Chapter 9

MICROBIAL SPHERES FROM MICROBIAL MATS

Ulrike BREHM, Wolfgang E. KRUMBEIN and Katarzyna A. PALINSKA
Carl von Ossietzky University of Oldenburg, ICBM, AG Geomicrobiology, PO Box 2503, Germany
ulibrehm@africa.geomic.uni-oldenburg.de

1. INTRODUCTION

Filamentous cyanobacteria from the LPP-group (*Lyngbya* sp., *Plectonema* sp., *Phormidium* sp.), diatoms and heterotrophic bacteria are the dominant microorganisms of North Sea microbial mats (Gerdes et al., 1986). Cultivated on solid or liquid media the cyanobacteria produce motile trichomes. They employ motility to optimize their positions in their microhabitat using a number of external factors. Light is the major factor in directing their search for a suitable survival and growth niche. Organic substances and their concentrations may be another trigger. However, chemotaxis is essentially very poorly described in cyanobacteria. A large potential area for research has therefore been left untouched (Kangatharalingam et al., 1991).

A description of the coordinated movement and to date unknown three-dimensional assemblage of *Phormidium* sp. filaments with other organisms is reported here. Moreover, the possible strategies for this new type of trichome agglomeration are presented and discussed.

2. MATERIALS AND METHODS

Filamentous cyanobacteria of the LPP-group belonging to the *Phormidium* sp., were isolated from North Sea microbial mat cultures and maintained in liquid as well as ASN III medium solidified with 0.5 % agar (Rippka et al., 1979). The cultures were found to be not axenic, as green algae and diatoms, from the genera *Navicula* and *Nereneis*, were present in the medium, however, filamentous cyanobacteria clearly dominated.

All samples were kept under ambient temperature and light conditions in the laboratory since February 2000. The petri dishes were viewed daily with a help of stereoscope to examine the growth of the organisms. Identification of the cyanobacteria was performed under light microscopy (Axiovert, Zeiss and Axioplan, Zeiss) and by Cryo-Scanning Electron Microscopy (Cryo SEM, Zeiss) described by Sargent (1988).

In further experiments following signal substances in final concentration of 10 mM (cAMP - cyclic Adenosin-Mono-Phosphat, BHL - Butyroyl-Homoserinlacton, OHHL - Oxohexanoyl-Homoserinlacton) were separately added to solid (0.5% agar) and liquid ASNIII media. BHL was additionally used at 1 and 5 mM concentrations.

3. RESULTS AND DISCUSSION

Microbial communities isolated from North Sea microbial mats showed a number of different assemblages in culture.

The most spectacular agglomerations were spheres. Tightly interwined filaments of cyanobacteria formed the surfaces of the spheres. The inner part of the spheres was composed of a varying number of diatoms.

3.1 Principles of movement

Three types of gliding trichomes were observed, displaying a different but very constant velocity as evidenced by repeated microscopic measurements. The majority of the cyanobacteria were sluggish and moved very slowly, packed close together and following the same direction. In contrast, the trichomes, which constructed the spheres, were constantly and actively moving on the surfaces of the spheres. A third group of filamentous cyanobacteria changed the position frequently by forming numerous loops. These, however, never built circles or spheres. Perhaps they were able to form spheres but not all of the needed biochemical parameters were fulfilled.

The velocity of the *Phormidium* sp. trichomes moving through 0.5 % agar medium at room temperature was about 8 μm per second.

From our observations we derive the theory that motility is a significant survival factor for the microorganism that possesses it. Simple gliding movement even without oriented response to the stimuli would allow exposure to a greater number of nutrient ions in a given time.

Motility in some cyanobacteria, e.g. the heterocystous forms, is occasional, and occurs only in some phases of their morphogenetic cycles (hormogonia). Movement and/or growth can serve as a mean of spreading an otherwise attached organism over a wider area.

Although steering by gliding movement of cyanobacteria has not yet been reported, some taxic responses are features, which could add at least the directional orientation to many of their movements.

Gliding is movement in contact with a solid or semi-solid surface without flagella-like organs (Castenholz 1973). This movement can be continuous in one direction for a longer time or can occur in frequent intervals.

Most members of the Oscillatoriaceae rotate when gliding. Trichomes moving on the surface of the agar often move in wide arcs, resulting in flat circles of many trichomes with non-uniform diameters. Castenholz (1973) explained circling of trichomes by a lateral force caused by a rotating trichome. In our studies this phenomenon was observed at the late stage of cultivation.

Such trichome ”deformations” or occurrence of unexpected colony forms may occur with collisions and with portions of the same trichome gliding together in opposing directions. However, this explanation does not accommodate for the formation of the three-dimensional structures observed and documented during these studies.

3.2 Assemblages of biofilm communities

The filamentous cyanobacteria studied here are characterized by single segments of 3μm width and 4-4.5μm length. Obviously they belong to the LPP group (*Lyngbya, Plectonema, Phormidium*) (Rippka et al., 1979).

Filamentous cyanobacteria isolated from North Sea microbial mats showed a number of different assemblages in culture.

The formation of these aggregates appeared to be deliberate. Twelve days after incubation cyanobacteria formed circles of microorganisms. During the next days the circles remained stationary but morphed into spheres composed of a small number of individuals which could be clearly identified. Cyanobacterial trichomes seemed to glide inside the spherical envelope, never penetrating into the center of the spheres. The spheres

themselves were located within the agar. The number of associated diatoms was found to be considerably higher inside the spheres than outside (Fig. 1).

Other spherical assemblages developed as well, but were considerably smaller in size (0.81 mm, 0.75 mm, 0.46 mm). It was evidenced in several culture sets that the spheres were the center from which a radial arrangement of cyanobacteria emerged (Fig. 2). The surfaces of the spheres were composed of permanently moving trichomes.

The position of the spheres did not change with time, but the number of trichomes involved was variable. After 4-6 weeks the appearance of the three-dimensional figures grew pale, favouring dense concentric circles. Beginning at the surface of the spheres the cyanobacteria moved to form dense rings growing on the centerpart. The number of individuals joining the circles increased with time (Fig. 3). In comparison with the number of cyanobacteria in the surrounding agar, the dark green colour of the rings was conspicious.

Trichomes of the studied ”sphere” can move with respect to other trichomes in the colony and, this can result in constant changes in the relative position of the trichomes and shape of the colony. ”Spheres” maintain their original shape and place, although the trichomes continuously move. The movement of the aggregated trichomes is clearly coordinated. This means: the trichomes move together as a ”sphere”. In cases, in which trichomes move away, the ”sphere” turns. According to our screening of the literature this pattern of aggregation and locomotion of aggregates has not been recorded before.

The size of the spheres did not change over the whole time of observation. Finally, the rings were reduced in width and number of trichomes.

3.2.1 Formation of spherical aggregates of trichomes

The steps of biofilm sphere formation are as follows:

A marine biofilm community kept in liquid or on solid media excretes various substances. These substances are accumulated in certain places and encircled by microscopically visible envelopes (Fig. 4).

These spherical appearances are recognized by some microorganisms. They approach the spheres and penetrate through the envelopes at specific places. The envelopes seem to be permeable only for specific microorganisms. According to our observations bacteria and diatoms can not penetrate through the flexible but resistant envelopes. As long as the trichomes of *Phormidium* sp. are penetrating the membranes, they still can

glide inward and outward. After they enter completly into the sphere, they do not glide back.

The colonization of the spheres leads to recognizable stratifications of:

a) trichomes of *Phormidium* sp. forming a balloon-like layer underneath the surface of the sphere and
b) loosely distributed masses of the diatom *Navicula perminota* rich in EPS, and located in the inner part of the sphere (Fig. 5)
c) chemoorganotrophic bacteria and sometimes
d) a few green algae.

Sometimes cyanobacteria leave the spheres and form hairy outer rims (Figs. 6 and 7). Chemoorganotrophic bacteria and diatoms cannot leave the mature spheres.

During the sphere formation the number of diatoms inside systematically (Figs. 4 and 8) increases.

Figures 9-10 document the penetration of *Phormidium* sp. trichomes through the envelope into the sphere.

The cryo-REM micrograph shows dense layer of thin *Phormidium* sp. trichomes (Figs. 11).

Surface as well as the inside of the sphere was always very rich in EPS. *Phormidium* sp. trichomes in an advance stage of sphere formation created a non-transparent cover. Diatoms inside the sphere were visible only in sections after having cut the sphere (Fig. 12).

Finally, when the growth conditions inside the sphere become less favourable the diatoms abandon this microbial community, however, leaving the cyanobacterial surface network intact in form and shape. Only the framework of the spheres stays visible. This phase remains permanent. Sometimes the number of cyanobacteria increases again, but never reaches the maximum of the initial spherical phase.

3.2.2 Initiation of sphere formation

The activity of the diatoms and the presence of the diatom-cyanobacterial community were conspicuous. To elucidate the role of the bacteria in this community typical signal substances were applied in further investigations. The chosen signal substances favour the metabolism of certain heterotrophic bacteria what results in emphasizing the organisms out of the community.

In order to enhance the sphere building a number of different signal substances were separately added to the media (cAMP, OHHL Oxohexanoyl-Homoserinlacton, BHL Butyroyl-Homoserinlacton). The best results were obtained with BHL. Samples without signal substances had only up to 4 spheres per plate. After adding BHL the number of the spheres raised up to 43, with the average of 25. Temperatures between 16 ° C and 27 °

(degree) C and varied light intensities were tested and revealed not to have influence on the sphere growing. The size of the spheres differed between 0.3 mm and 1.2 mm.

Bacterial chemotaxis is a motile response to chemical gradients and may explain many consortia of cyanobacteria and bacteria. Direct evidence for the role of chemotaxis in forming such consortia is incomplete. Also quorum sensing (Surette et al., 1999) may be an important factor. Formation of spherical aggregates of trichomes could provide evidence for cyanobacterial chemotaxis to bacterial and algal products (e.g. calcite formation, data not shown), implying that chemotactic responses play a role in the formation of certain microbial consortia in nature.

A kind of trichome communication was observed in *Pseudanabaena galeata* (Castenholz, 1982). He observed the formation of swarms of trichomes, and called them ”comets”. Similar to the phenomenon observed there, an elaborate gliding of aggregates took place in our cultures as well. In case of these comets, light triggered complex movements. In case of sphere formation light has to be excluded as a factor causing movement. The origin of the spheres can not be connected with the existence of air bubbles, as no differences in density inside and outside the sphere within the agar were visible. Similar to our ”spheres”, the ”comets” formed and maintained their integrity during their movement. The manner in which trichome-to-trichome communication is formed with the respect to the movement is, in both cases entirely unknown.

Aggregates of other simpler geometry have been often observed in planktonic *Aphanizomenon flos-aquae* (Janson et al., 1994). The integrity of large flakes in this case is influenced by chemotactic phenomena and is probably maintained by some type of trichome-trichome interaction. Similar reactions could also be involved in the formation of ”spheres”, in the case of benthic biofilm LPP-forms.

4. CONCLUSIONS

Filamentous microorganisms isolated from North Sea microbial mats showed spherical assemblages in culture. The formation of ”spheres” is certainly a result of elaborate gliding of trichome aggregates. The integrity of large ”spheres” is most probably influenced by chemotactic phenomena and maintained by some type of trichome-trichome interaction. In the early stage of culture cyanobacteria move radially towards the sphere, later in older culture stages, the direction of the movement turns to the opposite

direction. This suggests the presence of methabolic, secondary products, which attract cyanobacteria and influence their movement.

Following hypothesis could be drawn from these studies:

Biofilms can organize themselves in spherical aggregates.
The functions of these aggregates are multiple

- Better organization of light, mineral and other resources
- Better protection from grazing
- Improved propagation
- Mutual symbiotic relationships
- Propagation by transport of spheres in the water column
- Organization of mineral deposits in spherical bodies (ooids).

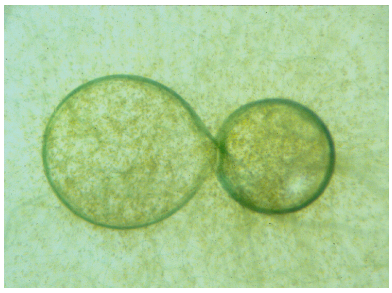

Figure 1. High diatom concentrations inside the spheres. Diameter of both spheres is about 1.2 mm. Light microscopy.

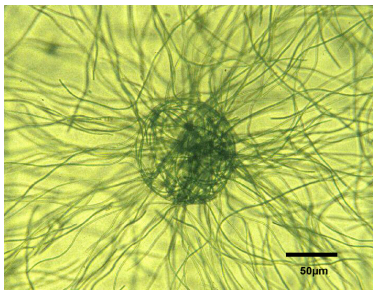

Figure 2. Radial movements of *Phormidium* trichomes towards and against the sphere

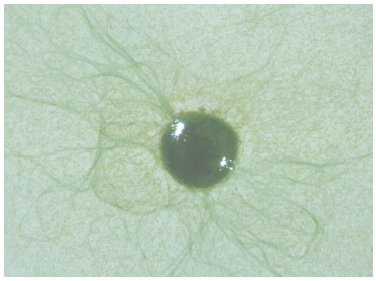

Figure 3. Sphere formation after 1 week. Diameter ca. 700 µm. Light microscopy.

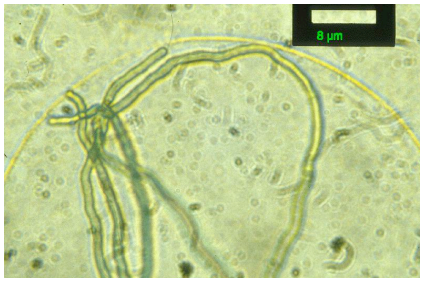

Figure 4. Envelope appearance of a sphere. Light microscopy.

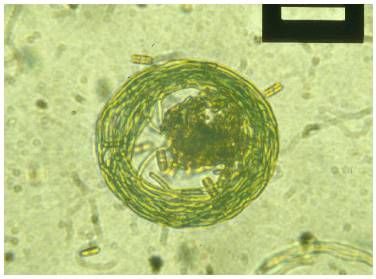

Figure 5. Sphere 8-12 hours after initiation. Diameter ca. 85 µm. Light microscopy

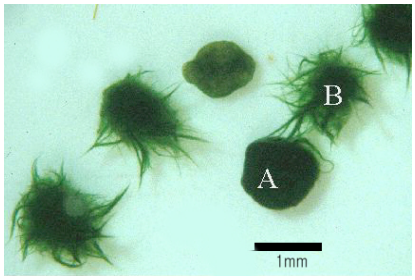

Figure 6. Two types of spheres: A) a smooth and B) a hairy one. Diameter of each ca. 1.0 mm. Light microscopy.

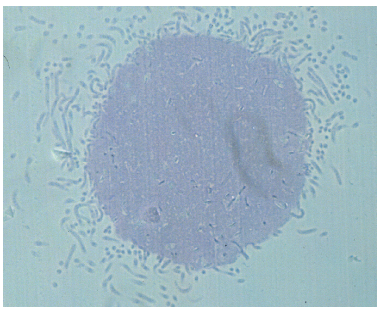

Figure 7. Thin section of a hairy sphere stained with Toluidin. Diameter ca. 1.0 mm. Light microscopy.

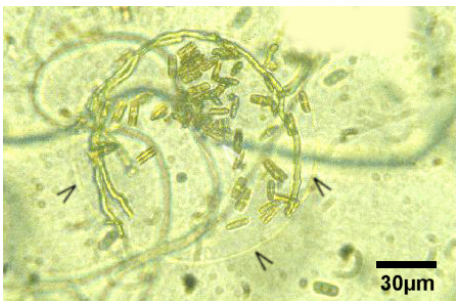

Figure 8. Sphere in an early stage. Diameter 120 µm. Light microscopy.

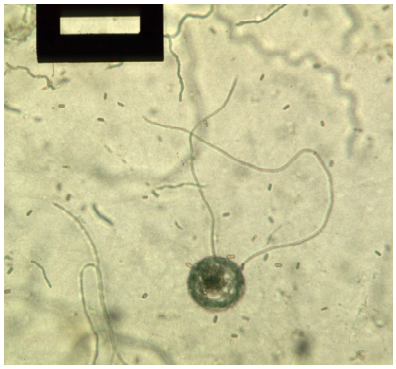

Figure 9. Trichomes of *Phormidium* sp. penetrate a sphere. Diameter ca. 84 µm. Light microscopy.

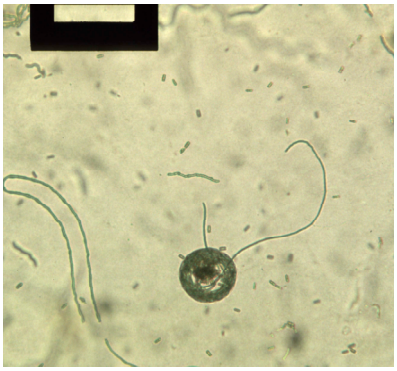

Figure 10. Trichome penetration eight minutes later. Light microscopy.

Figure 11. Surface detail of a sphere. Interwinded *Phormidium* trichomes are clearly visible. Cryo-SEM.

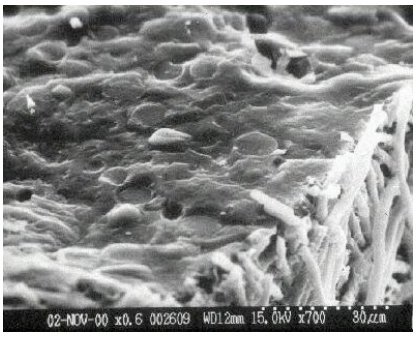

Figure 12. Section detail of a sphere. Cryo-SEM.

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