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CONFERENCE PROCEEDINGS

Behavior of Filamentous Cyanobacteria in Laboratory Culture

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Abstract—The observations of a laboratory culture of filamentous cyanobacteria revealed a complex of behavioral responses of their community, which maintain their activity as an integrated entity. A number of structures formed in the course of filament regrouping were revealed and described; their possible structural and functional analogues in eukaryotic organisms were determined. It is assumed that the behavioral reactions of the filaments help to maintain the integrity of the community at the stage prior to the formation of the structural bonds between its elements.

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Key words: cyanobacteria, community, group behavior of filaments, integrity

Although the complex behavior of communities of filamentous cyanobacteria in laboratory cultures had been noticed by many authors, it was considered a purely laboratory artifact not deserving investigation. Few publications described the interaction between filaments [1, 2]. The laboratory observations of the behavior of filamentous cyanobacteria presented in this article were performed due to the fact that these organisms are considered an actualistic model for the discussion of the nature of stromatolites, fossils of organic origin.

The behavioral aspects of filament interaction are discussed in the present paper. The investigation of molecular mechanisms of the interaction was not the goal of this work.

Cyanobacterial communities, both in nature and in laboratory cultures, form leathery films (Fig. 1). The filaments there are in constant motion and the structure of the film is in a kind of dynamic equilibrium. In the present work, the term "community" designates a compact aggregation of cyanobacteria of two different genera. Their trophic interactions are not considered.

Damage to the film, its interaction with various surfaces and sediments, weak vibration, and changes in the direction of illumination were used in the present work as initiating factors.

A cyanobacterial community from Kamchatka thermal springs was used for the experiments. It contains *Oscillatoria terebriformis* (Ag.) Elenk. emend. as the major component and *Phormidium angustissimum* W. et G.S.West as the minor component. The culture of *Microcoleus chthonoplastes* was used for some experiments.

Community response to disruptions of its mechanical integrity. The increased activity of the filaments after mechanical damage was shown to be directed towards the restoration of the community as a whole and was not caused by actions against individual filaments. This was evident from the behavior of the filaments. Disruption of the leathery film resulted in contraction of its fragments by the threads formed of filaments. The integrity of a torn film was restored by the "regenerative" action of the filaments; their most active motility was detected in the direction of the nearest disrupted zones of the film. The filaments join into threads in order to reach the opposite side of the rupture. The movement of the filaments in opposite directions leads

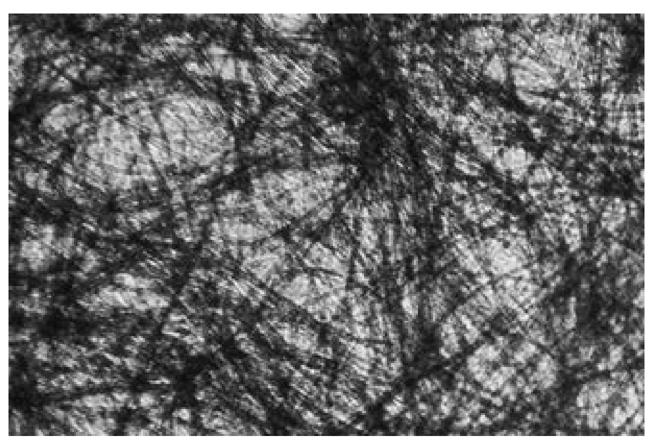

Fig. 1. Positioning of filaments within the film.

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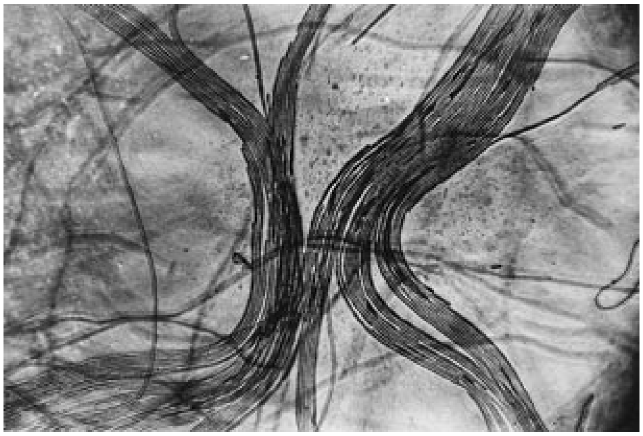

Fig. 2. Bending of filaments on an agarized medium.

to the curing of the rupture. Both whole and damaged filaments exhibited such activity.

A decrease in the area occupied by the culture was observed in the course of the reparation of the mechanically disrupted film. Similar behavior was reported for the culture of *O. terebriformis* from Oregon hot springs [3]; it may indicate that a community can maintain a certain degree of stability under changing environmental conditions, a manifestation of a certain homeostasis.

These behavioral responses are possibly related to the ability of the filaments to receive information concerning the condition of the community as a whole; i.e., this feature is similar to the competence of the cells of multicellular eukaryotes.

The culture was illuminated from above in the course of this experiment. In the disrupted leathery film illuminated from below, the behavioral reactions of the filaments resulted in the formation of a netlike structure. Colonization of space, rather than a flat surface, occurs in this case.

These differences in community structure caused by the direction of illumination indicate that these communities are able to differentiate between two independent factors, the direction of gravity and of illumination.

A community requires a relatively short time (1–1.5 h) to recognize the real incubation conditions. Afterwards, the structure conforms to its real position in space.

Thus, a community exhibits a broad range of behavioral responses; its appearance is determined by an integral and selective reaction to various environmental factors.

Community response in the course of movement through different substrata. According to our observations, leathery film is not the only form of community organization. It is not a result of random independent movement of filaments, but rather the final phase of a directional process. This can be observed after pouring a layer of calcium carbonate powder over the film. The initial structure of the film is restored via a strict sequence of stages: the stellar stage, the stage of subparallel filaments, and the uniform leathery structure. The three different stages are probably related to the filament positioning, which is optimal for the sediment immobilization at a given film thickness. The integrated behavioral reaction indicates that the informational relations are not disrupted when the community dissociates into individual filaments in order to penetrate through the sediment.

The group behavior of the filaments resulted in different structures when the "crawling substrates" with other structural characteristics were used (sand, cotton wool). A net of relatively thick threads with big cells was formed on sand. On the surface of cotton wool, the filaments aligned along the cotton fibers.

Thus, depending on the sediment structure, different film structures were formed by the community under the same conditions. This finding may indicate the existence of a community mechanism for the "evaluation" of the surface properties of the substratum.

Filament propagation over the surface of different substrates. A number of specific structures was revealed in the course of colonization of free space; their existence indicated that the filaments were able to create a favorable environment for the community as a whole even under objectively unfavorable conditions. The propagation of filament along a horizontal surface below the surface of the medium occurs as a front, which can be subdivided into several zones with different filament positioning. When a vertical glass surface is colonized, bent aggregations of parallel filaments are formed along the air–medium boundary. On the agar surface, the filaments form bends, loops, braids, and rings consisting of several individuals (Fig. 2).

The form of the spatial structures probably depends on the humidification of the surface. Dry surfaces are colonized by formations that probably perform the function of capillary systems in order to deliver liquids to the filaments located above the medium surface. Agar surface is colonized by structures of complex or closed contour; they are probably used to conserve moisture and not to provide its constant inflow.

Thus, the filaments react to different conditions by coordinated response, forming the structures required to satisfy the needs of the community.

Community response to weak vibration. Two specific responses were experimentally revealed in the community. They indicated the ability of the community to form specialized structures out of unspecialized components (filaments). Coordinated interaction of the structures aimed at a common result was observed. Since the results of coordination of filament movement are evident in spite of the absence of specific regulatory structures, these data lead to the conclusion that an "information field" exists in the community, a feature resembling competence of eukaryotic cells. When the community existed as a compact aggregate, its behavioral response to vibration was complex. The aggregate was raised and removed from the zone of unfavorable effects by coordinated action of multiradiate structures (see below) and retractable threads. The multiradiate structures possibly performed the information function within the volume of possible mechanical movements, while the threads played the mechanical part. Even in the absence of physical relations between structures, the correlation between all the structures was detected.

The ability of a community to maintain or change its position in space. The position of a film in space is maintained by coordinated filament movement. For instance, in spite of the production of photosynthetic oxygen, different reactions occurred when the film was illuminated from above or from below under the same light intensity. If the film was attached to the bottom, it remained there when illuminated from below and rose to the surface when illuminated from above.

Thus, the film is capable of regulating the amount of gas within its volume and its degree of adhesion, thereby changing its buoyancy depending on the direction of illumination.

The ability of a community to form structures incorporating gas bubbles. The community can redistribute photosynthetic oxygen and regulate its accumulation within the structures formed by the group behavior of filaments. Small, chaotically spaced bubbles are found in mechanically disintegrated films. Big bubbles associated with threads are present in undamaged films. The bubble formation commenced on the formation of a small gas-filled cavity within a thread; it then increased in volume and became spindle-shaped (Fig. 3).

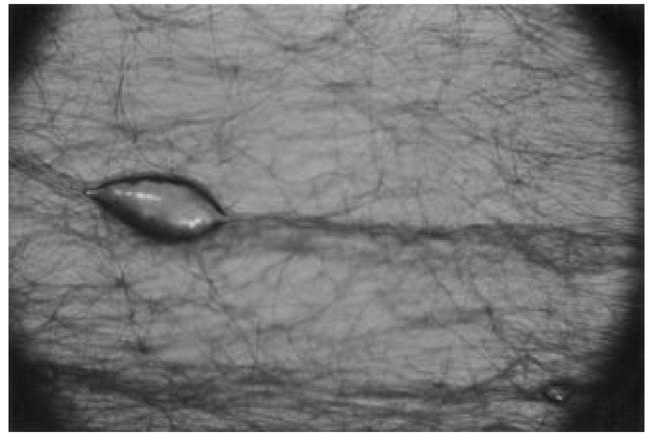

Fig. 3. Formation of an oxygen bubble within a thread; a spindle-shaped cavity and a gas channel are visible.

Thus, the envelopes of gas bubbles are specific structures formed as a result of the specific behavior of filaments.

A number of interacting structures of different shape and function formed via complex group behavior of filaments were revealed in the film. Their interactions and transformations have a clear functional sense. Some of these interactions and transformations are described below.

Film. *General appearance.* A flat, multilayered two-dimensional structure. The filaments in the film are interwoven in a manner similar to the collagen fibers of the skin. A boundary of three zones with different placement of filaments is formed at its edges. "Flows" of filaments of various shapes—featherlike, spiral, etc.—can also be present in the film. *Formation conditions.* Flat, usually horizontal surfaces of substrata or media. *Mode of formation.* Depending on conditions, the film spreads over substrata in different ways (see description above). *Functions.* Combines filaments in a structural and functional unity. The filaments within the film can maintain a favorable spatial orientation, fix the sediment which can bury it, and resist environmental factors insurmountable for individual filaments. Metabolic processes and the interaction of specialized structures occur within the film. *Structural and functional analogues.* Analogous to the surface structures, with a number of features characteristic of a highly organized integral whole (the ability to create and maintain an internal environment, to differentiate into the parts of varying structure and function, and to coordinate their activity).

Three-dimensional nets. *General appearance and structure.* Films with multiple perforations located horizontally, vertically, or at an angle within a layer of liquid (Fig. 4). *Formation conditions.* They are formed in big volumes of liquid under dispersed illumination. *Mode of formation.* Formed by long thin threads and multiradiate aggregates. The latter combine individual threads into a net, due to their capability of directional movement. In the course of further filament accumulation within the threads, they blend together and the intermediate cells disappear. Some of the cells remain free of filaments. *Functions.* Enable the film to colonize the available volume. *Structural and functional analogues.* They are to some degree analogous to the leaves of tropical plants growing under limited illumination, perforated or with light spots.

Multiradiate aggregates. *Structure.* Spherical units comprised of a limited number of filaments. The free ends of the filaments are directed externally along the radii (Fig. 5). A dense nucleus is present in the center. *Formation conditions.* Are formed in the initial phase of colonization of new volumes. *Mode of formation.* The mode of formation was not observed. The structures can become separated from the film and move within the volume of the liquid. *Functions.* "Collection of information" concerning the surrounding space and its "transfer" to the community is their main function. Move the free ends of threads. Create the spatial structure of the community. Form new colonies. *Structural and functional analogues.* The organs which perceive

Fig. 4. Initial stage of net formation.

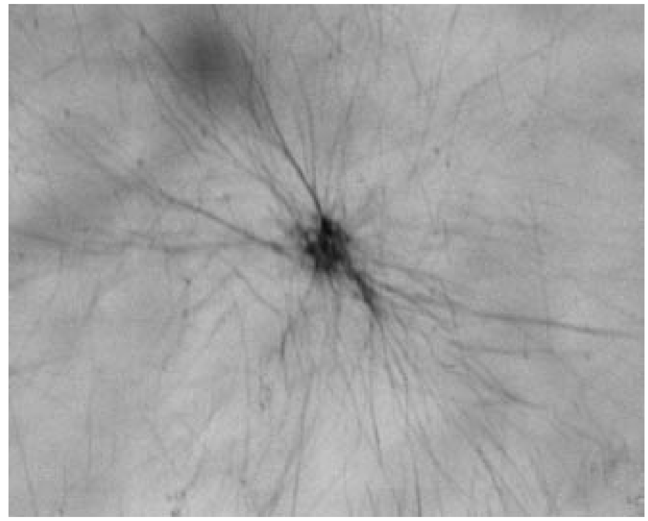

Fig. 5. A multiradiate aggregate.

Fig. 6. Threads in *Microcoleus chthonoplastes* culture.

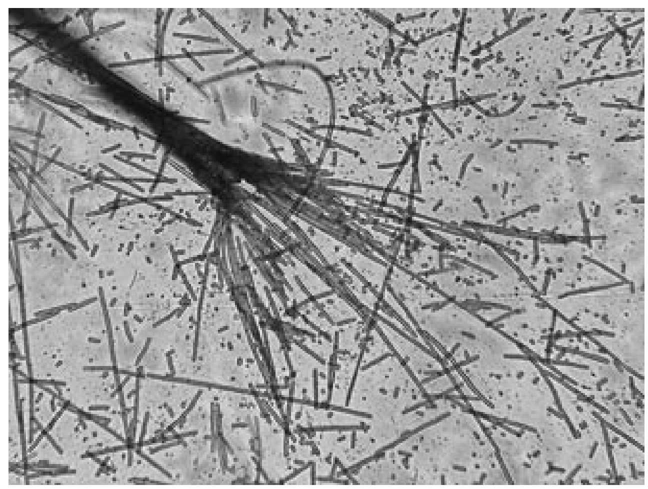

Fig. 7. Thread; the middle part and a fanlike end are visible.

and transfer information. Organs of vegetative reproduction.

Threads. *General appearance.* Aggregation of threads (up to 10 cm in length) located in parallel and touching each other with their lateral sides. *Composition.* The middle and terminal parts can be discerned. In the middle, the filaments are parallel and densely packed; in the terminal parts, they are located in a fanlike arrangement (Fig. 7). *Formation conditions.* Forms when the film must move in space or heal mechanical damage. *Mode of formation.* A loose band of filaments becomes segregated within a film and then transforms into a thread. *Functions.* Maintaining or changing the spatial position of the community. Colonization of new regions of space (together with multiradiate aggregates). The threads are capable of contraction (Figs. 6, 8) and of regeneration of damaged films. *Structural and functional analogues.* Muscles and extremities.

Walls of gas bubbles. *General appearance.* The regions of leathery film around the bubbles of photosynthetic oxygen. *Structure.* Similar to the film. *Mode of formation.* Small bubbles form within the threads that reinforce the film. They increase in size and acquire an oblong shape. *Functions.* Regulation of the spatial position of the film; oxygen storage for its utilization in the dark period of the day. *Structural and functional analogues.* The structures used to change an organism's buoyancy (swimming bladder of fish, gas chambers of cephalopods).

Rings. *General appearance.* Macroscopic structures with the border seldom consisting of one contour. *Structure.* They are comprised of concentric aggregations of filaments (Fig. 9). *Formation conditions.*

Under experimental conditions, rings were formed only on coarse-grained sand or on agarized media. *Mode of formation.* A ring-shaped structure with a fuzzy contour, which then becomes clearer, is formed within the film. *Functions.* Unknown. In *Anabaena cylindrica* Lemm., similar structures are the sites of akinete germination [4]. *Structural and functional analogues.* Probably, the reproductive organs.

Capillary structures. *General appearance.* Dense loop-shaped aggregations both on horizontal and vertical surfaces (Fig. 10). *Formation conditions.* Surfaces above the surface of the liquid. Open, loop-shaped structures are formed on drier surfaces and closed contours are formed on humidified ones. *Mode of formation.* Was not observed. *Functions.* Elevation of liquid by capillary forces. *Structural and functional analogues.* Intercellular spaces in plants.

The results of our observations lead to some preliminary conclusions. Communicative relations can probably be expressed both in behavior and in morphology (by formation of structures). The cells in the filaments of multicellular (filamentous) cyanobacteria have achieved the highest degree of integration. They are bound with cytoplasmic bridges, which perform the metabolic and informational functions (excitation transfer and movement of compounds from cell to cell). They are also differentiated within the filament; this can be considered a communication phenomenon. Within the population–communicative paradigm, heterocysts, one of the kinds of specialized cyanobacterial cells, are "altruistic" cells. They have sacrificed a number of important functions for the ability to fix atmospheric nitrogen and to transport it to other cells via the plasmodesms [5]. The film comprised of filaments is the next hierarchical level; the filaments act as its elements. Our experimental results give reason to believe that the film is characterized by integral features, since its characteristics as a whole cannot be reduced to the sum of the characteristics of its components. The film has structures and functions not present at the filament level. The choice of structural or functional analogues for interpretation of the film-derived structures seems justified, especially because similar experience exists in the study of microbial populations. For example, the air cavities in bacterial colonies were interpreted as the analogues of a primitive circulatory system [6]; the so-called "waveguides" were compared to axons of the nerve cells [7]. Communicative relations between the elements of the film are not, however, structurally formed; they are manifested only on behavioral level. Collective behavior of the filaments within the film can be seen in filament regrouping which leads to formation of the structures used to maintain the viability of the community as a whole. The mucous matrix is probably the only morphologically expressed component participating in communication. One of its functions is to serve as the medium used for signal and compound

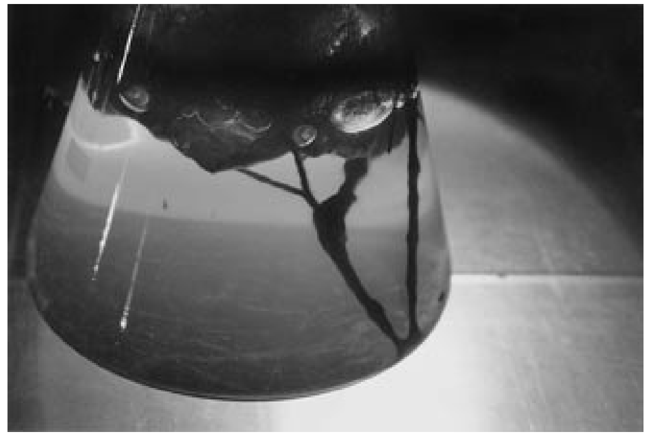

Fig. 8. Contraction of threads.

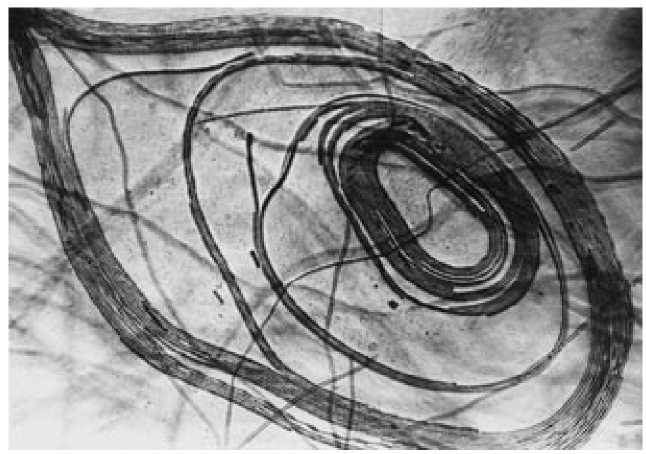

Fig. 9. Rings on agarized medium.

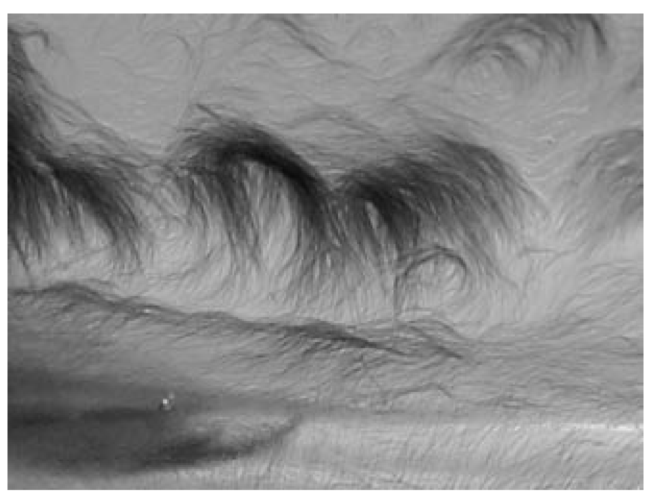

Fig. 10. Loop-shaped structures on the vertical glass surface above the level of the medium.

exchange in order to achieve the interactions within the population.

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