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NOTE

Bacterial Aggregates Formation After Addition of Glucose in Lake Baikal Water

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For determining the process of bacterial aggregation, glucose was added into water from Lake Baikal which had been stored for seven months. In the presence of a higher concentration of glucose, the abundance of single bacteria and aggregates were higher, but the biovolumes of both bacteria were similar. Theses results mean that both free-living and aggregated bacteria have similar maximum sizes and that aggregates are forming with available organic materials. With available organic materials, the biovolume of aggregates becomes larger.

***Key words*:** Bacterial aggregates, Bacterial biovolume, Glucose addition, Lake Baikal, Lake snow

Macroscopic organic aggregates in the ocean are important in the process of the cycling and flux of particulate organic materials (POM) from the surface to the depths (Alledge and Silver, 1988). Large organic aggregates, dubbed 'lake snow', have also been found in lakes. These particular organic materials carry a trophic significance similar to that of 'marine snow' in the ocean (Grossart and Simon, 1993).

There are some possible hypotheses related to bacterial aggregation. In large lakes and in the ocean, bacterial aggregation occurs on the surface of zooplankton fecal pellets and on phytoplankton debris (Turner, 2002). Due to the condition of the substratum, the bacterial attachment mechanism and community are distinctly different. Epiphytic bacteria on living plants and attached bacteria on cellulose films were observed to be morphologically and genetically different (Hong *et al*., 1999). Another possible means of aggregation is physical turbulence. By rolling a cylinder according to the dark-light cycle of 12-12 hrs, synchronous formations of aggregates >3 mm in length were formed in a culture tank (Weiss *et al*., 1996). Diatom bloom can be a causative agent leading to bacterial aggregation. When a surface for attachment and physical movement were not provided, bacteria aggregation was found during the diatom bloom (Riemann *et al*., 2000). Bacterial aggregation can begin at microcolony formation and synthesis of exopolymer should follow in order to advance enlargement (Marshall *et al*., 1989).

However, no united opinion exists as to the nature of bacterial aggregates. Some contributions related to the solving of this problem may be of a systematic, statistical approach, such as dimensional diversity, typical of the microbial community. Indeed, a number of papers have previously demonstrated that the abundance of bacterial aggregates is always considerably lower than that of single bacteria. On the other hand, larger bacterial aggregates have a lower relative abundance in the microbial association (Inkina and Ostapenya, 1984).

The purpose of this work is to reveal, under laboratory experiment, the difference between the dimensional structures of the elements of a microbial community in two contrary states: in terms of a relative state of rest, and in terms of a state of active functioning, in order to establish the general trend of changes of dimensional spectra. We expected that such an approach would allow us to come to an understanding of the self-organizing process of aggregation in a closed system, as well as in an open ecosystem. On the basis of this initiative, an investigation of the two cases was carried out to assess the dimensional spectra of elements of microbial communities. The first one examined natural water which had been sustained in an isolated state for an extended period. The second instance assessed the same water subsequent to the addition of glucose. The dimensional spectra of single cells and micro-

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colonies observed in these samples were then estimated.

In order to authenticate the processing of aggregation data, three differently amended Baikal waters in 3 liter volumes were used. First sample consisted of 3 μm filtered Baikal Lake water stored for 7 months in total darkness. This water was used to define the stable dimensional structure of microcolonies. The second and third samples were amended with the daily addition of glucose for 4 days and 11 days, respectively.

As for the additives, glucose was used as an energy substrate. Periodic additions of axenic glucose solution into the water of experimental vessels were done once a day in the amount of 5 mg/l for 4 days and 5 mg/l for 11 days. Throughout the course of the experiment, the water was exposed to darkness at an ambient temperature of 20-22°C.

Filtration of each sample was followed by microscopic observation. Ten ml of each sample was concentrated on a membrane filter (SYNOP). The single cells and aggregates on the filter were both counted and regarded as elements of the microbial community. The biovolumes and abundance of both elements were analyzed by microscope (Kuznetsov and Dubinina, 1989). The whole filter area was observed by maintaining the vertical and horizontal positions of the knobs of the microscope (X1000). Bacterial abundance was counted and the bacterial biovoume was measured according to the length and width of each bacteria. The biovolume of aggregates was obtained by tallying the number of aggregated bacteria and their mean biovolume.

With the addition of glucose, some of the individual tiny bacteria can grow, enlarge, multiply, and form microcolonies (Fig. 1). This means that the bacteria exposed to darkness for 7 months were dormant during that period, but the addition of glucose should return them to an active state.

The statistical changes of bacterial abundance, biovolume and mean volumes of single and aggregated bacteria following glucose addition are shown in Table 1. The numbers of bacterial elements of the microbial community increased 2-2.5 times after glucose addition. On average, both the volume of single cells and that of the aggregated bacteria increased 5-6 times after the addition of glucose. On average, the volume of bacterial aggregates, or microcolonies, of the sample to which glucose was added for 4 days was 7 times greater than that of the untreated sample. The volume of bacterial aggregates in the sample to which glucose was added for 11 days was 17 times larger than that of bacterial aggregates in untreated water. The total cumulative biomass of microbial elements was vastly increased to levels almost 20 times that of untreated water. The increase in biovolume was connected, in part to the increment of bacterial element abundance and also to the amplification of individual bacteria and bacterial aggregates. The mean biovolumes of single and aggregated bacteria were not different from one another, though the final concentrations of both tanks differed (20 mg/l for 4 days addition tank and 55 mg/l for 11 days addition tank). This means that

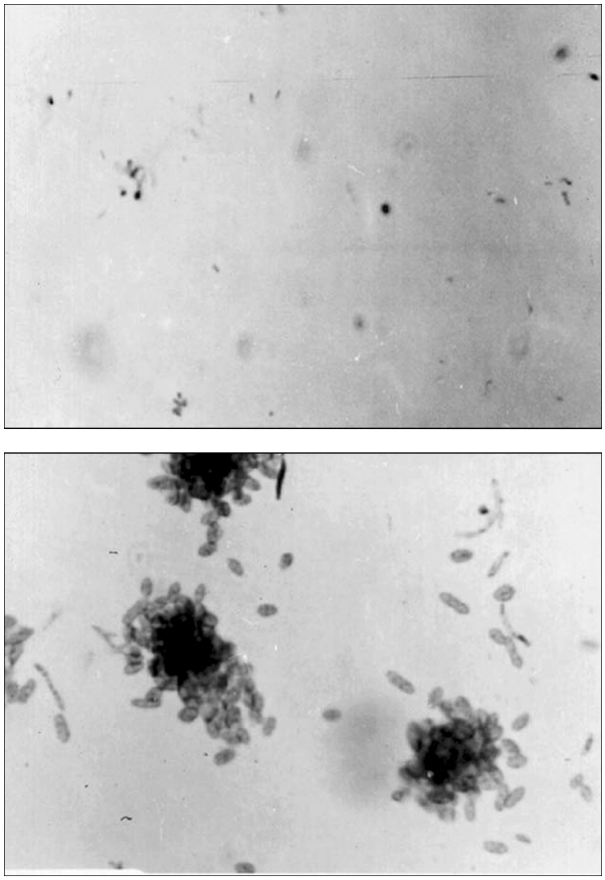

Fig. 1. Microphotography of aggregates formation (X 320). (top): After 7 months storage. Only tiny bacteria can be observed. (bottom): After glucose addition. The bacterial cell size is enlarging and number is increasing and forming microcolonies.

Table 1. Statistical parameters of the microbial community in Baikal water stored for 7 months and in the same water with daily added glucose

Indices investigated	Baikal water stored for 7 months	Same water under the condition of daily addition of glucose (5 mg L^{-1} d^{-1})	
		4 days	11 days
Total amount of elements of microcenosis in 1 mL	290,000 (14,500)*	640,000 (56,000)	810,000 (39,000)
Total volume of microbial biomass, μm^3/ml	69,000 (4,500)	1,400,000 (241,000)	1,300,000 (94,000)
Mean volume of single bacteria, μm^3	0.22 (0.12)	1.15 (0.98)	1.24 (1.05)
Mean volume of cells in aggregation, μm^3	0.2 (0.01)	1.34 (0.17)	1.28 (0.14)
Mean volume of bacterial aggregates, μm^3	0.7 (0.2)	5.0 (2.2)	12.0 (4.9)

*standard deviation

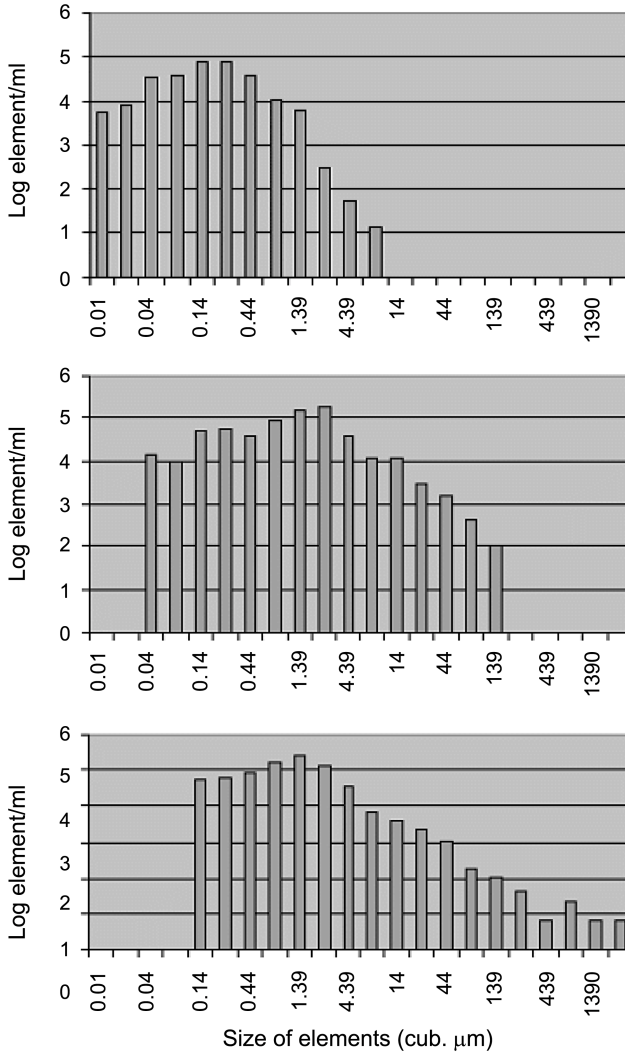

Fig. 2. Size spectra of microbial elements (single cells and aggregates). (top); Control, (middle); glucose addition for 4 days, (bottom); glucose addition for 11 days.

both categories of bacteria might be enlarging to maximum volumes of about 1.2 μm^3 in the enriched condition. On the other hand, the mean volume of bacterial aggregates of the 11 days addition tank was 2.4 times greater than that of 4 days addition tank. These results suggest that addition of organic materials causes the bacterial cells to expand to maximum size. Bacteria are then adsorbed to aggregates, forming larger aggregates, called 'lake snow'.

The dimensional spectra were moved to the enlarged side with the addition of glucose (Fig. 2). With 11 days of glucose addition, larger aggregates appeared in small numbers. Approximately 3,000 μm^3 of aggregates were 100,000 times larger than a single, tiny bacterium of 0.01 μm^3. Single cells were also enlarged by the glucose addition. In all three spectra, the numbers of bacterial elements larger than 1.2 μm^3 gradually dropped lower by 2-4 orders that of single bacteria in dominating dimensional groups. In terms of the increment of total biovolume, their

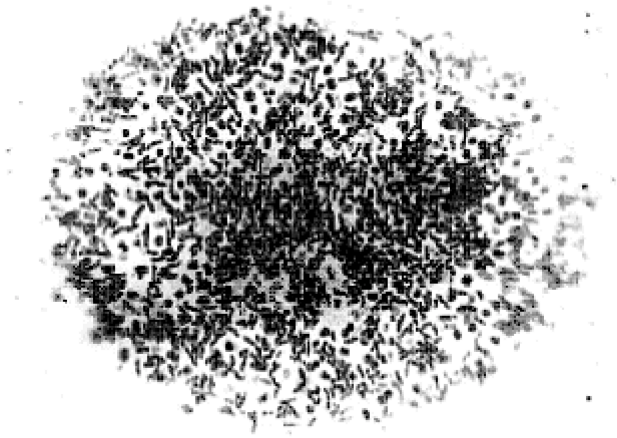

Fig. 3. Microphotograph of typical aggregated bacteria in Lake Baikal (X800).

relative abundance was reduced. This phenomenon is characteristic to the natural conditions of Lake Baikal (Spiglazov, 1999).

An example of the typical large aggregates in Lake Baikal can be seen in Fig. 3. Similar aggregates can be easily found in Lake Baikal, an oligotrophic lake. By means of the results above, assumptions can be made in regard to the aggregate production and size regularity. During the first step, phytoplanktons on the surface later release exudate during the process of photosynthesis. Exudates then stimulate the growth of single and aggregated bacteria to a maximum size of 1.2 μm^3. In the pelagic area of a lake, diatom blooming can cause bacterial growth and aggregation. In the artificial diatom blooming experiment, attached bacterial abundance increased after blooming. Prior to the peak of bloom, attached bacteria occupied from 7% to 27%, but post bloom phase, aggregations occupied between 42% and 85% (Riemann *et al*., 2000). These results suggest that diatom exudate can stimulate bacterial multiplication, enlargement, and aggregation. In Lake Constance, the highest numbers of aggregates was found between the surface and 5 m in summer and from late August until early October, during the cyanobacteria blooming period (Grossart and Simon, 1993). This means that bacterial growth and aggregation are stimulated by organic substance released from phytoplankton. The second step is enlargement of aggregates due surplus organic material. When microcolonies or small aggregates are forming, the bacteria produce exopolymer to prevent desorption of daughter cells (Vandevivere and Kirchman, 1993). With self produced organic materials and concentrated nutrients, the aggregates will become larger and larger by means of adsorption of other bacteria and aggregates. The third step of this process is act of sinking down to deep layer of aggregates. During the sinking, the bacterial community structure of the aggregates is changed (Weiss *et al*., 1996) and aggregates can avoid zooplankton grazing

(Juergens and Guede, 1994). A variety of biological and physical interactions cause the aggregates to eventually enlarge until they are of macroscopic size (>3 mm). These macroscopic aggregates are abundant in the middle layer of Lake Constance, for example (Grossart and Simon, 1998), and these aggregates are large and visible enough to be captured by scuba divers (Schweitzer *et al*., 2001). These large aggregates are composed of highly active bacteria (Karner and Herndl. 1992), which have a hand in the production of the oxygen depleted zone (Alldredge and Cohen, 1987). In this zone, methanogens are sometimes found (Bianchi *et al*., 1992).

In conclusion, our results show that in Lake Baikal, the biovolume of both free living and aggregated bacteria are at their maximum sizes and aggregates are form using available organic materials. If a higher concentration of available organic materials exists, the biovolume of aggregates is greater. This finding could be valuable in the study of aggregates, lake snow formation, and nutrient flux in lakes.

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