Surface orientation affects the direction of cone growth by *Leptolyngbya* sp. C1, a likely architect of Octopus Spring coniform structures.

Supplemental Information

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Materials and Methods

Enrichment of heterotrophs from Octopus Spring cones

Heterotrophs were isolated by plating on Bacto nutrient agar (Difco), R2A agar (Difco), MacConkey agar (Difco), starch agar (Difco), antibiotic medium 2 (Difco) in the absence of antibiotics, bismuth sulfite agar (Difco), malic acid media (10 g malic acid, 0.02 g magnesium sulfate, 0.02 g potassium chloride, 3 g potassium phosphate dibasic, 2 g sodium phosphate monobasic, 0.005 g ferric citrate, 0.1 g calcium carbonate, 1.5 mg boric acid, 0.8 mg manganese sulfate, 0.6 mg zinc sulfate, 0.1 mg cupric sulfate, 0.2 mg ammonium molybdate, and 0.01 mg cobaltous sulfate per liter) and on media prepared by autoclaving agar cultures of well grown *Leptolyngbya* sp. C1 enrichments grown on D media, homogenized, and then re-poured into plates (cyano soup media). All single colonies were transferred a minimum of three times. Inoculated plates were incubated at 37° C in the dark.

Isolate identification

Isolates were identified by 16S rRNA gene sequencing. 16S rRNA genes were amplified with 8F and 1492R as described (23). The sequence was determined using the same primers. Primer 515F was also used if necessary for more complete coverage of the 16S rRNA gene. Gene sequences were deposited in Genbank with the following accession numbers KC182742-KC182752.

Motility and exopolysaccharide production

Soft agar (4 g/l) was used to detect motility. Motility was assayed in plates and tubes using the media of isolation. Cells were positive for motility if they were able to spread away from the original streak line or stab. EPS production was determined by calcofluor binding and fluorescence of cultures grown on their respective agar plates with 0.02 % calcofluor added (18).

Results and Discussion

Microbial Isolation

In order to identify more of the heterotrophic diversity than may have been captured using the clone library, and to identify specific intrinsic physiological capabilities that may influence cone formation, cultures were initiated to enrich and isolate cyanobacteria and aerobic heterotrophs. Ten different heterotrophs were isolated and 1 cyanobacterium was enriched from the Octopus Spring cones (Table S1).

Sequencing of the 16S rRNA gene was used to identify close relatives of the isolates. There was no overlap between the heterotrophs identified in the clone library and the isolates. This is not necessarily surprising since many clones with sequences corresponding to known heterotrophs identified in the library were only present once, suggesting that heterotrophs are not likely to be abundant, are much more diverse than the cyanobacterial population, and that saturation in the clone library

was not achieved. Thus, the isolation of heterotrophs is quite complementary as this approach was able to identify additional organisms. OS1 is a *Staphylococcus* sp. isolate that has close relatives associated with the filamentous cyanobacterium *Arthrospira platensis* (10). It also appears to be ubiquitous including insect guts (7, 24), which may have served as its original mechanism of dispersal to the cone in Octopus Spring. *Aeromonas* OS5 and OS6 have also been identified in insect guts (Accession JN644562 and (13), respectively). OS2 is an *Arthrobacter* sp. isolate. Related clones and isolates have been identified in metal rich environments (8, 19). They have also been identified in association with a decaying Microcystic bloom (Accession HQ904194) and in algal-bacterial consortia (Accession AB552850), which would suggest this organism may be able to thrive growing in close association with cyanobacteria. The 16S rRNA gene from *Agrobacterium* sp. OS3 has 98% identity to a clone from a library derived from bacteria growing on *Microcystis* sp. exudates (12). *Chryseobacterium* sp. OS7 is also related to isolates living on *Microcystis* (Accession HQ896846). *Pseudomonas* sp. OS4 is closely related to aquatic bacteria (Accession EF575036) and a clone identified in association with coral (Accession JQ347400). *Paracoccus* sp. CS4 is ubiquitous being found in dust (1), sediment (17), and seawater (Accession FJ96075). *Microbacterium* CS2 is closely related to isolates from alkaline groundwater (22), a symbiont of the cyanobacterium *Chlorella sorokiniana* IAM C-212 (15), and cyanobacterial lysing bacteria (Accession AB610601).

As noted above, many of the isolates identified have relatives that are also found in close associations with cyanobacteria. As evident from the microscopy (Figure 2), the cyanobacteria in the cones are living in physical proximity with non-chlorophyll *a* containing cells which could benefit from this association by metabolizing the cyanobacterial exudates (3, 4). This is evident in the *Leptolyngbya* C1 enrichment. Although the *Pseudomonas* sp. OS4 can grow on nutrient rich agar, it can only grow on the autotrophic D media when *Leptolyngbya* sp. C1 is also present. Isolates enriched on the cyanosoup media are also able to thrive on *Leptolyngbya* produced products. Interspecies carbon flow may be important in determining coniform morphology.

Characterization of Isolates and Cyanobacterial Enrichment

The isolates were examined for traits that are suspected to be important in cone formation such as phototaxis, motility, and exopolysaccharide production (Table S1). The possession of these traits by different strains may suggest their potential role in cone formation. Not surprisingly, only *Leptolyngbya* sp. C1 was able to positively phototax. Also, under the conditions tested (low % agar), only three of the heterotrophic isolates, *Staphylococcus* sp. OS1, *Arthrobacter* sp. OS2, and *Pseudomonas* sp. OS4 were motile. Motility would be required to ultimately aid in the formation of the cone, move from the base to the tip of the cone, and potentially escape lithification. Several of the strains did produce EPS under the test conditions (Table 1). Genes involved in the biosynthesis and degradation of EPS have been identified in the metagenomes of microbialites (6) and stromatolites (16). EPS likely plays a role in the structural formation of the cone, protecting the structure

from shear stress, precipitation of silica during cooling and evaporation of water (9, 14), and subsequent trapping and binding of sand grains (20) and/or other particulate matter. It can act as a source of nutrients for a community of microbes serving as an intermediate in the flow of carbon from autotrophs to heterotrophs, but it is interesting to note that the heterotrophic isolates also produced EPS. Heterotrophic production of EPS could play a role in lithification and formation of cones since not all cyanobacteria can form cones (5). The physical and chemical properties of the EPS may affect the ability to form cones thus EPS from different organisms may render different morphologies and different degrees of lithification. Although not relevant to these cones found in a siliceous spring, EPS has also been shown to mediate carbonate precipitation (2, 11, 21) by the ability to trap calcium and then release calcium upon its degradation by heterotrophs such as fermenters and sulfate reducing bacteria. EPS may also aid carbonate mineralization by acting as a precipitation template (11).

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Table S1. Isolates and enrichment culture from coniform structure.

Isolate	Media	Top Genbank Hit	% Identity	Motility	EPS	Shape
OS1	R2A	*Staphylococcus saprophyticus*	99	+	+	Cocci
OS2	R2A	*Arthrobacter oxydans*	99	+	+	Rod
OS3	Malic Acid	*Agrobacterium albertimagni*	99	-	-	Rod
OS4	Starch	*Pseudomonas sp.*	99	+	+	Rod
OS5	Antibiotic	*Aeromonas fluvialis*	99	-	+	Rod
OS6	Bismuth	*Aeromonas hydrophila*	100	-	+	Rod
OS7	Antibiotic	*Chryseobacterium sp.*	99	-	-	Rod
CS2	Cyanosoup	*Microbacterium oxydans*	100	-	-	Rod
CS3	Cyanosoup	*Afipia sp.*	99	-	-	Rod
CS4	Cyanosoup	*Paracoccus sp.*	99	-	+	Rod
C1 enrich-ment	D	*Leptolyngbya sp.* (also contains *Pseudomonas sp. OS4*)	97	+	+	Filament