

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324 A MAG closely affiliated (100% RpoB identity) with *Psychroflexus* sp. WDS2C27, a
325 member of the bacterial order Flavobacteriales, was detected in the GSL metagenome where it
326 comprised 2.29% of the total reads (**Fig. 3A**). The detection of sequences affiliated with
327 *Psychroflexus* in hypersaline GSL is consistent with the detection of related strains in
328 hypersaline lakes (28), salt pans (29), among other brine habitats [e.g., (30)]. The GSL
329 *Psychroflexus* MAG encodes near complete glycolysis and TCA pathways and lacks evidence
330 for autotrophic metabolism (**Supp. Dataset 2**). The MAG also encodes cytochrome *c* oxidase
331 (complex IV) but did not encode other canonical protein complexes that would allow it to respire
332 other oxidants such as nitrate or sulfate. Additionally, the organism encodes a homolog of a
333 rhodopsin, allowing the bacterium to use light to generate/supplement an electrochemical
334 gradient that could be used to drive ATP synthesis or to perform work. The MAG encodes a
335 group 2a [NiFe] Hydrogenase, suggesting a generalized ability to metabolize H₂ as a source of
336 reductant (4). Together, these data suggest that the organism is an aerobic photoheterotroph

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338 **Bin 11: *Henriciella* (Proteobacteria; Caulobacterales)**

339 Estimated genome size = 2.49 Mb; completeness = 87.9%; contamination = 0.0%; abundance
340 within community = 1.6%.

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342 A MAG that is related (88% RpoB identity) to *Henriciella pelagia* accounted for 1.6% of
343 the total reads (**Fig. 3A**). Like *H. pelagia* which was classified as oxidase positive (31), the GSL
344 MAG encodes cytochrome *c* oxidase (complex IV) (**Supp. Dataset 2**). Homologs of protein
345 complexes allowing for anaerobic respiration (e.g., nitrate reductase, bisulfite reductase) were
346 not identified. The MAG encodes a full glycolytic and TCA cycle but does not encode
347 autotrophic pathways. Together, these observations suggest that the bacterium is an aerobic
348 heterotroph. The MAG encodes alpha-amylase that converts starch into glucose and maltose as
349 well as maltase that converts maltose into glucose. These data suggest that the organism utilizes
350 starch. Interestingly, the MAG encodes for xanthorhodopsin, a retinal-based green-light
351 absorbing unidirectional proton pump (8). Xanthorhodopsin is a retinal protein/carotenoid
352 complex (8) and the MAG encodes β -carotene 15,15'-dioxygenase that is involved in the
353 synthesis of the carotenoid salinixanthin (15), suggesting that one of the ways that the organism
354 is generating an electrochemical gradient is via light energy. Overall, the bacterium represented
355 by this MAG is likely an aerobic photoheterotroph.

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357 **Bin 12: *Rhodohalobacter* (Bacteroidota; Balneolales)**

358 Estimated genome size = 3.81 Mb; completeness = 66.8%; contamination = 6.0%; abundance
359 within community = 1.5%.

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361 A MAG closely affiliated (91% RpoB) with *Rhodohalobacter halophilus* that represented
362 1.5% of the total reads was identified in the GSL microbial mat (**Fig. 3A**). *Rhodohalobacter*
363 *halophilus*, the first species identified for this genus, was isolated from seawater and is reported
364 as a halophilic heterotroph and facultative anaerobe capable of fermenting carbohydrates (32).
365 The GSL *Rhodohalobacter halophilus* MAG encodes cytochrome *c* oxidase (complex IV),
366 suggesting the ability to respire O₂ (**Supp. Dataset 2**). A homolog for nitrous-oxide reductase,
367 which converts N₂O to N₂, was also identified suggesting the possibility for anaerobic respiration
368 (33). Partially complete TCA, glycolytic, and pentose phosphate pathways were also identified,
369 which may be due to the incompleteness of the MAG (66.8% complete). Homologs for
370 phospholipid and ribose transporters were also identified. The former could be degraded by the
371 nearly complete fatty acid degradation pathway while the latter could be metabolized through the
372 pentose phosphate pathway. The ability to ferment pyruvate to acetate is suggested by the
373 detection of homologs of phosphotransacetylase and acetate kinase. Homologs for autotrophic
374 carbon fixation pathways were not detected. A fragment of a halorhodopsin homolog was
375 identified in this MAG. Halorhodopsins are retinal-based green/yellow light-absorbing
376 unidirectional chloride pumps that might enable this organism to maintain cytoplasmic osmotic
377 balance (12). Thus, these observations suggest that *Rhodohalobacter halophilus* MAG is a
378 photoheterotroph and possibly a facultative anaerobe, pending the strains ability to respire N₂O
379 or ferment organic compounds.

380

381 **Bin 13: *Rhodohalobacter* (Bacteroidetes; Balneolales)**

382 Estimated genome size = 3.79 Mb; completeness = 93.1%; contamination = 0.0%; abundance
383 within community = 1.3%.

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385 A MAG closely affiliated (96% RpoB identity) to *Rhodohalobacter halophilus* (bacterial
386 order Balneolales) represented 1.3% of the total reads (**Fig. 3A**). The MAG encodes nearly
387 complete glycolysis, gluconeogenesis, pentose phosphate, and TCA cycle pathways (**Supp.**
388 **Dataset 2**). It also encodes cytochrome *c* oxidase (complex IV), a key enzyme complex involved
389 in respiration of O₂. The MAG lacks evidence for autotrophic pathways or protein complexes
390 that allow for anaerobic respiration. However, the MAG encodes alcohol dehydrogenase and five
391 other key enzymes required for fermentation, suggesting the possibility that it can ferment
392 organic carbon substrates. These findings are consistent with the previous characterizations of
393 members of this genus as facultative anaerobes (32) and supports the notion that this GSL
394 population can survive under oxic and anoxic conditions. The ability to ferment and thus
395 metabolize organic carbon substrates under anoxic conditions potentially differentiates this
396 bacterium from the other putatively aerobic heterotrophs in the mat community. This MAG also
397 encodes halorhodopsin, a retinal-based green/yellow-light absorbing chloride pump that could
398 support cytoplasmic osmotic balance through the use of light (12). These observations suggest
399 this organism is a facultative anaerobic photoheterotroph.

400

401 **Bin 14: *Thiohalocapsa* (Proteobacteria; Chromatiales)**

402 Estimated genome size = 4.28 Mb; completeness = 98.3%; contamination = 0.0%; abundance
403 within community = 1.3%.

404

405 A MAG closely related (93% RpoB identity) to *Thiohalocapsa* sp. ML 1 represented
406 1.3% of total reads in the GSL mat metagenome (**Fig. 3A**). *Thiohalocapsa* is commonly

407 identified in anoxic, sulfidic environments (34). The GSL *Thiohalocapsa* shows many genomic
408 similarities to *Thiohalocapsa* sp. ML1 (34, 35) including encoding complete glycolytic and TCA
409 cycles (**Supp. Dataset 2**). Consistent with the genus *Thiohalocapsa* comprising anoxygenic
410 phototrophs (36), the GSL MAG encodes photosynthetic reaction centers proteins L, M, and H,
411 as well as genes required to synthesize bacteriochlorophyll (BchLNBXYZ). The MAG also
412 encodes phosphoribulokinase which is involved in the Calvin Cycle. However, homologs of
413 Type I or Type II ribulose-1,5- bisphosphate carboxylase oxygenase (CbbS and CbbM,
414 respectively) were not detected in the MAG. A BLASTp search of unbinned contigs identified
415 homologs of CbbSL and CbbM that exhibited close identities (95%, 86%, and 85%, respectively)
416 to homologs from *Thiocapsa* sp. ML 1, suggesting that the GSL *Thiohalocapsa* strain is capable
417 of CO₂ fixation via the Calvin Cycle. Based on genomic data, the most likely electron donor
418 fueling anoxygenic photosynthesis is sulfide, which can be oxidized by sulfide:quinone
419 oxidoreductase encoded in the GSL MAG. Sulfide is likely supplied to this bacterium by sulfate
420 reducing bacteria that are widely distributed in sediments and anoxic zones in GSL (37, 38). The
421 GSL MAG also encodes Mo-nitrogenase and all of the proteins needed for dissimilatory nitrate
422 reduction/denitrification (i.e., NapAB). Cytochrome *cbb3* oxidase, which is typically only
423 expressed in microorganisms living in microaerobic or anoxic conditions (39), was also
424 identified in the MAG. Additionally, the MAG contains the enzymes for the fermentation of
425 pyruvate to acetate. These enzymes include pyruvate dehydrogenase, phosphotransacetylase, and
426 acetate kinase. Thus, the organism is hypothesized to be either an anaerobe or to be facultatively
427 anaerobic and is capable of anoxygenic photosynthesis.

428

429 **Bin 15: *Coralimargarita* (Verrucomicrobia; Puniceococcales)**

430 Estimated genome size = 2.71 Mb; completeness = 93.1%; contamination = 8.6%; abundance
431 within community = 1.2%.

432

433 A MAG closely related (80.6% RpoB identity) to *Coralimargarita akajimensis* that
434 represented 1.2% of the total reads was identified in the GSL microbial mat (**Fig. 3A**). Previous
435 physiological characterizations indicate *C. akajimensis* is an obligately aerobic heterotroph (26).
436 Unlike the genome of *C. akajimensis*, the GSL MAG does not encode a complete glycolytic
437 pathway but might be able to assimilate glycerate-3P via a complete gluconeogenesis pathway
438 (**Supp. Dataset 2**). This population may obtain glycerate-3P from CO₂ fixed via the Calvin
439 cycle. The GSL MAG encodes complete TCA and pentose phosphate pathways. Like *C.*
440 *akajimensis*, the GSL MAG appears capable of utilizing fructose based on encoded homologs for
441 fructokinase, phosphofructokinase, and xylose isomerase. This MAG can also convert lactose to
442 glucose and galactose via β -galactosidase. This MAG encodes a sodium/glucose cotransporter
443 and a suite of subunits for the ABC oligopeptide transport system. This MAG encodes several,
444 but not all, subunits for a cytochrome *c* oxidase making it unclear if it can respire O₂ like *C.*
445 *akajimensis* (26). The GSL population appears capable of fermentation by the encoded alcohol
446 dehydrogenase, lactate dehydrogenase, and malate dehydrogenase. This organism can also
447 convert acetate to acetyl-CoA by acetyl-CoA synthetase. In addition, a group 3d (bidirectional)
448 NiFe hydrogenase is encoded in the MAG suggesting the ability to reversibly transform H₂. The
449 fitness of the organism is likely enhanced by xanthorhodopsin, a retinal-based green-light
450 absorbing unidirectional proton pump that it may use to support the generation of an
451 electrochemical gradient (8). In addition, this MAG encodes the full suite of genes for the
452 glycine betaine/proline ABC transport system, as well as a chlorine/proton antiporter for

453 maintaining osmotic balance. Together, these observations suggest this GSL *Coralimargarita*-
454 affiliated population to be a facultative autotroph capable of fermentation and phototrophy.

455

456 **Bin 16: *Wenzhouxiangella* (Proteobacteria; Chromatiales)**

457 Estimated genome size = 1.88 Mb; completeness = 51.7%; contamination = 5.2%; abundance
458 within community = 1.2%.

459

460 A MAG closely related to *Wenzhouxiangella sediminis* (97% ribosomal protein S1
461 identity) was identified that accounted for 1.2% of the total GSL microbialite metagenome
462 sequences (**Fig. 3A**). The MAG encodes two out of three subunits of the cytochrome *bd*
463 complex, *CydA* and *CydB* (**Supp. Dataset 2**). The MAG is 51.7% complete which means it
464 could encode the missing protein (*Cydx*) as well as a complete cytochrome *c* oxidase but these
465 were just not sequenced or they were excluded from the bin during assembly. Indeed, the bin 5
466 MAG, which is closely related to the bin 16 MAG, encodes a complete cytochrome *c* oxidase
467 and is inferred to be an aerobe (described above). The less complete MAG (bin 16) does not
468 encode pathways allowing for respiration of other electron acceptors such as nitrate. When
469 combined with the finding that the more complete bin (bin 5) appears to be from an aerobe, it is
470 plausible that the less complete MAG also corresponds to an aerobic organism. Like bin 5, the
471 bin 16 MAG encodes an incomplete glycolytic pathway and lacks homologs of glucose-6-
472 phosphate isomerase, phosphofructokinase, and diphosphate-fructose-6-phosphate 1-
473 phosphotransferase. This suggests that the organism cannot metabolize sugars. Interestingly, the
474 MAG encodes endoglucanase that is involved in the hydrolysis of cellulose for use in glycolysis.
475 However, for reasons mentioned above, it is not clear if the organism is capable of glycolysis.

476 The MAG encodes isocitrate lyase and malate synthase involved in the glyoxylate cycle and this
477 could provide substrates for gluconeogenesis. The MAG encodes a complete TCA cycle but has
478 incomplete valine, leucine, and isoleucine degradation pathways. The MAG encodes most of the
479 genes for phospholipid transport but lacks most of the genes for beta oxidation of fatty acids.
480 Similar to the more complete bin 5, bin 16 encodes for photosystem II including the reaction
481 center proteins PufM, PufL, PufC, PucC, PuhE, PuhA and alpha/beta subunits of an antenna
482 complex. It also encodes most of the proteins required for the biosynthesis of the carotenoid,
483 spheroidene, which forms a complex with the reaction center in *R. sphaeroides* (19). It is
484 unlikely that the organism can synthesize bacteriochlorophyll, however, since the MAG lacks
485 homologs of COR and DPOR (20). In addition, the MAG encodes a fragment of a
486 xanthorhodopsin homolog and a homolog of β -carotene 15,15'-dioxygenase, a protein involved
487 in the synthesis of the carotenoid salinixanthin (15). This suggests that xanthorhodopsin could
488 function to support the generation of an electrochemical gradient across the membrane of the cell
489 using light energy. Intriguing, the MAG encodes the large subunit of RuBisCO but not the small
490 subunit. Thus, the organism could be a facultative aerobe that can perform anoxygenic
491 photoheterotrophic carbon assimilation.

492

493 **Bin 17: Rhodothermaceae (Bacteroidetes; Bacteroidetes Order II, *Incertae sedis*)**

494 Estimated genome size = 4.07 Mb; completeness = 82.8%; contamination = 3.6%; abundance
495 within community = 1.2%.

496

497 A MAG distantly affiliated (77% RpoB identity) with *Rhodothermaceae* bacterium RA
498 was identified that represented 1.2% of the GSL mat community (**Fig. 3A**). *Rhodothermaceae*

499 bacterium RA is halo- and thermophilic aerobic heterotroph isolated from a saline hot spring
500 (40). The GSL *Rhodothermaceae* MAG encodes near complete glycolysis, TCA cycle, and
501 pyruvate metabolism pathways and lacks homologs of key proteins for anaerobic respiration or
502 autotrophy (**Supp. Dataset 2**). This suggests aerobic heterotrophy and is consistent with a
503 previous characterization of a member of this family (40). Further, this MAG encodes for a
504 sodium ion-pump rhodopsin (11) and for halorhodopsin, a retinal-based green/yellow-light
505 absorbing chloride pump (12). This suggests this organism could be a photoheterotroph that uses
506 light to support the generation of an electrochemical gradient across its membrane and maintain
507 its cytoplasmic osmotic balance.

508

509 **Bin 18: *Inquilingus* (Proteobacteria; Inquilingaceae)**

510 Estimated genome size = 4.23 Mb; completeness = 98.3%; contamination = 0.0%; abundance
511 within community = 1.2%.

512

513 A MAG distantly related (86% RpoB identity) to the bacterium *Inquilingus limosus* (α -
514 proteobacteria; Inquilingaceae) was detected in the GSL mat metagenome (**Fig. 3A**). *I. limosus*
515 was originally isolated from the lung of a cystic fibrosis patient but related sequences have since
516 been identified in hypersaline mats (41). The GSL MAG encodes for glycolysis, the TCA cycle,
517 and pentose phosphate pathways but does not encode for known autotrophic pathways. This
518 suggests the organism is a heterotroph. The MAG encodes for Mo-nitrogenase suggesting it can
519 reduce atmospheric nitrogen for biosynthetic purposes. The MAG also encodes the dissimilatory
520 nitrate reductase suggesting an ability to respire nitrate. A homolog for xanthorhodopsin, a
521 retinal-based green-light absorbing proton pump (8), and a homolog for β -carotene 15,15'-

522 dioxygenase, a protein involved in the synthesis of the carotenoid salinixanthin (15), were
523 identified. This suggests an ability to use light energy to support the generation of an
524 electrochemical gradient across its cytoplasmic membrane. When combined with the presence of
525 genes encoding cytochrome *c* oxidase (complex IV), the organism is predicted to be a facultative
526 anaerobe photoheterotroph.

527

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