Supplementary File

1. Characterization of Microbial Community Composition in Laboratory Enrichment Cultures

1.1. Methods

Genomic DNA from ~0.5 g of all samples was extracted using UltraClean soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol for maximum DNA yield. The DNA concentrations were measured using a NanoDrop 1000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

Universal bacterial primers 27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-TACGG(C/T)TACCTTGTTACGACTT-3') [1] were used to amplify bacterial 16S rRNA genes. Amplification used an initial denaturation step at 95 °C for 4 min, followed by 30 cycles of denaturation at 95 °C for 35 s, annealing at 52 °C for 55 s, elongation at 72 °C for 55 s, and a final 3 min elongation step at 72 °C in a MyCyclerTM Thermal Cycler (Bio-Rad, Hercules, CA, USA). PCR mixtures contained (per 50 µl): 0.4 µM of primers, 2 µl of DNA template (or 2 µl of purified 16S rRNA gene PCR product), 1× Ex Taq™ buffer (TAKARA Bio Inc., Mountain View, CA, USA), 2 mM MgCl2, 1.25 U TaKaRa ExTaq™ (TAKARA Bio Inc., Mountain View, CA, USA), 0.25 mM of each dNTP. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA, USA).

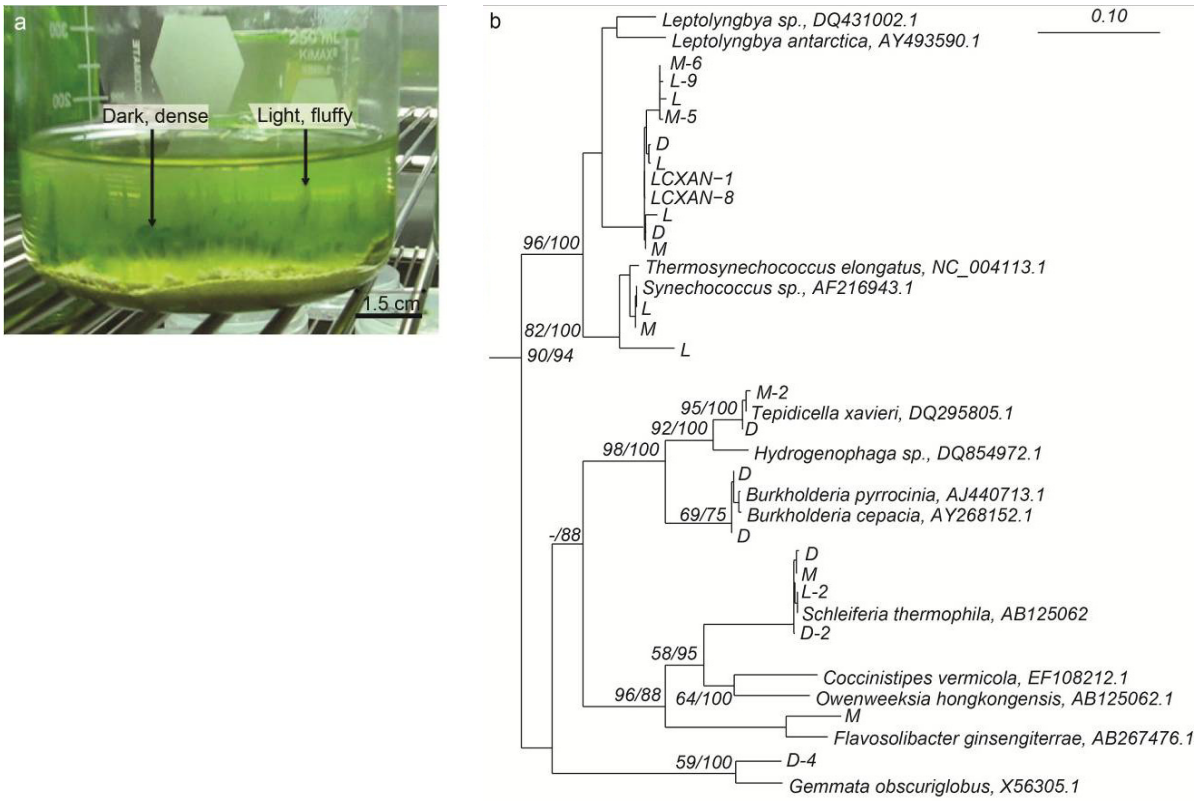
PCR purified products were electroporated at 2500 V with One Shot TOP10 electrocompetent *E. coli* (Life Technologies, Grand Island, NY, USA). For each transformation, 20 µL and 50 µL of the transformation were plated on LB agar plates containing 50 µg/ml ampicillin and incubated at 37 °C. After 24 hours, isolated colonies were resuspended in 3 mL of LB broth with 50 µg/ml ampicillin and incubated on orbital shaker at 37 °C for 14 hours. Plasmid DNA was extracted from *E. coli* using QIAPrep Spin Miniprep Kit (Qiagen Inc., Valencia, CA, USA) and eluted in 30 µL of water.

Clones containing nearly full length 16S rRNA gene fragments from light tufts, dark cones and light tufts with dark and cohesive centers (mixed morphology) were sequenced in both directions using T3 (5'-AATTAACCCTCACTAAAGG-3') and T7 (5'-TAATACGACTCACTATAGG-3) primers. Sequences were assembled in Aligner (CodonCode Corporation, Dedham, MA, USA), aligned to a global alignment using Greengenes NAST-aligner [2,3] and screened for putative chimeras using Bellerophon accessed through the Greengenes website [4]. Eight potential chimeric candidates were excluded from the data set. The remaining sequences (a total of 58) were imported into the Greengenes October 2010 ARB database using the ARB software package [5]. Affiliations of nearly full-length 16S rRNA gene sequences were confirmed by the addition to a Greengenes CoreSet tree consisting of full-length sequences. This was done without changing the branch topology using the parsimony insertion tool in ARB. The filter "lanemaskPH" was used to ignore hypervariable regions of the 16S rRNA molecule provided with the ARB database. Additional trees were calculated by using maximum likelihood method RAxML-VI-HPC under the GTRCAT model of evolution [6] and neighbor-joining method using Jukes-Cantor correction. The robustness of inferred neighbor-joining and maximum likelihood tree topologies was evaluated by bootstrap analysis using 1000 replicates. *Symbiobacterium thermophilum*, str. IAM 14863 was used as an outgroup.

1.2. Results

Bacterial 16S rRNA gene sequences in compact cones, fluffy, tufted aggregates and mixed morphologies (Figure S1) suggested the presence of expanded niches for non-photosynthetic organisms in small, compact cones. Clone libraries from tufts, compact cones and mixed morphologies contained similar 16S rRNA gene sequences of thin filamentous non-heterocystous cyanobacteria from Subsection III [7]. These sequences clustered with the 16S rRNA gene sequences of subsequently enriched cone-forming cyanobacteria (XAN-1, Figure 1) and cyanobacteria that did not form cones under the same experimental conditions (XAN-8 in Figure 7; [8]). Cyanobacterial clones comprised 87% of all clones in light, tufted aggregates, but only 16% in the dark compact structures (Figure S1). Most sequences in the dark, compact cones were instead similar to non-photosynthetic heterotrophic organisms and were more diverse than the sequences of non-photosynthetic organisms in tufts (Figure S1). These data provide a rough outline of the microbial communities in the laboratory enrichment cultures, and will be examined in more detail in the future.

Figure S1. Composition of microbial communities in fluffy tufts and dark, compact, gelatinous cones. (**a**) Photograph of the culture containing tufts (light green) and compact cones (dark green); (**b**) Phylogenetic relationships based on near-full length 16S rRNA gene sequences of clones from fluffy tufts and dark compact cones.



In Figure S1, D indicates clones present in dark, compact cones, L indicates clones present in light, fluffy tufts and M indicates clones present in mixed morphologies (dark compact cones overgrown by light tufts). Each sequence represents a 98% identity cluster. The number of clones belonging to each 98% cluster present in the libraries from D (dark, compact) or L (light, fluffy) aggregates, respectively, is indicated by numbers separated by dashes. Dashes were omitted when only one clone was present.

Sequences similar to *Schleiferia* sp. [9] were detected both in light and dark aggregates, but the dark, compact aggregates and aggregates with a mixed morphology contained other non-cyanobacterial sequences as well. The phylogenetic tree was constructed by adding clone sequences to Greengenes CoreSet tree consisting of full-length sequences, without changing branch topology and using the parsimony insertion tool in ARB. Bootstrap values with >50% support are indicated at the nodes in the order of neighbor-joining/maximum likelihood.

2. Short-Term Observations of Cyanobacterial Motility in Anaerobic and Aerobic Media

2.1. Methods

Small clumps were obtained by homogenizing the same cone taken from a batch cone-forming enrichment culture grown on silica sand in the medium that was initially equilibrated with the atmospheric air. Growth medium and conditions are described in the main article. The material was homogenized aerobically by repeated passing through a 24-gauge needle attached to a 5 mL syringe. Half of the homogenized material was resuspended into 500 L of fresh medium equilibrated with the atmospheric air, and the remaining half into 500 L of fresh medium that had been bubbled with nitrogen for 10 min. A few small visible clumps were placed onto a microscope slide and covered by a coverslip. To avoid contact with air, clumps that were resuspended in the anaerobic medium were placed onto the slide that was continuously flushed by a stream of pure N_2 and covered by a coverslip. Cyanobacterial clumps and filaments were visualized using chlorophyll A autoflorescence, as described in the main text. Images of the same fields of view were taken every minute for 1 hour. During the imaging experiment, the samples were kept at room temperature. The samples were kept in the dark between individual time points.

3. Movies S1 and S2

Motility of cyanobacterial filaments in clumps as a function of oxic and anoxic conditions. Movie S1 (anoxic_migration.mp4) shows a bacterial aggregate placed in anoxic media. Initially bent filaments within the aggregates become horizontal and glide away. The aggregates become less dense (visible as a decrease in brightness in the centers of clumps). Some reversals and bending also occur. Movie S2 (oxic_migration.mp4) shows the motion of cyanobacteria placed in a medium that was equilibrated with the atmospheric air. The horizontally gliding filaments at the edges of the clump reverse their directions and do not migrate far from the clump, or bend (e.g., the filament in top center). Bent filaments within the aggregate mostly remain bent. These experiments show the behavior of filaments in clumps at 1-min intervals during 1 hour. In contrast, Figure 5 of the main text shows the same O_2-dependent trends of clump dispersal or persistence, but only at the beginning and at the end of a 2-hour experiment.

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