

1 **Title:** Community structure of phototrophic co-cultures from extreme environments
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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 $\sim 3 \times 10^{14}$ 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

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

Culture collections provide the possibility of preserving microbial isolates over extended periods of time without introducing significant genetic changes¹⁵ and they facilitate open access to these isolates and their associated metadata¹⁶. Hence co-culture repositories represent a promising starting point for developing and testing techniques to study and manipulate uncultivated microbes. Although culture collections hold enormous potential for capturing and preserving microbial biodiversity and for improving cultivation techniques, there are numerous challenges in maintaining these biological depositories and the individual samples they contain. A detailed understanding of the make-up of individual co-cultures is essential to assess their true value and ultimately to develop strategies that will be suitable to address challenges associated with long-term sample maintenance. With recent advances in DNA sequencing technologies and the accessibility of 16S rRNA gene-based microbial community profiling, we are now well positioned to re-inventory and evaluate existing culture collections. Standardized inventories will facilitate sample documentation for deposits maintained by individual laboratories and large culture collections alike, which will be essential for cataloguing, preserving and surveying the planet’s microbial biodiversity.

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

(CCMEE). While some of the samples, and their dominant phototrophs were studied previously using 16S rRNA profiling and morphological characterization¹⁷⁻²⁴ the diversity of the photosynthetic and non-photosynthetic organisms and the overall community assemblage of these co-cultures have not yet been characterized. To add further value to this study, we selected samples that originated from diverse extreme environments across the globe; with properties suggesting each co-culture would yield a unique microbial consortium. An enhanced understanding of the microbial diversity that is preserved within environmental co-cultures available through public culture collections will contribute to a better understanding of global microbial biodiversity.

Materials and Methods

Sample collection & sample description

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

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

FECB1 (CCMEE ID 5011) and FECB3 (CCMEE ID 5034) were collected from saline and brackish melt ponds in Antarctica respectively and were dominated by phototrophic cyanobacteria classified as *Oscillatoria* sp.¹⁸. FECB2 (CCMEE ID 5019) was collected from a small freshwater pond (Pinnacle Pond) in the Ice Pinnacle area near Bratina Island, Antarctica, and was phylogenetically uncharacterized prior to our efforts. FECB4 (CCMEE ID 5047; AP1) and FECB5 (CCMEE ID 5049; AO21) were isolated from Lake Arcas, Spain and the dominant photosynthetic organisms within these samples were classified by 16S rRNA sequence analysis as being related to *Pseudanabaena limnetica* and *Oscillatoria* cf. *tenuis*, respectively²⁴. FECB6 (CCMEE ID 5051), FECB14 (CCMEE ID 5093; WT-97 Cal), FECB15 (CCMEE ID 5083), and FECB19 (CCMEE ID 5091; Y-97) were collected from diverse hot springs and a warm hot spring outflow (warm creek) within Yellowstone National Park (YNP) (Table 1). FECB10 (CCMEE ID 5056; M88-VD (1)) was collected as epiliths from the Viscaino Desert in Mexico²³. FECB17 (CCMEE ID 5085; RC-97 Cal) and FECB36 (CCMEE ID 6076) were isolated from Rabbit Creek and a crust in the Sentinel Spring Meadows in YNP respectively and dominant phototrophs of these co-cultures were characterized previously as *Calothrix* spp.²². FECB22 (CCMEE ID 5097; HW-91) and FECB26 (CCMEE ID 5099; B77-scy,j,) were collected from a tree trunk in Hawaii and a wooded fence in Bermuda respectively. FECB24 (CCMEE ID 5098; AN-90) was obtained from a shallow melt pond (~10 m²) in the Victoria Valley, Antarctica, whereas FECB28 (CCMEE ID 5102) was collected from a saline melt pond on Bratina Island, Antarctica¹⁹. FECB32 (CCMEE ID 6031), FECB34 (CCMEE ID 6069) and FECB38 (CCMEE ID 6083) were endoliths collected from

subsurface (1-5 mm depths) travertine deposits in YNP²⁰. FECB53 (CCMEE ID 5610) was collected from Sylvan Springs in YNP. Temperature and pH at the sampling site of FECB53 were determined to be 40°C and pH4. The dominant phototropic strain in FECB53 was identified previously as the thermo-acidophilic *Cyanidioschyzon*²¹. FECB58 (CCMEE ID 5216; OH-9-45C) and FECB68 (CCMEE ID 5240; OH-2-55C) were collected from Hunter's Hot Spring in Oregon and the phototroph dominating these samples was determined to be a thermophilic member belonging to the genus *Synechococcus*¹⁷.

Growth of co-cultures

To obtain sufficient biomass for subsequent DNA analysis, 100 µL of each co-culture were transferred to 25 mL of sterile BG11 media²⁵. For FECB52 and FECB53 BG11 was substituted by Cyanidium medium²⁶. Co-cultures were subjected to a 12 hr diurnal light/dark cycle while grown at the temperatures indicated in Table 1.

DNA extraction and 16S rRNA gene amplification

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

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

Sequence data analysis

Raw sequencing data were downloaded from the JGI's Genome Portal (<http://genome.jgi.doe.gov/>) under the project ID 1032475. Data were decompressed and de-interleaved using the 7-zip software (www.7-zip.org) and an in-house script, respectively. De-interleaved files were subsequently processed using MOTHUR version 1.38.1^{27,28}. Paired-end reads were combined using the *make.contigs* command. Sequences with ambiguous base calls and sequences longer than 325 bp were removed using *screen.seqs*. Duplicate sequences were merged using *unique.seqs*, and the resulting unique sequences were aligned to the V4 region of the SILVA database (v123)²⁹. Chimeras were removed using UCHIME³⁰ and quality filtered sequences were taxonomically classified at 80% confidence to the GreenGenes reference taxonomy (release gg_13_5_99)³¹. Non-prokaryotic sequences were removed and the *dist.seqs* command was used to calculate pairwise distances between the aligned sequences. The resulting pairwise distance matrix was used to cluster sequences into operational taxonomic units (OTUs) with a 97% sequence identity cut-off using UCLUST³². The most abundant sequence of each OTU was picked as the representative sequence. OTUs were taxonomically classified using the *classify.otu* command using the

GreenGenes reference taxonomy (release gg_13_5_99). Shannon, Simpson, and Chao1 estimators were calculated in MOTHUR²⁷.

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

Availability of data and material

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

Results & Discussion

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

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

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

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

The McMurdo Dry Valley (MDV) is one of the most extreme deserts on Earth, and although the importance of the microbial communities for the biogeochemical cycles of this region is widely accepted, the microbial ecology of the MDV remains poorly understood³⁵. FECB3, originating from a brackish pond on Bratina Island, was dominated by OTU000003, which recruited 80.3% of all reads (Supplemental Table S2). OTU000003 was classified as the cyanobacterium *Phormidium pseudopriestleyi*, previously reported to dominate microbial mats of the anoxic zone of Lake Fryxell, Antarctica³⁶. The second and third most abundant OTUs in FECB3 were OTU000015 and OTU000061 respectively (Supplemental Table S2). Both OTU000015 and OTU000061 were classified as

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

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

Omnipresence of Cyanobacteria and Proteobacteria within photosynthetic co-cultures

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

non-classified phyla might contribute to the separation of these samples into distinct clusters (i.e. cluster XII, IX, and IV) (Figure 2). In addition to their omnipresence, *Cyanobacteria* and *Proteobacteria* also recruited the majority of the reads in all but four (i.e. FECB2, FECB12, FECB58, and FECB68) of the samples under investigation (Figure 3 and Supplemental Table S3). In FECB2 and FECB12 the majority of the reads were recruited by OTUs classified as members of the phylum *Bacteroidetes* (recruiting 50.6% and 72% of the reads respectively), whereas within FECB58 and FECB68, *Armatimonadetes* (38.3%) and *Chloroflexi* (25.9%) were identified as the most abundant phyla (Figure 3 and Supplemental Table S3). The fact that these samples were dominated by phyla other than the *Cyanobacteria* or *Proteobacteria* may also help to explain why these samples (Figure 2) form distinct clusters (cluster I, XIV and V, IV respectively).

Firmicutes dominate photosynthetic co-cultures from hot springs

Firmicutes abundances calculated for co-cultures from hot spring samples were higher compared to those calculated for co-cultures from other environments studied during this project. OTUs assigned to the *Firmicutes* phylum were detected above the applied cut-off level of 0.1% in only four of the twenty-six co-cultures under investigation (Table 3). Interestingly, these samples (i.e. FECB34, FECB52, FECB58 and FECB68) are co-cultures collected from hot springs or deposits within hot springs, with FECB52, FECB58 and FECB68 being maintained in culture at temperatures >40°C. OTU000073 (classified as *Alicyclobacillus tolerans*), OTU000082 (classified as members of the genus *Paenibacillus*), OTU000154 (classified as *Geobacillus vulcani*), and OTU000158 (classified as a member of the *Bacillaceae* family) recruited 5.9%, 3.4% , 0.5% and 0.4%

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

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

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

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

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

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

Photosynthetic co-cultures containing the deep-branching candidate phylum Melainabacteria

Extreme environments, similar to those on early Earth, are often proposed to hold critical information about the historical progression of life on our planet and one niche that encompasses those physical stresses is the endolithic environment of rocks²⁰. Phylogenetic analysis of the heterotrophic population associated with FECB32, which was isolated from travertine deposited by hot springs in YNP, found that sequences from MLE-12 (OTU000109) recruited ~2% of the sample specific sequences (Supplemental Table S2). This rendered MLE-12, previously assigned to the deep-branching candidate phylum *Melainabacteria*⁵², as the eleventh most abundant organism in this photosynthetic co-culture. It has been proposed previously that *Melainabacteria*, which is commonly found in aquatic habitats, separated from the cyanobacteria before the latter acquired photosynthetic capabilities⁵². Hence FECB32 might be a particularly valuable co-culture to generate new insights into the evolution of and relationship between the phylogenetically closely related *Cyanobacteria* and *Melainabacteria*. In addition, this sample might provide the opportunity to enhance our understanding of the origin of oxygenic photosynthesis and aerobic respiration in *Cyanobacteria*, an area that is currently still poorly understood⁵³.

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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).

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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

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

FECB68 was dominated by 6 OTUs (i.e. OTU000028, OTU000030, OTU000036, OTU000049, OTU000065, and OTU000014) recruiting ~25.7%, 23.1%, 20.4%, 14.3%, 7.6%, and 4.9% of the reads respectively. OTU000028 was classified as belonging to the genus *Chloroflexus*, whereas OTU000030 and OTU000036 were classified as representative of the genus *Meiothermus* and *Gloeobacter*, respectively. *Chloroflexus* is an anoxygenic phototrophic bacterium that grows at temperatures up to 70°C⁵⁴ and forms yellow-orange-greenish mats in association with cyanobacteria⁵⁵. Members of the cyanobacterial genus *Gloeobacter* lack thylakoids, and have been proposed to host the earliest ancestors, or a missing link, in the cyanobacteria lineage⁵⁶. Thus, FECB68 offers a unique opportunity to investigate interspecies interaction between a member of these basal cyanobacteria and the thermophilic phototroph *Chloroflexus*, represented by OTU000028 in this co-culture. As outlined in a recent review⁵⁴, Hunter's Hot Spring located in Oregon is one of the most studied hot springs in the world, and has a large repertoire of work conducted over the last 40 years⁵⁴. However, most of this work was performed prior to the advent of recent molecular techniques. Hence, the sequencing data generated from FECB58 and FECB68 during this study will complement previous work performed using traditional microbiology techniques and facilitate new insights into the microbiology of this unique ecosystem.

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

FECB22 and FECB26 are mesophilic co-cultures collected from similar habitats (i.e. from tree bark and a wooden fence) from two locations (i.e. Hawaii and Bermuda) approximately 9,000 kilometers apart from each other (Figure 1 & Table 1). Diversity index calculation placed these two samples in the mid-range of the diversity spectrum of the 26 co-cultures analyzed for this study. The inverse Simpson, Chao1, and Shannon index was calculated at 4.46, 937.11 and 2.08 for FECB22 and 2.29, 722.94, and 1.32 for FECB26, respectively (Table 2). Within FECB22, 23 OTUs were identified as individually recruiting more than 0.1% of the generated reads. In contrast, FECB26 contained only 16 OTUs that recruited more than 0.1% of the reads each (Table S2). FECB22, scraped from tree bark in Hawaii, was dominated by 11 OTUs, each recruiting >1% of the reads. The most abundant OTU (OTU000017) was classified as a member of the *Mycoplana*, a genus that contains bacteria capable of aromatic compound degradation⁵⁷, and it recruited 40.2% of the reads. OTU000042 (classified as *Rhizobium leguminosarum*), OTU000045 (classified as *Acetobacteraceae*), and OTU000072 (classified as *Cyanobacteria*), were the next most abundant OTUs, recruiting 17.1%, 16.3%, and 5.5% of the reads generated from FECB22 respectively. *Rhizobium leguminosarum* is a well-studied α -proteobacterium capable of N₂-fixation and “rhizobia” have been suggested repeatedly to facilitate more sustainable agricultural practices through their symbiosis with legumes, reducing the need for nitrogen fertilizer⁵⁸. It remains to be seen if OTU000042 provides N₂ to the other organisms in this co-culture or if it consumes all of the fixed N₂ itself. *Acetobacteraceae* are α -proteobacteria often associated with low pH environments and are known for their ability to efficiently synthesize biological cellulose^{59,60}. Furthermore, *Acetobacteraceae* have been reported

before as some of the dominant players in photosynthetic consortia during soil formation⁶¹. It would be interesting to explore the agricultural and chemical potential of a minimalistic co-culture composed of the 4 OTUs (i.e. OTU000017, OTU000042, OTU000045 and OTU000072) that dominated FECB22, as they may combine the ability to degrade aromatic compounds and synthesize cellulose while removing nitrogen from the atmosphere. FECB26, on the other hand, was dominated by OTU000010, which recruited 63.2% of the reads generated and classified as an unclassified member of the *Nostocales*; a phylogenetic group known for their functional and morphological diversity. Members of the *Sphingomonadaceae* (i.e. OTU000041 and OTU000062), phototropic α -proteobacteria often found in high abundance in environments previously thought to support mostly the growth of cyanobacteria⁶², contributed to a total of 25.6% of the generated reads. Most interestingly, OTU000017 was also identified within FECB26 recruiting ~1.6% of the reads. It is possible that OTU000017 facilitates a metabolic reaction, in which aromatic compounds typically associated with the decomposition of woody material under aerobic conditions are utilized. Further characterization of this organism in co-culture and eventually in axenic culture might provide further clarity if this is the case.

Conclusion

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

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

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

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

measurements, such as carbon and nitrogen utilization capabilities, the scientific community would have immediate access to the phylogenetic and functional diversity available through the existing culture collections. The identification of **Minimum Information about a Co-Culture Sample (MICCS)** would be a significant step in standardizing sample acquisition and maintenance, increasing the value of current and future microbial samples collected from the environment. Developing MICCS and applying them to co-cultures currently available from existing culture depositories is beyond the scope of the work presented here, but we hope that the results presented here will contribute to the initiation of this process and stimulate broad involvement and support from the scientific community and various funding agencies.

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

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

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

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765

766 **Acknowledgements:** This work was funded by the College of Agricultural and
767 Environmental Science and the Microbiology & Biochemistry, Molecular, Cellular, and
768 Developmental Biology Graduate Group at University of California Davis (Davis, CA) and
769 the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) in Walnut Creek, CA.
770 Work conducted by the JGI, a DOE User Facility, is supported by DOE's Office of
771 Science under Contract No. DE-AC02-05CH11231. We would also thank Drs. Jorge
772 Rodrigues and John Meeks from UC Davis for providing valuable comments and
773 suggestions on how to improve this manuscript.

774 We would like to dedicate this publication to Professor Dr. Richard Castenholz who
775 passed away during the completion of this work after a long and satisfying journey in the
776 world of Cyanobacteria. He was, and will remain, a great inspiration to many of us.

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

787 del Rio, Miranda Harmon-Smith, Erik Hawley, Matthias Hess, and Nicole Shapiro

788 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 CCME.

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.**

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