Title: Community structure of phototrophic co-cultures from extreme environments

Charles Brooke[1], Morgan P. Connolly[2], Javier A. Garcia[3], Miranda Harmon-Smith[4], Nicole Shapiro[4], Erik Hawley[5], Michael Barton[4], Susannah G. Tringe[4], Tijana Glavina del Rio[4], David E. Culley[6], Richard Castenholz[7] and *Matthias Hess[1, 4]

[1]Systems Microbiology & Natural Products Laboratory, University of California, Davis, CA
[2]Microbiology Graduate Group, University of California, Davis, CA
[3]Biochemistry, Molecular, Cellular, and Developmental Biology Graduate Group, University of California, Davis, CA
[4]DOE Joint Genome Institute, Walnut Creek, CA
[5]Bayer, Pittsburg, PA
[6]LifeMine, Cambridge, MA
[7]University of Oregon, Eugene, OR

***Corresponding author:**

Matthias Hess
University of California, Davis
1 Shields Ave
Davis, CA 95616, USA
P (530) 530-752-8809
F (530) 752-0175
mhess@ucdavis.edu

ABSTRACT

Cyanobacteria are found in most illuminated environments and are key players in global carbon and nitrogen cycling. Although significant efforts have been made to advance our understanding of this important phylum, still little is known about how members of the cyanobacteria affect and respond to changes in complex biological systems. This lack of knowledge is in part due to the reliance on our ability to maintain pure cultures when determining the metabolism and function of a microorganism. To fill this knowledge-gap, we selected 26 photosynthetic co-cultures from the Culture Collection of Microorganisms from Extreme Environments (CCMEE) for 16S rRNA gene sequencing. We assessed if samples readily available from the CCMEE could contribute valuable insights to advance applied and fundamental science in the areas of global and local carbon and nitrogen cycling, without growing individual members of these co-cultures axenically. Results from this work will aid in determining whether culture depositories in general hold the potential to advance fundamental and applied research. Since maintaining culture depositories is resource intensive, such an assessment will be of great value in guiding future funding decisions.

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that are found in the majority of illuminated habitats and are known to be some of the most morphologically diverse prokaryotes on our planet [1]. The global cyanobacterial biomass is estimated to total ~$3x10^{14}$ g of carbon [2] and cyanobacteria may account for 20–30% of Earth's primary photosynthetic productivity [3]. The efficient photosynthetic machinery of cyanobacteria has inspired

growing interest in the utilization of axenic cyanobacteria as well as cyanobacteria containing co-cultures in microbial fuel cells [4,5]. In addition to having a global effect on the carbon cycle, cyanobacteria-mediated nitrogen fixation has been estimated to supply 20–50% of the nitrogen input in some marine environments [6]. A detailed comprehension of cyanobacteria and their contribution to global carbon and nitrogen cycling is therefore necessary for a multi-scalar understanding of these globally important nutrient cycles and ultimately for our ability to build accurate models to predict future climate patterns.

Besides their ecological relevance, cyanobacteria have potential applications in biotechnology. The photosynthetic metabolism of cyanobacteria facilitates the assimilation of carbon dioxide, a cheap and abundant substrate, to synthesize a variety of value-added compounds with industrial relevance [7]. Numerous strains of cyanobacteria have been investigated for their potential to produce bioactive compounds, biofertilizer, biofuels, and bioplastics [8]; and interactions of cyanobacterial strains with other bacteria have been found to improve desirable cyanobacterial phenotypes [9]. Genes encoding enzymes capable of catalyzing reactions that result in unique products, such as modified trichamide, a cyclic peptide suggested to protect the bloom-forming *Trichodesmium erythraeum* against predation [10], and prochlorosins, a family of lanthipeptides with diverse functions that are synthesized by various strains of *Prochlorococcus* and *Synechococcus* [11,12], have been identified from cyanobacterial genomes [13,14]. It is very likely that *de novo* genome assembly from metagenomic data will facilitate the discovery of novel enzymes from cyanobacteria for which we currently lack the appropriate isolation and cultivation techniques. Although metagenome-derived genomes hold great potential to enhance our knowledge about genomic dark matter, ultimately, improved techniques to isolate and enable axenic culturing of microorganisms that are currently characterized as

74 “unculturable”, as well as new genetic tools to work with non-axenic cultures will be
75 necessary in order to fully access the biotechnological potential of cyanobacteria.

76

77 Culture collections provide the possibility of preserving microbial isolates over extended
78 periods of time without introducing significant genetic changes [15] and they facilitate open
79 access to these isolates and their associated metadata [16]. Hence co-culture repositories
80 represent a promising starting point for developing and testing techniques to study and
81 manipulate uncultivated microbes. Although culture collections hold enormous potential
82 for capturing and preserving microbial biodiversity and for improving cultivation
83 techniques, there are numerous challenges in maintaining these biological depositories
84 and the individual samples they contain. A detailed understanding of the make-up of
85 individual co-cultures is essential to assess their true value and ultimately to develop
86 strategies that will be suitable to address challenges associated with long-term sample
87 maintenance. With recent advances in DNA sequencing technologies and the accessibility
88 of 16S rRNA gene-based microbial community profiling, we are now well positioned to re-
89 inventory and evaluate existing culture collections. Standardized inventories will facilitate
90 sample documentation for deposits maintained by individual laboratories and large culture
91 collections alike, which will be essential for cataloguing, preserving and surveying the
92 planet’s microbial biodiversity.

93

94 To explore the potential of culture collections, specifically those that maintain samples of
95 microbial co-cultures, to provide reference genomes from environmentally or industrially
96 relevant microorganisms, we reexamined the biodiversity of 26 historical phototrophic
97 samples from the Culture Collection of Microorganisms from Extreme Environments

98 (CCMEE). While some of the samples, and their dominant phototrophs were studied
99 previously using 16S rRNA profiling and morphological characterization [17-24] the diversity
100 of the photosynthetic and non-photosynthetic organisms and the overall community
101 assemblage of these co-cultures have not yet been characterized. To add further value to
102 this study, we selected samples that originated from diverse extreme environments across
103 the globe; with properties suggesting each co-culture would yield a unique microbial
104 consortium. An enhanced understanding of the microbial diversity that is preserved within
105 environmental co-cultures available through public culture collections will contribute to a
106 better understanding of global microbial biodiversity.

107

108 **Materials and Methods**

109

110 *Sample collection & sample description*

111 Co-cultures selected for this study are part of a larger culture collection and were
112 collected from different locations (Table 1) between 1988 and 2002. Isolates were
113 collected using sterile techniques, kept in the dark and stored on ice as quickly as
114 possible. Samples were transported to the laboratory where aliquots were prepared for
115 cultivation and preservation at -80°C. For this study, co-cultures were selected from the
116 CCMEE to cover a variety of geographical locations (Figure 1) as well as a range of
117 different ecosystems (Table 1). Due to the lack of a consistent terminology historically
118 used to describe the sampling sites, we categorized co-cultures according to the
119 geographical location (e.g. Antarctica, Bermuda, Denmark, Mexico and Spain) and
120 based on a general description of the habitat (i.e. creek, crust, freshwater, hot spring,
121 marine, saline pond, terrestrial, travertine, and tree bark) from where the co-cultures

122 were collected. In addition, we used the growth medium and temperature (i.e. 12°C,
123 23°C, 40°C, 45°C, 55°C) at which available co-cultures have been maintained
124 historically in the laboratory to categorize the co-cultures used in this study.

125 FECB1 (CCMEE ID 5011) and FECB3 (CCMEE ID 5034) were collected from saline and
126 brackish melt ponds in Antarctica respectively and were dominated by phototrophic
127 cyanobacteria classified as *Oscillatoria* sp [18]. FECB2 (CCMEE ID 5019) was collected
128 from a small freshwater pond (Pinnacle Pond) in the Ice Pinnacle area near Bratina
129 Island, Antarctica, and was phylogenetically uncharacterized prior to our efforts. FECB4
130 (CCMEE ID 5047; AP1) and FECB5 (CCMEE ID 5049; AO21) were isolated from Lake
131 Arcas, Spain and the dominant photosynthetic organisms within these samples were
132 classified by 16S rRNA sequence analysis as being related to *Pseudanabaena limnetica*
133 and *Oscillatoria* cf. *tenuis,* respectively [24]. FECB6 (CCMEE ID 5051), FECB14 (CCMEE
134 ID 5093; WT-97 Cal), FECB15 (CCMEE ID 5083), and FECB19 (CCMEE ID 5091; Y-97)
135 were collected from diverse hot springs and a warm hot spring outflow (warm creek)
136 within Yellowstone National Park (YNP) (Table 1). FECB10 (CCMEE ID 5056; M88-VD
137 (1)) was collected as epiliths from the Viscaino Desert in Mexico [23]. FECB17 (CCMEE ID
138 5085; RC-97 Cal) and FECB36 (CCMEE ID 6076) were isolated from Rabbit Creek and
139 a crust in the Sentinel Spring Meadows in YNP respectively and dominant phototrophs
140 of these co-cultures were characterized previously as *Calothrix* spp. [22]. FECB22
141 (CCMEE ID 5097; HW-91) and FECB26 (CCMEE ID 5099; B77-scy,j,) were collected
142 from a tree trunk in Hawaii and a wooded fence in Bermuda respectively. FECB24
143 (CCMEE ID 5098; AN-90) was obtained from a shallow melt pond (~10 m^2) in the
144 Victoria Valley, Antarctica, whereas FECB28 (CCMEE ID 5102) was collected from a
145 saline melt pond on Bratina Island, Antarctica [19]. FECB32 (CCMEE ID 6031), FECB34
146 (CCMEE ID 6069) and FECB38 (CCMEE ID 6083) were endoliths collected from

147 subsurface (1-5 mm depths) travertine deposits in YNP [20]. FECB53 (CCMEE ID 5610)
148 was collected from Sylvan Springs in YNP. Temperature and pH at the sampling site of
149 FECB53 were determined to be 40°C and pH4. The dominant phototropic strain in
150 FECB53 was identified previously as the thermo-acidophilic *Cyanidioschyzon* [21].
151 FECB58 (CCMEE ID 5216; OH-9-45C) and FECB68 (CCMEE ID 5240; OH-2-55C) were
152 collected from Hunter's Hot Spring in Oregon and the phototroph dominating these
153 samples was determined to be a thermophilic member belonging to the genus
154 *Synechococcus* [17].

155

156 *Growth of co-cultures*

157 To obtain sufficient biomass for subsequent DNA analysis, 100 µL of each co-culture
158 were transferred to 25 mL of sterile BG11 media [25]. For FECB52 and FECB53 BG11
159 was substituted by Cyanidium medium [26]. Co-cultures were subjected to a 12 hr diurnal
160 light/dark cycle while grown at the temperatures indicated in Table 1.

161

162 *DNA extraction and 16S rRNA gene amplification*

163 Total microbial DNA was extracted from 500 µL of each photosynthetic co-culture using
164 the FastDNA SPIN Kit for Soil (MP Biomedical, Solon, OH) according to the
165 manufacturer's instructions. Extracted DNA was quantified via fluorescence (Qubit;
166 Thermo Scientific, USA) and the hypervariable V4 region of the 16S rRNA gene was
167 amplified from extracted DNA using the primer set 515F/805R (515F: 5′-
168 GTGCCAGCMGCCGCGGTAA-3′ and 805R: 5′-GGACTACHVGGGTWTCTAAT-3′). The
169 forward primer included an 11 bp barcode to allow multiplexing of samples during
170 sequencing. The barcode sequence for each sample is listed in Supplemental Table S1.

171 Subsequent PCR reactions were performed using the 5PRIME HotMasterMix
172 amplification mix (QIAGEN, Beverly, MA) with the following PCR conditions: initial
173 denaturation for 90 sec at 94°C, followed by 30 amplification cycles (45 sec at 94°C, 60
174 sec at 60°C, and 90 sec at 72°C) followed by a final extension step of 72°C for 10 min.
175 Amplification products were cooled to 4°C. Samples were sequenced at the Department
176 of Energy's Joint Genome Institute (JGI; http://www.jgi.doe.gov) according to JGI's
177 standard operating procedure using Illumina's MiSeq platform and v3 chemistry.

178

179 *Sequence data analysis*

180 Raw sequencing data were downloaded from the JGI's Genome Portal
181 (http://genome.jgi.doe.gov/) under the project ID 1032475. Data were decompressed
182 and de-interleaved using the 7-zip software (www.7-zip.org) and an in-house script,
183 respectively. De-interleaved files were subsequently processed using MOTHUR version
184 1.38.1 [27,28]. Paired-end reads were combined using the *make.contigs* command.
185 Sequences with ambiguous base calls and sequences longer than 325 bp were removed
186 using *screen.seqs*. Duplicate sequences were merged using *unique.seqs*, and the
187 resulting unique sequences were aligned to the V4 region of the SILVA database (v123)
188 [29]. Chimeras were removed using UCHIME [30] and quality filtered sequences were
189 taxonomically classified at 80% confidence to the GreenGenes reference taxonomy
190 (release gg_13_5_99) [31]. Non-prokaryotic sequences were removed and the *dist.seqs*
191 command was used to calculate pairwise distances between the aligned sequences.
192 The resulting pairwise distance matrix was used to cluster sequences into operational
193 taxonomic units (OTUs) with a 97% sequence identity cut-off using UCLUST [32]. The
194 most abundant sequence of each OTU was picked as the representative sequence.
195 OTUs were taxonomically classified using the *classify.otu* command using the

196 GreenGenes reference taxonomy (release gg_13_5_99). Shannon, Simpson, and
197 Chao1 estimators were calculated in MOTHUR [27].

198

199 In order to visualize the overall compositional differences between the co-cultures, an
200 uncorrected pairwise distance matrix was generated using the *dist.seqs* command in
201 MOTHUR and a tree was generated using *Clearcut* (version 1.0.9*)* [33]. A cladogram from
202 the resulting tree file was constructed and visualized using iTOL (https://itol.embl.de;
203 accessed on October 16th, 2016; [34]). Cluster designations were assigned at a branch
204 length of 0.05. Samples whose branches split at a distance >0.05 were considered as
205 part of the same cluster (Figure 2).

206

207 **Availability of data and material**

208 Co-cultures subject to this study are publicly available through the CCMEE and the
209 UTEX Culture Collection of Algae at the University of Texas at Austin upon request
210 using the corresponding FECB ID (Table 1). Co-cultures can also be obtained from the
211 Hess Lab at UC Davis. Sequences generated during this project have been deposited
212 and are publicly available at NCBI's SRA under the BioProject ID PRJNA401502. All
213 other data is included in this published article and its supplementary information files.

214

215 **Results & Discussion**

216

217 A total of 3,357,905 raw reads (mean (SD) = 129,150 (±15,845) reads per sample) were
218 generated from the V4 region of the 16S rRNA gene (Table 2). Quality filtering removed
219 ~3.8% (±0.57%) of the raw reads from each sample due to insufficient quality. The

220 remaining reads were assigned to a total of 5,785 distinct Operational Taxonomic Units
221 (OTUs) based on 97% sequence identity (Table S2).

222

223 To estimate the microbial diversity within each sample, rarefaction analyses were
224 performed (Supplemental Figure S1) and diversity indices were calculated (Table 2). The
225 inverse Simpson index of the samples ranged between 1.52 and 9.24 with the lowest and
226 highest indices calculated for FECB3 and FECB32 respectively (Table 2). Not surprisingly,
227 the diversity in the co-cultures under investigation appeared to be negatively correlated
228 with the proportion of reads recruited by the dominant OTU of each sample (Pearson r =-
229 0.8806; p <0.01). Although samples ranked slightly differently based on their diversity
230 when Chao1 or Shannon indices were calculated, the overall trend remained the same
231 (Table 2).

232

233

234 *The McMurdo Dry Valley Lake System, a physically highly stable lacustrine system*

235 The McMurdo Dry Valley (MDV) is one of the most extreme deserts on Earth, and although
236 the importance of the microbial communities for the biogeochemical cycles of this region
237 is widely accepted, the microbial ecology of the MDV remains poorly understood [35].
238 FECB3, originating from a brackish pond on Bratina Island, was dominated by
239 OTU000003, which recruited 80.3% of all reads (Supplemental Table S2). OTU000003
240 was classified as the cyanobacterium *Phormidium pseudopriestleyi*, previously reported
241 to dominate microbial mats of the anoxic zone of Lake Fryxell, Antarctica [36]. The second
242 and third most abundant OTUs in FECB3 were OTU000015 and OTU000061 respectively
243 (Supplemental Table S2). Both OTU000015 and OTU000061 were classified as

244 Rhodobacteriaceae and recruited 9.2% and 8.2% of the reads generated for FECB3.
245 Whereas a taxonomic classification of OTU000015 was not possible at a resolution higher
246 than the family level, OTU000061 was classified as *Paracoccus marcusii,* a Gram negative
247 organism that displays a bright orange color due to the synthesis of carotenoids such as
248 astaxanthin [37].

249

250 While the microbial ecology of melt ponds and lakes in the MDV, habitats covered year-
251 round with an ice sheet, have been studied in great detail; most of the insights regarding
252 the microbial community assemblage in these waters are based primarily on microscopy
253 [36]. Molecular data, like those presented here and those that could theoretically be
254 generated from other MDV samples that are readily available from the CCMEE and other
255 culture collections, will be of great value to extend our knowledge framework of the
256 microbial ecology of this unique ecosystem.

257

258 *Omnipresence of Cyanobacteria and Proteobacteria within photosynthetic co-cultures*
259 While the microbial communities of the co-cultures under investigation varied greatly,
260 cyanobacteria and proteobacteria co-occurred in all 26 of the community assemblages.
261 Community composition analysis revealed that each of the co-cultures contained at least
262 one OTU (mean (SD) = 2 ($\pm$1.23)) that recruited >0.1% of the co-culture specific reads
263 and that was classified as *Cyanobacteria* (Table 3). The only other phylum present in
264 each of the individual 26 co-cultures and represented by at least one OTU recruiting
265 >0.1% of the reads was the *Proteobacteria* phylum (Table 3). In contrast, only three
266 samples, namely FECB5, FECB30 and FECB68, contained OTUs that recruited >0.1%
267 of the sample specific reads and that could not be classified at the phylum level or at a
268 higher taxonomic resolution (Table 3). It is possible that the relatively high abundance of

269 non-classified phyla might contribute to the separation of these samples into distinct
270 clusters (i.e. cluster XII, IX, and IV) (Figure 2). In addition to their omnipresence,
271 *Cyanobacteria* and *Proteobacteria* also recruited the majority of the reads in all but four
272 (i.e. FECB2, FECB12, FECB58, and FECB68) of the samples under investigation
273 (Figure 3 and Supplemental Table S3). In FECB2 and FECB12 the majority of the reads
274 were recruited by OTUs classified as members of the phylum *Bacteroidetes* (recruiting
275 50.6% and 72% of the reads respectively), whereas within FECB58 and FECB68,
276 *Armatimonadetes* (38.3%) and *Chloroflexi* (25.9%) were identified as the most abundant
277 phyla (Figure 3 and Supplemental Table S3). The fact that these samples were
278 dominated by phyla other than the *Cyanobacteria* or *Proteobacteria* may also help to
279 explain why these samples (Figure 2) form distinct clusters (cluster I, XIV and V, IV
280 respectively).
281
282
283 *Firmicutes dominate photosynthetic co-cultures from hot springs*
284 *Firmicutes* abundances calculated for co-cultures from hot spring samples were higher
285 compared to those calculated for co-cultures from other environments studied during this
286 project. OTUs assigned to the *Firmicutes* phylum were detected above the applied cut-
287 off level of 0.1% in only four of the twenty-six co-cultures under investigation (Table 3).
288 Interestingly, these samples (i.e. FECB34, FECB52, FECB58 and FECB68) are co-
289 cultures collected from hot springs or deposits within hot springs, with FECB52, FECB58
290 and FECB68 being maintained in culture at temperatures >40°C. OTU000073 (classified
291 as *Alicyclobacillus tolerans*), OTU00082 (classified as members of the genus
292 *Paenibacillus*), OTU000154 (classified as *Geobacillus vulcani*), and OTU000158
293 (classified as a member of the *Bacillaceae* family) recruited 5.9%, 3.4% , 0.5% and 0.4%

294 of the reads generated from FECB52, FECB34, FECB68 and FECB58 respectively
295 (Supplemental Table S2). *Alicyclobacillus tolerans* and *Geobacillus vulcani* have been
296 described previously as aerobic spore-forming thermophiles and have been isolated
297 from lead–zinc ores [38] and hot springs [39] in Russia, respectively. Members of the genus
298 *Paenibacillus* have been isolated from a wide variety of environments and some
299 *Paenibacillus* species have been found to promote crop growth directly via biological
300 nitrogen fixation, phosphate solubilization, production of the phytohormone indole-3-
301 acetic acid and they have been identified as a potential source of novel antimicrobial
302 agents [40]. Although it is difficult to make a reliable prediction of the metabolic capacities
303 of the organism associated with OTU000082 solely based on 16S rRNA data, it is
304 certainly possible that this organism might possess the ability to promote or inhibit plant
305 and microbial growth respectively.

306

307 *Photosynthetic co-cultures from Antarctica and YNP to study adaptation to increased*
308 *radiation, low temperatures and oligotrophic growth conditions*

309 Microbial adaptation to extreme environments and the molecular framework that allows
310 microorganisms to survive and thrive in the presence of increased rates of radiation, low
311 temperatures and in the absence of nutrients has fascinated the scientific community for
312 decades and remains poorly understood. In an attempt to provide a better basis of the
313 taxonomic make-up of co-cultures that were collected from ecosystems that are
314 characterized by these extremes we included co-cultures from Antarctica and YNP in
315 this study (Table 1). OTU-based comparison of Antarctica and YNP co-cultures revealed
316 between 197 (FECB2) and 549 (FECB6) distinct OTUs (mean (SD) = 342 ($\pm$87.2)
317 OTUs), based on 97% sequence similarity (Table 2). The number of OTUs that recruited
318 >0.1% of all reads ranged from 3 to 29 OTUs, with FECB2 and FECB32 having the

319 lowest and highest OTU count respectively (Table 2). FECB2 was dominated by an OTU
320 classified as *Hymenobacter,* which recruited all *Bacteroidetes*-specific reads generated
321 from this sample (Tables 3 & 4). The genus *Hymenobacter* contains several pigmented
322 bacteria that have been isolated from Antarctica and have been reported to possess
323 increased resistance to radiation [41,42], which might explain their increased abundance in
324 FECB2, a co-culture isolated from an environment known to possess increased levels of
325 UV radiation. Taking this into consideration, FECB2 and its individual community
326 members could be a potential target for future studies to enhance our understanding of
327 processes that infer resistance to radiation and DNA damage. The second most
328 abundant OTU in FECB2, recruiting 48% of the generated samples, was classified as
329 *Phormidium* sp (Supplemental Table S2), a cyanobacterial genus that has been reported
330 to dominate aquatic microbial mats from Antarctica [43,44]. Representative isolates from
331 this genus have been proposed previously as cost-effective options for industrial
332 carotenoid production [45], suggesting that FECB2 may hold the potential for industrial
333 carotenoid production.

334

335 FECB32 is a mixed culture isolated from an ancient travertine at Mammoth in YNP. Our
336 analysis indicated that FECB32 contained 29 OTUs that each accounted for >0.1% of all
337 the generated reads (Table 2). Fifteen of these OTUs recruited >1% of all reads and 4
338 OTUs collectively accounted for ~60% of the reads generated from this co-culture
339 (Supplemental Table S4). These 4 OTUs were classified as *Sphingopyxis alaskensis,*
340 *Chelativorans sp.* and as members of the *Chitinophagaceae* and *Comamonadaceae*
341 *families,* recruiting ~19%, 13%, 17%, and 11% of the reads respectively (Supplemental
342 Tables S2 & S4). *Sphingopyxis alaskensis* is a Gram negative bacterium found in
343 relatively high abundance in oligotrophic regions of the ocean [46,47] and it has been

344 studied in great detail as a model marine bacterium, specifically to understand microbial
345 adaptation to cold or oligotrophic environments [48,49]. The *Chitinophagaceae* family
346 contains a wide phylogenetic diversity with many of its members being mesophilic.
347 However, *Chitinophagaceae* have been reported to grow optimally at temperatures of
348 55°C and higher [50,51].

349

350 *Photosynthetic co-cultures containing the deep-branching candidate phylum*
351 *Melainabacteria*

352 Extreme environments, similar to those on early Earth, are often proposed to hold critical
353 information about the historical progression of life on our planet and one niche that
354 encompasses those physical stresses is the endolithic environment of rocks [20].
355 Phylogenetic analysis of the heterotrophic population associated with FECB32, which
356 was isolated from travertine deposited by hot springs in YNP, found that sequences from
357 MLE-12 (OTU000109) recruited ~2% of the sample specific sequences (Supplemental
358 Table S2). This rendered MLE-12, previously assigned to the deep-branching candidate
359 phylum *Melainabacteria* [52], as the eleventh most abundant organism in this
360 photosynthetic co-culture. It has been proposed previously that *Melainabacteria*, which is
361 commonly found in aquatic habitats, separated from the cyanobacteria before the latter
362 acquired photosynthetic capabilities [52]. Hence FECB32 might be a particularly valuable
363 co-culture to generate new insights into the evolution of and relationship between the
364 phylogenetically closely related *Cyanobacteria* and *Melainabacteria*. In addition, this
365 sample might provide the opportunity to enhance our understanding of the origin of
366 oxygenic photosynthesis and aerobic respiration in *Cyanobacteria*, an area that is
367 currently still poorly understood [53].

368

369 Interestingly, OTU000109 was also detected in FECB36 and FECB38 (Supplemental
370 Table S2), although at significantly lower abundance (<0.001%). FECB36 and FECB38
371 were similar to FECB32 in that they were isolated from sites in YNP. Interestingly,
372 FECB32 and FECB38 cluster together (cluster IX) suggesting a similar overall microbial
373 community profiles, but separately from FECB36 (Figure 2). The only additional samples
374 that contained OTUs classified as *Melainabacteria,* recruiting >0.1% of the generated
375 reads, were FECB58 and FECB68 with ~0.9% and ~0.2% of their reads to this deeply
376 branched phylum, respectively (Supplemental Table S2). It seems noteworthy that
377 FECB58 and FECB68 were also isolated from hot springs and clustered closely together
378 based on their overall microbiome composition (Clusters V and IV respectively; Figure
379 2).

380

381 *Photosynthetic co-cultures from Hunter's Hot Spring, Oregon*

382 Hunters Hot Spring continues to be a source of interesting microbial activity. FECB58
383 and FECB68 were both isolated from Hunters Hot Spring in Oregon, USA and they
384 shared similar microbial community members. Despite their similar community profile,
385 abundances of the dominant OTUs associated with these two hot spring co-cultures
386 were remarkably different. FECB58 was dominated by 3 OTUs (OTU000014,
387 OTU000024, and OTU000033). OTU000014 was classified as OS-L, an uncultured
388 representative of the phylum *Armatimonadetes*, OTU000024 which was classified as
389 belonging to the *Bacteroidetes* phylum, and OTU000033 which was classified as
390 *Thermosynechococcus*. These OTUs contributed 38%, 29% and 20% of the reads
391 generated from FECB58 respectively. Whereas OTU000014 recruited ~4.9% of all reads

392 generated from FECB68, representing the sixth most abundant OTU in the FECB68
393 community, OTU000024 and OTU000033 were only present at an abundance <0.0001%
394 in FECB68 (Supplemental Table S2).

395

396 FECB68 was dominated by 6 OTUs (i.e. OTU000028, OTU000030, OTU000036,
397 OTU000049, OTU000065, and OTU000014) recruiting ~25.7%, 23.1%, 20.4%, 14.3%,
398 7.6%, and 4.9% of the reads respectively. OTU000028 was classified as belonging to
399 the genus *Chloroflexus*, whereas OTU000030 and OTU000036 were classified as
400 representative of the genus *Meiothermus* and *Gloeobacter*, respectively. *Chloroflexus* is
401 an anoxygenic phototrophic bacterium that grows at temperatures up to 70°C [54] and
402 forms yellow-orange-greenish mats in association with cyanobacteria [55]. Members of the
403 cyanobacterial genus *Gloeobacter* lack thylakoids, and have been proposed to host the
404 earliest ancestors, or a missing link, in the cyanobacteria lineage [56]. Thus, FECB68
405 offers a unique opportunity to investigate interspecies interaction between a member of
406 these basal cyanobacteria and the thermophilic phototroph *Chloroflexus*, represented by
407 OTU000028 in this co-culture. As outlined in a recent review [54], Hunter's Hot Spring
408 located in Oregon is one of the most studied hot springs in the world, and has a large
409 repertoire of work conducted over the last 40 years [54]. However, most of this work was
410 performed prior to the advent of recent molecular techniques. Hence, the sequencing
411 data generated from FECB58 and FECB68 during this study will complement previous
412 work performed using traditional microbiology techniques and facilitate new insights into
413 the microbiology of this unique ecosystem.

414

415 *Photosynthetic co-cultures from lignocellulosic surfaces with potential to fix nitrogen and*
416 *degrade aromatic compounds.*

417 FECB22 and FECB26 are mesophilic co-cultures collected from similar habitats (i.e.
418 from tree bark and a wooden fence) from two locations (i.e. Hawaii and Bermuda)
419 approximately 9,000 kilometers apart from each other (Figure 1 & Table 1). Diversity
420 index calculation placed these two samples in the mid-range of the diversity spectrum of
421 the 26 co-cultures analyzed for this study. The inverse Simpson, Chao1, and Shannon
422 index was calculated at 4.46, 937.11 and 2.08 for FECB22 and 2.29, 722.94, and 1.32
423 for FECB26, respectively (Table 2). Within FECB22, 23 OTUs were identified as
424 individually recruiting more than 0.1% of the generated reads. In contrast, FECB26
425 contained only 16 OTUs that recruited more than 0.1% of the reads each (Table S2).
426 FECB22, scraped from tree bark in Hawaii, was dominated by 11 OTUs, each recruiting
427 >1% of the reads. The most abundant OTU (OTU000017) was classified as a member of
428 the *Mycoplana*, a genus that contains bacteria capable of aromatic compound
429 degradation [57], and it recruited 40.2% of the reads. OTU000042 (classified as *Rhizobium*
430 *leguminosarum*), OTU000045 (classified as *Acetobacteraceae*), and OTU000072
431 (classified as *Cyanobacteria*), were the next most abundant OTUs, recruiting 17.1%,
432 16.3%, and 5.5% of the reads generated from FECB22 respectively. *Rhizobium*
433 *leguminosarum* is a well-studied α-proteobacterium capable of N_2-fixation and "rhizobia"
434 have been suggested repeatedly to facilitate more sustainable agricultural practices
435 through their symbiosis with legumes, reducing the need for nitrogen fertilizer [58]. It
436 remains to be seen if OTU000042 provides N_2 to the other organisms in this co-culture
437 or if it consumes all of the fixed N_2 itself. *Acetobacteraceae* are α-proteobacteria often
438 associated with low pH environments and are known for their ability to efficiently
439 synthesize biological cellulose [59,60]. Furthermore, *Acetobacteraceae* have been reported

440 before as some of the dominant players in photosynthetic consortia during soil formation
441 [61]. It would be interesting to explore the agricultural and chemical potential of a
442 minimalistic co-culture composed of the 4 OTUs (i.e. OTU000017, OTU000042,
443 OTU000045 and OTU000072) that dominated FECB22, as they may combine the ability
444 to degrade aromatic compounds and synthesize cellulose while removing nitrogen from
445 the atmosphere. FECB26, on the other hand, was dominated by OTU000010, which
446 recruited 63.2% of the reads generated and classified as an unclassified member of the
447 *Nostocales*; a phylogenetic group known for their functional and morphological diversity.
448 Members of the *Sphingomonadaceae* (i.e. OTU000041 and OTU000062), phototropic α-
449 proteobacteria often found in high abundance in environments previously thought to
450 support mostly the growth of cyanobacteria [62], contributed to a total of 25.6% of the
451 generated reads. Most interestingly, OTU000017 was also identified within FECB26
452 recruiting ~1.6% of the reads. It is possible that OTU000017 facilitates a metabolic
453 reaction, in which aromatic compounds typically associated with the decomposition of
454 woody material under aerobic conditions are utilized. Further characterization of this
455 organism in co-culture and eventually in axenic culture might provide further clarity if this
456 is the case.

457

458 **Conclusion**

459

460 Culture collections can provide easy access to biological samples without the need for
461 extensive resources by the requesting individual, subsequently facilitating new studies
462 and ultimately advancing our understanding and appreciation of phylogenetic and
463 functional biodiversity. The 16S rRNA based community fingerprints of the 26

464 photosynthetic co-cultures described here provide us with a first glimpse into the
465 taxonomic and functional diversity of communities from extreme environments that were
466 considered for a long time as too harsh to support the growth of complex microbial
467 communities. The extreme conditions that are associated with the habitats from where
468 these co-cultures were collected offer the unique opportunity to study the molecular
469 mechanisms that support the growth of these extremophilic co-cultures and their role in
470 global carbon and nitrogen cycling. Furthermore, an in-depth understanding of these
471 extreme co-cultures holds the potential to discover novel microbial proteins that might
472 render current agricultural, industrial and medical processes more economical and
473 sustainable. The relatively low diversity and complexity of these co-cultures make them
474 ideal subjects to investigate symbiotic relationships. By determining the chemical and
475 physical requirements of individual community members in the low complexity co-cultures
476 described here, the goal of enabling their axenic growth is promising. Advancing our ability
477 to infer the metabolic requirements of individual hitherto uncultivable microorganisms is of
478 great importance as has been shown by a large body of work including the "Genomic
479 Encyclopedia of Bacteria and Archaea" (GEBA) and CyanoGEBA projects, multi-
480 investigator projects spearheaded by the Kerfeld and Eisen group in collaboration with
481 Drs. Krypides and Woyke from the DOE's Joint Genome Institute [63-65]. However,
482 bioinformatics and wet-lab tools to dissect complex microbial communities and processes
483 into their individual components are still in their infancy and obtaining pure isolates from
484 complex microbial communities still represents a major challenge. The photosynthetic co-
485 cultures utilized in this work represent excellent model systems for tool development and
486 verification due to their relatively low community complexity and their public availability via
487 culture collections such as the CCMEE and UTEX.

488

489 This study highlights a major challenge (i.e. standardization of protocols) associated with
490 environmental samples and sample data obtained during independent sampling efforts.
491 Fortunately, with recent advances in data technologies, the task of data acquisition and
492 dissemination has become less of a challenge. Recording standardized geographical and
493 environmental data, such as latitude, longitude, elevation, and temperature, can now be
494 performed with relatively high accuracy on a range of electronic devices - from most cell
495 phones, to inexpensive handheld devices developed specifically for accurate data
496 acquisition under field conditions. In order to make the best use of these technologies and
497 of biological samples that will be collected, defining a set of minimal information
498 parameters to be recorded during the collection of an environmental sample is of great
499 importance. Similar efforts have been successfully implemented by the Genomic
500 Standards Consortium (GSC) for microbial genomes and metagenomes in the form of the
501 "minimum information about a genome sequence" (MIGS) [66] and are enforced when
502 describing a novel microbial species [67]. It is understandable that characteristics suitable
503 as "minimal information" for environmental samples might differ from those that have been
504 established for axenic isolates and their genomes. However, as long as there is
505 consistency, the reported data will provide a valuable starting point for future efforts to
506 retroactively study archived samples.

507

508 16S rRNA gene profiling has become a commodity and easily generated phylogenetic
509 fingerprints provide a useful starting point to classify and categorize environmental
510 samples of microbial co-cultures. Due to the wide availability and continuing decline in
511 cost, this technique provides an ideal approach to re-examine the phylogenetic makeup
512 of legacy samples before they disappear due to continuous decline in funding for
513 maintaining small and non-centralized culture collections. In combination with biochemical

514 measurements, such as carbon and nitrogen utilization capabilities, the scientific
515 community would have immediate access to the phylogenetic and functional diversity
516 available through the existing culture collections. The identification of **M**inimum
517 **I**nformation about a **C**o-**C**ulture **S**ample (MICCS) would be a significant step in
518 standardizing sample acquisition and maintenance, increasing the value of current and
519 future microbial samples collected from the environment. Developing MICCS and applying
520 them to co-cultures currently available from existing culture depositories is beyond the
521 scope of the work presented here, but we hope that the results presented here will
522 contribute to the initiation of this process and stimulate broad involvement and support
523 from the scientific community and various funding agencies.

524

525 Another noteworthy aspect of samples readily available through existing culture
526 collections, including the consortia discussed in this work, is their educational value. More
527 specifically, samples that can be acquired and maintained without the need of significant
528 resources and for which basic phylogenetic and functional information is available. These
529 co-cultures provide a unique opportunity for exciting undergraduate research, in
530 combining microbial diversity, microbial ecology and biotechnology. Techniques for basic
531 biochemical and physiological characterizations of these samples could be learned and
532 conducted by dedicated undergraduate students within a few weeks. A research program
533 based on these co-cultures would provide students with the unique opportunity to develop
534 laboratory skills and to learn firsthand about biogeochemical processes that shape our
535 environment and climate. Additional publicly available *omics* data, such as metagenomics
536 and metatranscriptomics generated from individual samples, would extend the scope of
537 these undergraduate research programs, in providing students the opportunity to learn

538 various *omics* analysis techniques using web-based tools or standalone scripts,
539 depending on the educational level and interest of each student.

540

541 In summary, culture collections that provide access to and standardized information
542 about microorganisms and microbial consortia provide opportunities for educational and
543 scientific progress. Therefore, it is of high importance that culture collections continue to
544 obtain the financial support necessary to provide this invaluable service to our society

545

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777

778 **Conflict of Interest:** The authors declare no conflicts of interest.

779

780 **Author Contributions**

781 Charles Brooke, Richard Castenholz, David E. Culley, Matthias Hess, and Susannah G.

782 Tringe wrote the manuscript. Richard Castenholz and Matthias Hess designed the

783 experiment. Erik Hawley and Matthias Hess performed experiment. Michael Barton,

784 David E. Culley, Tijana Glavina del Rio, Miranda Harmon-Smith, Erik Hawley, Matthias

785 Hess, Nicole Shapiro, and Susannah G. Tringe generated the data. Michael Barton,

786 Charles Brooke, Morgan P. Connolly, David E. Culley, Javier A. Garcia, Tijana Glavina

del Rio, Miranda Harmon-Smith, Erik Hawley, Matthias Hess, and Nicole Shapiro analyzed the data.

789 **Figures & Tables Legends**

790 **Figure 1: Geographical locations of co-cultures analyzed.** 1.) Antarctica. McMurdo
791 Ice Shelf; Bratina Island; 2.) Spain. Lake Arcas; 3.) USA. Yellowstone National Park; 4.)
792 Mexico. Vizcaino Desert; 5.) USA. Eugene, Oregon. 6.) USA. Hawaii; 7.) Bermuda,
793 Somerset; 8.) Denmark. Limfjord Shallows; 9.) USA. Hunter's Hot Spring, Oregon. (Map
794 downloaded and adapted from
795 https://commons.wikimedia.org/wiki/File:ColoredBlankMap-World-10E.svg#file)

796

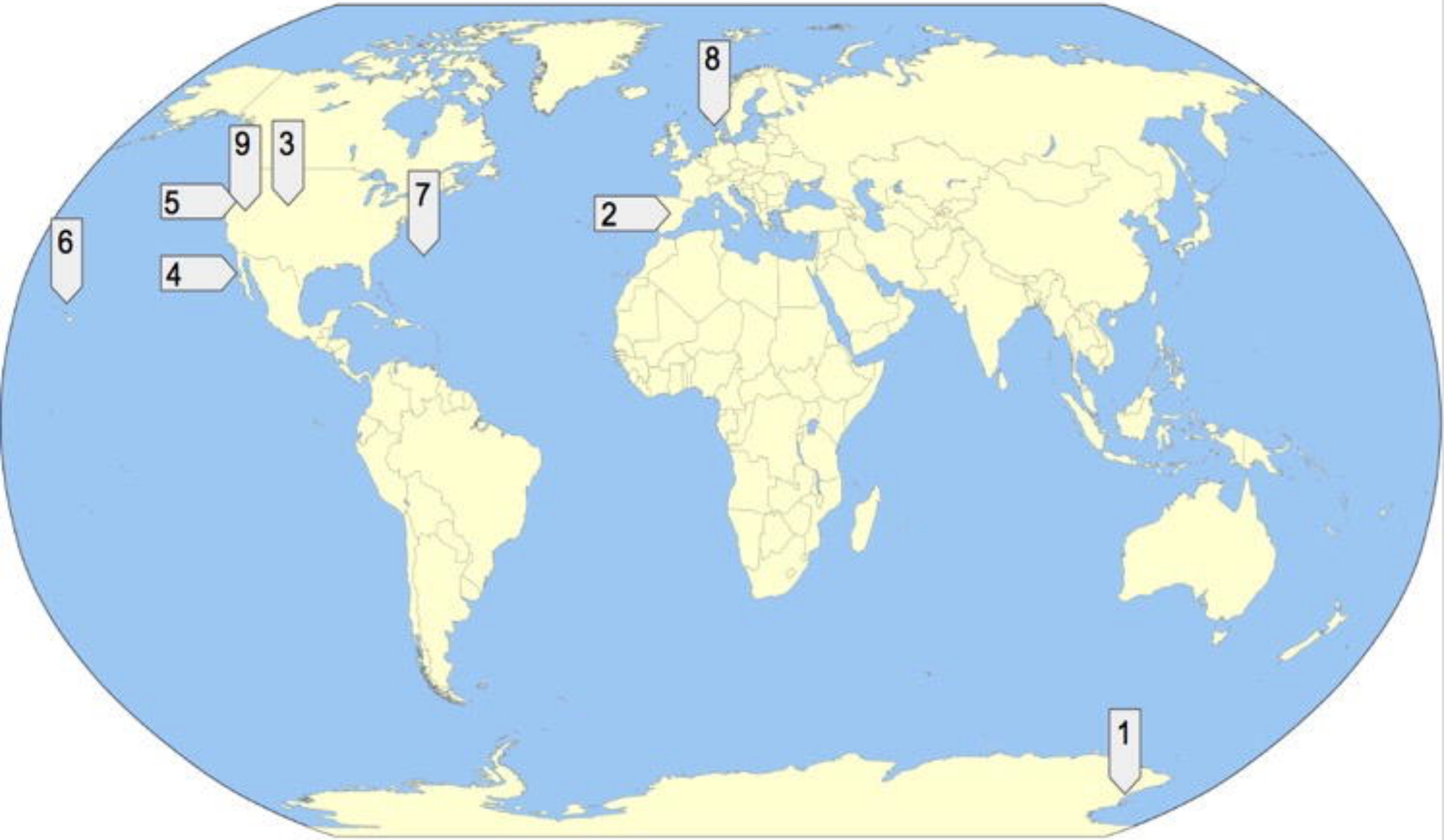
797 **Figure 2: Cladogram of 16S rRNA based community composition of co-cultures**
798 **under investigation.** FECB ID are provided for each co-culture. Sample location is
799 indicated on the branch. Roman numerals on the right indicate the clusters identified at a
800 branch cutoff of 0.05. Symbols (i.e. circles and squares) next to the sample ID indicate
801 habitat type and their color indicates the temperatures at which samples were historically
802 maintained in the CCMEE.

803

804 **Figure 3: Relative abundance of phyla associated with phototrophic co-cultures.**
805 **16S rRNA based community profile.** Only phyla recruiting >1% of the reads in at least
806 one of the co-cultures are shown.

807

808 **Table 1: Summary of photosynthetic co-cultures for which 16S rRNA gene profiles**
809 **were generated.**
810
811 **Table 2: Sequencing statistics and diversity indices for co-cultures investigated in**
812 **this study.**
813
814 **Table 3: Count and phylogenetic classification of identified OTUs at the phylum**
815 **level.** Only OTUs recruiting >0.1% of the co-culture specific reads are shown.
816
817 **Table 4: Taxonomy relative abundance of dominant OTU identified in each co-**
818 **culture.**
819
820
821
822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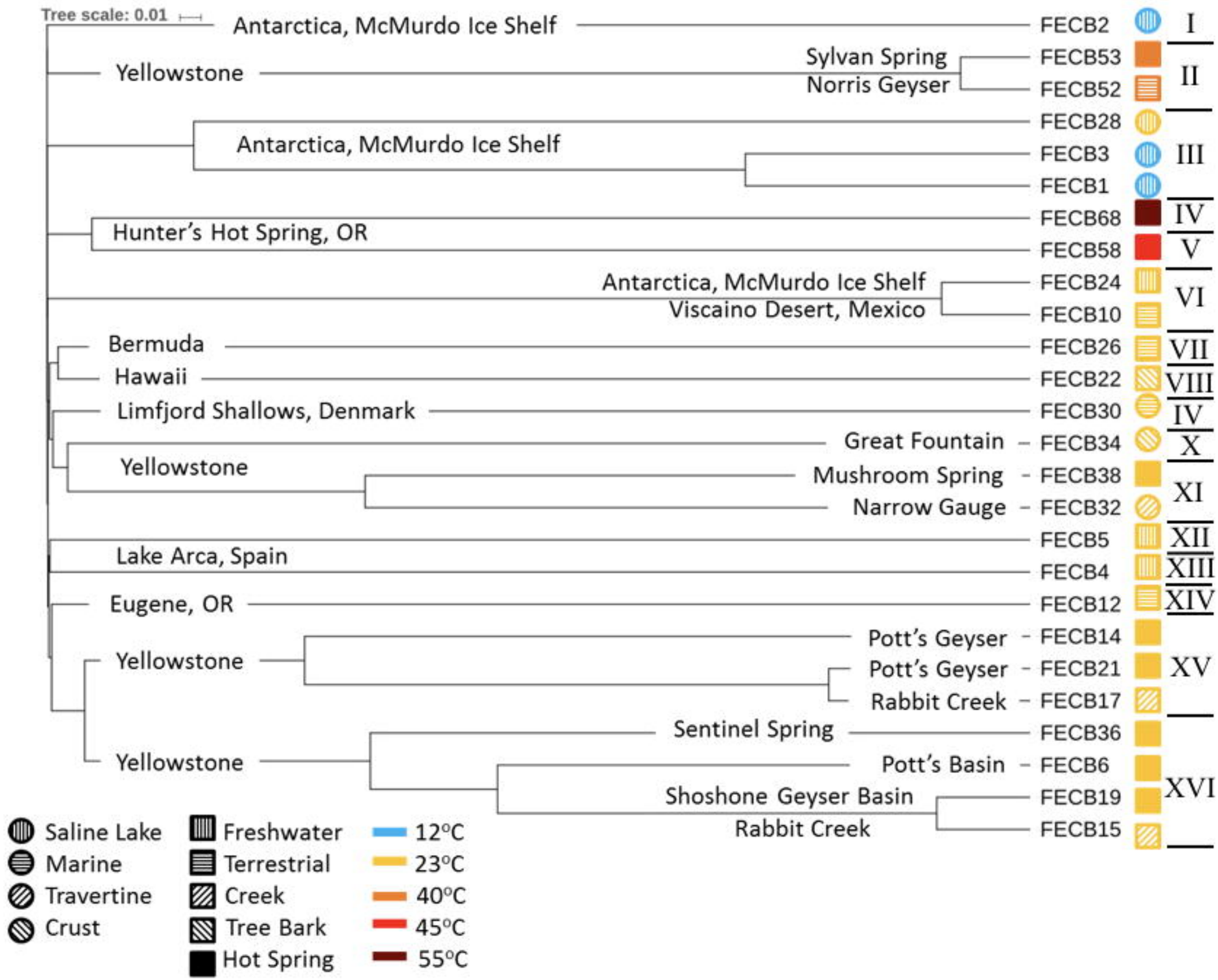
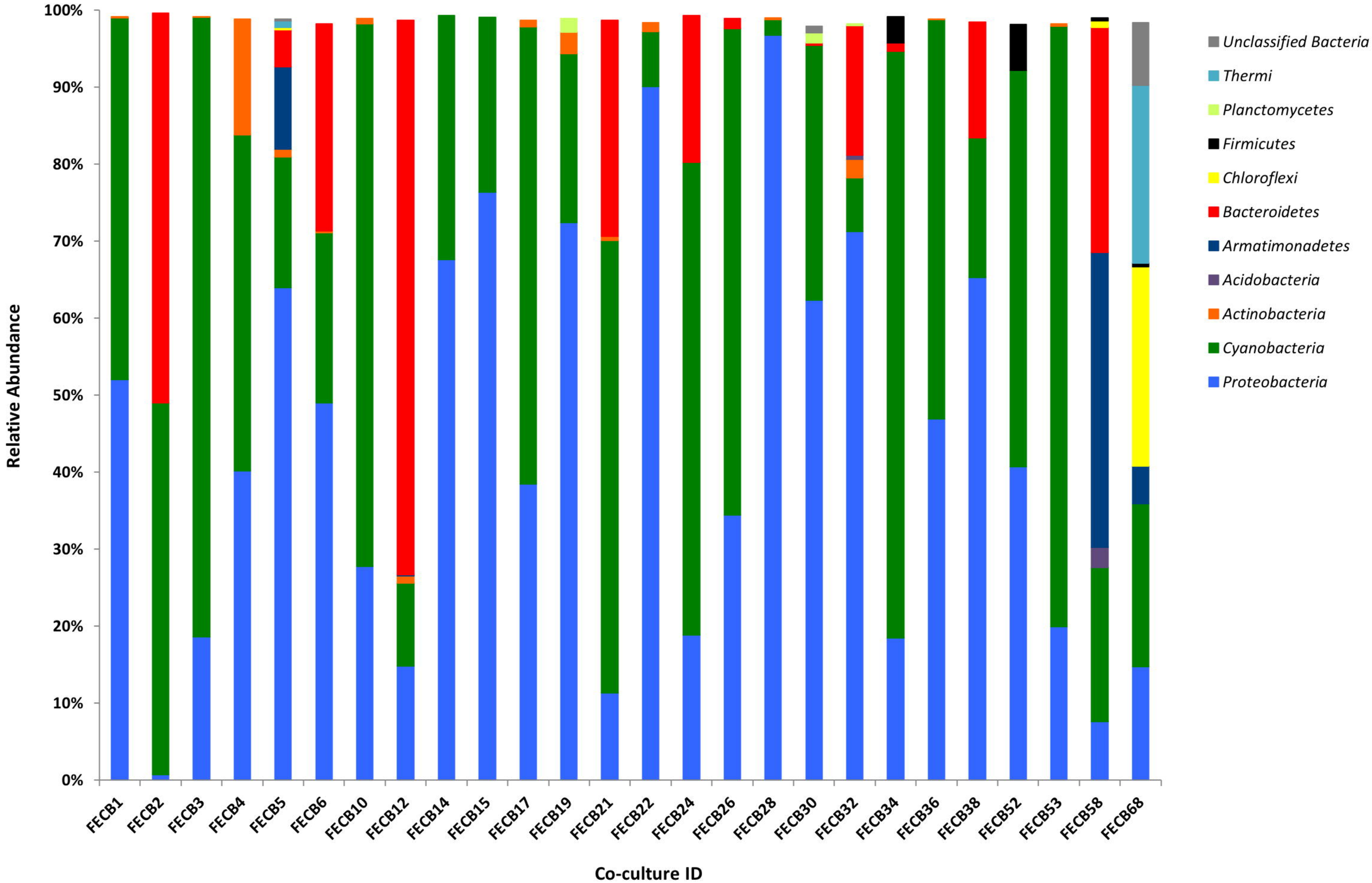

Tree scale: 0.01
Antarctica, McMurdo Ice Shelf
FECB2
I
Yellowstone
Sylvan Spring
Norris Geyser
FECB53
FECB52
II
FECB28
Antarctica, McMurdo Ice Shelf
FECB3
FECB1
III
Hunter's Hot Spring, OR
FECB68
IV
FECB58
V
Antarctica, McMurdo Ice Shelf
Viscaino Desert, Mexico
FECB24
FECB10
VI
Bermuda
FECB26
VII
Hawaii
FECB22
VIII
Limfjord Shallows, Denmark
FECB30
IV
Yellowstone
Great Fountain
FECB34
X
Mushroom Spring
FECB38
Narrow Gauge
FECB32
XI
Lake Arca, Spain
FECB5
XII
FECB4
XIII
Eugene, OR
FECB12
XIV
Yellowstone
Pott's Geyser
FECB14
Pott's Geyser
FECB21
Rabbit Creek
FECB17
XV
Yellowstone
Sentinel Spring
FECB36
Pott's Basin
FECB6
Shoshone Geyser Basin
FECB19
Rabbit Creek
FECB15
XVI
Saline Lake
Marine
Travertine
Crust
Freshwater
Terrestrial
Creek
Tree Bark
Hot Spring
12°C
23°C
40°C
45°C
55°C


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative Abundance
FECB1
FECB2
FECB3
FECB4
FECB5
FECB6
FECB10
FECB12
FECB14
FECB15
FECB17
FECB19
FECB21
FECB22
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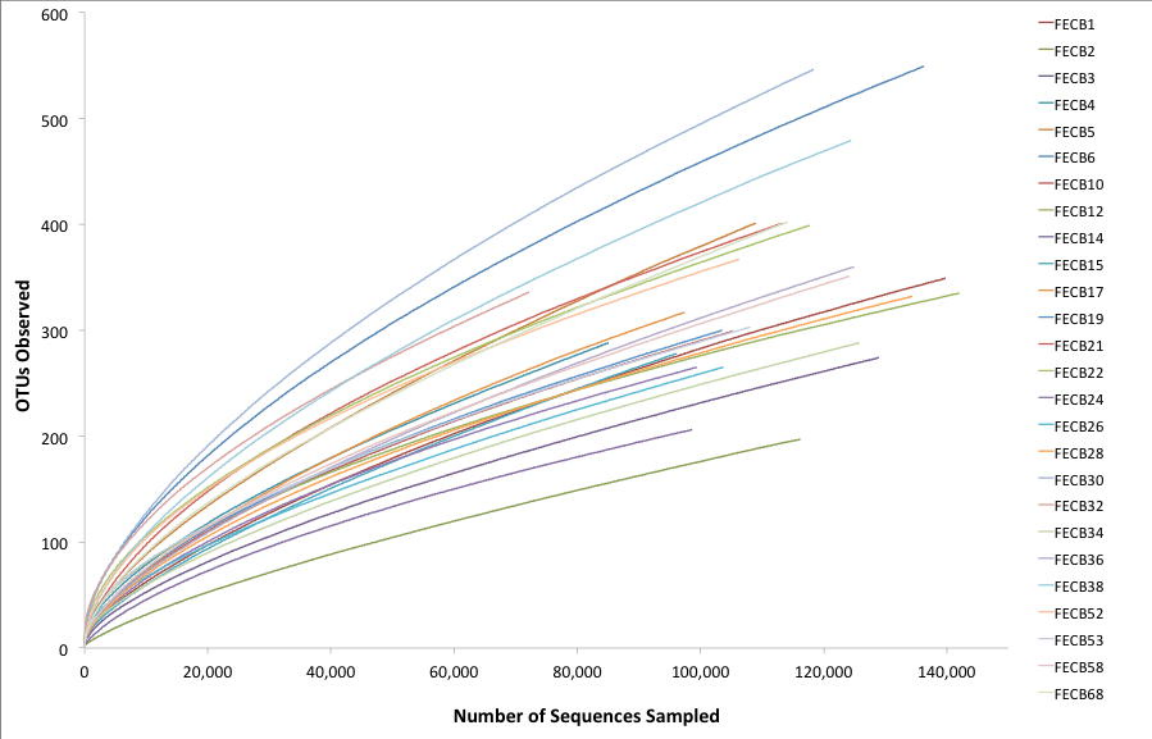
FECB28
FECB30
FECB32
FECB34
FECB36
FECB38
FECB52
FECB53
FECB58
FECB68
Co-culture ID
Unclassified Bacteria
Thermi
Planctomycetes
Firmicutes
Chloroflexi
Bacteroidetes
Armatimonadetes
Acidobacteria
Actinobacteria
Cyanobacteria
Proteobacteria


OTUs Observed
600
500
400
300
200
100
0
0
20,000
40,000
60,000
80,000
100,000
120,000
140,000
Number of Sequences Sampled
FECB1
FECB2
FECB3
FECB4
FECB5
FECB6
FECB10
FECB12
FECB14
FECB15
FECB17
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FECB21
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