Gregarious Cyanobacteria

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Abstract

Huber and collaborators reported in this issue of *Environmental Microbiology* about freshwater picocyanobacteria that showed phenotypic plasticity in the sense that they appeared as single cells as well as in aggregates. The authors suggested that aggregation might be an inducible defense as a response to the presence of grazers. This has been described for eukaryotic phytoplankton and for the cyanobacterium *Microcystis* but thus far not for picocyanobacteria. Although inducible defense as an explanation is an attractive possibility, it is also problematic. Aggregation is common among cyanobacteria and it offers many advantages as compared to a free-living lifestyle. Here these advantages are highlighted and the possibility of inducible defense is critically assessed.

Huber *et al.* (2017) investigated the phenotypic plasticity of freshwater picocyanobacteria, *i.e.* on the one hand their appearance as free-living single cells and on the other hand as more or less structured colonies and aggregates. Picocyanobacteria (by definition unicellular cyanobacteria smaller than 2 µm) have often been considered to occur and grow as free-living single cells (Pcy). However, it has become clear that these organisms have also been recorded as ‘microcolonies’ (<50 cells), ‘short (pseudo)trichomes’ (<10 cells), and ‘large colonies’ (up to several hundreds of cells) (CPcy) (Fig. 1a). The latter have frequently been observed in warm, shallow and nutrient-rich lakes, while Pcy are more abundant in deep oligo- to mesotrophic lakes (Callieri *et al.*, 2012).

Single cells may produce sticky extracellular polymeric substances (EPS) causing the cells to...
stay together after division. This EPS formation is usually the result of unbalanced growth
caused by nutrient limitation (Crosbie et al., 2003). While the small size of picoplankton and
the consequent large surface-to-volume ratio render these organisms a high affinity for
nutrients and is considered to be an adaptation for growth under oligotrophic conditions,
aggregation seems to undo this advantage. In shallow nutrient-rich waters with a high
standing stock of Pcy, the chance that cells encounter each other and stick together must be
greater than in deep lakes with more mixing. In shallow waters, the formation of aggregates
may also increase the transparency of the water column, while the colony protects the cells
inside from excess irradiation. In deep water, aggregates would sink leaving relatively more
Pcy in the euphotic zone. Aggregates may also have an important advantage as they
establish a self-sustaining microcosm in which nutrients are effectively recycled and provide
for instance a habitat for O₂-sensitive diazotrophic (N₂-fixing) microorganisms (Klut and
Stockner, 1991). The EPS matrix may absorb and retain metabolites as well as immobilize
nutrients and toxins. The mucilage in which the cells in the aggregate are embedded may
also be difficult to digest and certainly represents an inferior food source because it is
composed mostly of carbohydrates and is low in other nutrients. Hence, there is a plethora
of reasons why living in aggregates is preferred above a life as free-living single cells.

Colony morphology has been thought to be species-specific. Although there is no doubt that
this is to some extend the case, Huber et al. detects >30 phylotypes but recognize only 6
major colony morphologies. These CPcy may have characteristic morphologies that have
been given taxonomic value and depending on the arrangement of the cells in the colony
they are assigned as ‘Cyanodictyon’ (irregularly shaped spherical colonies with cells
irregularly arranged), ‘Aphanocapsa’ (regular spherical colonies with cells regularly
arranged), and ‘Eucapsis’ (colonies cubic with cells arranged in groups of 4). Many taxa of
unicellular cyanobacteria grow as multicellular organisms in well-organized and structured
colonies that have been given taxonomic value. This may be problematic because the
colonial growth form is sometimes lost in culture (Komarek et al., 2011). The question
addressed by Huber et al. was whether Pcy and CPcy may in fact belong to the same
genotype that show a phenotypic plasticity. And if this would be the case, which factors
would trigger these organisms to change from one morphotype into another.
Huber et al. investigated the temperate lake Chascomús in the Pampa region of Argentina to answer these questions. Lake Chascomús is a shallow (1.9 m) and hypertrophic, turbid and fully mixed lake. The most common CPcy morphotype that was present in the plankton was ‘Cyanodictyon’ and the occurrence of this morphotype correlated with Pcy, including the microcolonies and short trichomes. 16S rRNA and ITS sequence analysis of flow-cytometric fluorescent-activated cell sorting (FACS) of Pcy and CPcy (which were only of the ‘Cyanodictyon’ type and also included the microcolonies and short trichomes) revealed no indication of genotypic difference between the two sorted groups. They were all related to *Cyanobium* and *Synechococcus* and belong to the ‘Cyanobium + Anathece’ cluster and matched the sequences that were obtained from clone libraries and DGGE from samples of the lake. Hence, when Pcy and CPcy are belonging to the same organism, what causes them to take either of the two morphologies? Huber et al. investigated the possibility that the CPcy morphology protects from grazing. Although they observed only weak correlations of the distribution of Pcy and CPcy with grazers, there was one exception: cladocerans. Pcy were negatively correlated with small cladocerans while the percentage of cells in colonies was positively correlated. This observation was experimentally tested by adding the cladoceran *Bosmina* sp. to lake water from which grazers were removed. While no changes were observed in the control, Pcy were less abundant and the number of cells in CPcy increased when *Bosmina* sp. was present. The CPcy, which was mostly of the ‘Cyanodictyon’ morphotype, doubled in size during the 6 days that the experiment lasted. While this is all pretty convincing and exciting, it leaves us with many unanswered questions about the mechanisms behind this behavior. What causes (pico)cyanobacteria to form aggregates? When indeed the formation of the colony is a response on the presence of a grazer, how is this grazer sensed? Are there infochemicals involved and if so, what is their nature, who produces them, and how are they released and sensed? The authors suggest that the colony grows at the expense of Pcy (single cells). Hence, the single cell joins the CPcy, rather that selective grazing diminishes Pcy. Do CPcy fall apart and take back the Pcy morphology when the danger has passed? These questions remain unanswered in this paper and would require further research. In the grazing experiment, there seems to be no statistical significant difference between the number of cells per milliliter between day 0 and at day 6 with or without *Bosmina* sp. Does this mean that there is no growth and grazing or are growth and grazing in equilibrium and in the control without the grazer would growth and lysis cancel...
out? Or is the presence of *Bosmina* sp. alone enough to cause the shift from Pcy to CPcy and hence lowers the chance of grazing the lower abundance of Pcy? Why would not all Pcy aggregate in the CPcy? Or is just getting the abundance of Pcy below a certain threshold enough to prevent effective grazing? There seems indeed to be an ‘ideal free distribution’ for the ratio of single cells to colonies that represents the optimum for growth under herbivory (DeAngelis *et al.*, 2007). Moreover, although the increase in size by aggregation of cells could help to avoid being grazed by a certain organism, it could become the preferred food for other, bigger predators (Van Donk *et al.*, 2011). Among zooplankters, the rotifers in particular seemed to feed preferentially on the CPcy (Callieri *et al.*, 2012). And if aggregation would be beneficial with regard to grazing, it has to outweigh the negative effect of sinking (Van Donk *et al.*, 2011). Hence, there are many questions that demand an answer in order to understand this proposed strategy to avoid grazing pressure.

The response of eukaryotic phytoplankton towards herbivores is well-known and described. The green alga *Scenedesmus* induces aggregate formation when exposed to grazer-released infochemicals in a dose-response manner (Verschoor *et al.*, 2004). However, much is still unclear about the nature of these infochemicals, how they are released and sensed. About the role that infochemicals may have for inducing aggregation in cyanobacteria even less is known.

For instance, the unicellular cyanobacterium *Microcystis aeruginosa* produces aggregates when treated with spent medium of *Daphnia* or by disrupted *Microcystis* cells (Becker *et al.*, 2010). The toxin microcystin produced by *M. aeruginosa* has been shown to induce aggregate formation (Sedmak and Elser, 2006) although also other infochemicals may have been released from the disrupted cells. *M. aeruginosa* induced colony formation when grazed by the flagellate *Ochromonas* (Yang *et al.*, 2006). However, these authors also indicated that the colony-inducing effect was weak, which they attributed to the fact that they used an axenic culture in their experiment, suggesting that associated (heterotrophic) bacteria also play a role. Yang *et al.* (2006) also admitted that other, abiotic, factors contribute to the formation of larger colonies found under natural conditions. Moreover, the cost of the increased sinking rates and the decreased effective quantum yield of...
photosystem II of herbivory-induced colonial *M. aeruginosa* should be taken into account to judge the effectiveness of the strategy (Yang *et al.*, 2009).

The formation of aggregates and, hence, the increase in size has advantages for phytoplankton. Many planktonic cyanobacteria (filamentous and unicellular) produce gas vesicles that provide these organisms with buoyancy. When these organisms are mixed deep in the water column, the speed with which they float to the surface is proportional with the square of the radius (Stokes’ Equation). Hence, the bigger size of a cell aggregate helps to get back quickly to the illuminated water surface after a deep mixing event, where a single cell would need many days and would not survive. This is the well-documented strategy of the unicellular *Microcystis* (Fig. 1b) as well as of the heterocystous cyanobacterium *Aphanizomenon* (Fig. 1c), and the non-heterocystous *Trichodesmium* (and many others), which all form aggregates.

Aggregates of cyanobacteria that do not contain gas vesicles such as the picocyanobacteria discussed in Huber *et al.* also obey Stokes’ equation by sinking out more rapidly than single cells. The sinking rate of single cells of picocyanobacteria is negligible. While staying in the illuminated surface water they deplete the nutrients (nitrogen and phosphorus) locally. This leads to the production of transparent extracellular polymers (TEP) (by overflow metabolism), that cause aggregation of the Pcy. These aggregates of picocyanobacteria (CPcy) may be >1 mm and sink 400 m d$^{-1}$ (Deng *et al.*, 2016). This represents an important carbon export, but would it also be a selective advantage for these picocyanobacteria? I think it probably would. Removing a large part of the picocyanobacterial biomass from the euphotic zone would increase the transparency of the water column and increase the relative availability of nutrients, which would increase the survival of the Pcy. Also, regeneration of cyanobacterial biomass in the euphotic zone would allow heterotrophic bacteria to scavenge the nutrients. Finally, on a larger scale, carbon export (the carbon pump) is essential for balancing the global carbon budget.

There are other, bigger, unicellular cyanobacteria that form more or less structured aggregates. *Gloeothece* forms irregular groups of sheathed cells. This genus is known to fix *N$_2$* aerobically in the light. The aggregates with their multilayered gelatinous envelopes may
be an adaptation to allow aerobic N₂ fixation in the light (Ortega-Calvo and Stal, 1991). Only when a certain size is achieved, it would be possible to establish O₂ gradients such that the oxygen-sensitive nitrogenase can be active. Moreover, such aggregates might also allow some differentiation between cells. Some cells in the aggregate may cease oxygenic photosynthesis and fix N₂, while exchanging fixed nitrogen and electrons with the oxygenic cells. This still needs to be demonstrated but it would explain the differences of *Gloeothece* with other unicellular N₂-fixing cyanobacteria such as *Cyanothecae* and *Crocosphaera*, which do not form such aggregates and fix N₂ during the dark (Compaoré and Stal, 2009).

*Merismopedia* is a genus of unicellular cyanobacteria that is characterized by rectangular colonies embedded in a mucilaginous matrix (Fig. 1d). Their colonies are regular shaped. The cells divide in two dimensions that produce a flat plate with a grid-like pattern, which is often lost when isolated and cultured in the laboratory. This has made the taxonomy of this organism difficult because when grown as single cells they would be assigned to *Synechocystis* (Palinska and Krumbein, 1998). However, the typical colony shape can be restored when silicate is added to the growth medium (as for diatom media). Hence, silicate seems to be a requirement to form this aggregate (Stal and Wollenzien, unpublished observations). *Merismopedia* is often found in intertidal microbial mats on fine-sandy sediments. The aggregates are motile (Stal, unpublished observations). Individual small cells released from *Merismopedia* aggregates have been reported to be motile (Palinska and Krumbein, 1998), but motility of the whole aggregate in a coordinated manner has hitherto not been reported. It appears that the whole single cell layer plate may be either oriented towards the light, so that all cells receive maximum light, or that the plate tumbles with the single cell side of the plate directed towards the light, resulting in the lowest light harvesting for the plate. Hence, this may be an aggregate behavior to acclimate to light.

The benthic cyanobacterium *Microcoleus chthonoplastes* (re-named *Coleofasciculus chthonoplastes*) is known to form microbial mats and bio-crusts. These cyanobacteria produce bundles of a few to many tens of trichomes enclosed by well-structured polysaccharide sheaths, a property that is usually lost upon isolation and culturing in the laboratory (Stal, 2012). The function of this growth form is not well understood. It has been suggested that it protects against desiccation which would come handy in intertidal
microbial mats that are often exposed or in terrestrial bio-crusts. The bundle could also
serve to maintain a consortium of microorganisms that provide this microcosm with
necessary growth factors. The sheath that surrounds the bundle of trichomes could protect
against external factors such as heavy metals and other (toxic) compounds, which may be
bound and immobilized by the polysaccharides. The sheath might even prevent grazing.

Microbial mats that are formed by cyanobacteria are multicellular aggregates that behave as
macroscopic living entities (Fig. 1e). The consortia of a plethora of different functional
groups of microorganisms perform ecosystem level functions that would be impossible at
the level of single organisms (Paerl and Pinckney, 1996). The activities of single organisms
are coordinated through biological clock and other rhythmic processes and by signaling
through info-chemicals and quorum-sensing systems, most of which are largely unknown.
Microbial mats are among the oldest systems known from the fossil record (stromatolites)
and therefore this form of aggregation has been proven very successful. Pcy are not
exclusively pelagic and are found in microbial mats and periphytic biofilms, but little is
known about these organisms. They may differ from their pelagic counterparts and be
typical benthic organisms, but it is also possible that they originate from the water column
and deposited in these benthic ecosystems.

It is likely that cyanobacteria evolved from a unicellular type that gave rise to filamentous
forms (Schirrmeister et al., 2011). Filamentous cyanobacteria have the advantage to
exchange metabolites and information directly between the cells in a trichome, which led to
the development of gliding motility and the orientation of the trichome in physical and
chemical gradients. This allowed the cells in a trichome to 'collaborate' and get the best of
both, often incompatible, worlds. To add to this, the multicellular filamentous cyanobacteria
also developed a distribution of tasks by cell differentiation, which is common among
Eukarya, but unique among Bacteria (and Archaea). Some cyanobacteria develop
'heterocysts', cells that have lost photosystem-I, do not fix CO2 and produce a thick glycolipid
cell wall, and that serve as the N2-fixing factories of the organism. Many heterocystous
cyanobacteria also differentiate a second cell type, the akinete, which serves the survival of
the organism. The formation of aggregates by unicellular cyanobacteria may represent a
similar evolutionary development. While the evolution of cyanobacteria from 'simple' single
cells to more complex filamentous organisms can be considered as the typical direction evolution takes, it has also become clear that the taxa of unicellular cyanobacteria are not monophyletic and that evolution has taken the opposite direction from filamentous forms back to unicellular organisms (Schirrmeister et al., 2011). Obviously, the cost of multicellularity does not always pay off.

What I tried to highlight here is that free-living microorganisms in general, and cyanobacteria in particular, seem to be rather an exception than the rule and that most microorganisms prefer to live as a consortium in benthic environments, attached to a substratum, or as aggregates. There are many advantages to live in such consortia compared to a free-living lifestyle. In the latter, the cell is surrounded by a diffusive boundary layer that is nutrient depleted, although this boundary layer decreases with smaller cell size. The small cell size may make picocyanobacteria more attractive to grazers and filter-feeders, but aggregates offer more benefits than only preventing grazing. Aggregation of picocyanobacteria as a response to the presence of grazers is a possibility but there are certainly many other factors that trigger the formation of colonies as is the case in larger unicellular- and filamentous cyanobacteria. Cyanobacteria are gregarious organisms.

References


Fig. 1a. Aggregates and single cells of picocyanobacteria in the Baltic Sea

Fig. 1b. Aggregate of the gas vacuolated unicellular cyanobacterium *Microcystis aeruginosa* (Culture Collection Yerseke, CCY, ccy.nioz.nl).
Fig. 1c. Colonies of the filamentous heterocystous and gas-vacuolated cyanobacterium *Aphanizomenon flos-aquae* in the Baltic Sea.

Fig. 1d. *Merismopedia* sp. (cells fluoresce red) embedded in EPS (fluoresces green) (Culture Collection Yerseke, CCY, ccy.nioz.nl).
Fig. 1e. An intertidal microbial mat; another form of aggregation of cyanobacteria.