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Sediment bacteria attached to sand grains — indications to a protection from feeding by ciliates

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Abstract

Microbiological investigations of a marine, brackish sandy sediment area in a water depth of 10 to 12 m showed relatively high numbers of bacteria (4×10^8 - 2.4×10^9 bacteria g^{-1} dry weight of sediment). Benthic ciliates were estimated too. Their numbers varied between 1 and 87, with a mean of 21 individuals per gram dry weight of sediment. In general, the overwhelming part (80 %), were small forms of about 30 μm length. 54 % of the ciliates were predominantly bacterivorous. In feeding experiments using a fluorescent dye (DTAF), stained bacteria could be seen directly within the food vacuoles of the small ciliates, but this was the case only when free suspended bacteria were present. No uptake was visible if sand grains were added, which were colonized with stained bacteria. From these results, indications can be seen that small benthic ciliates cannot remove attached sediment bacteria. Taking into account that ciliates choose distinct size classes and certain strains of bacteria, less than a fraction of the total mass of bacteria is probably available as a potential food. This fact, together with a permanent reduction of ciliates by metazoa, ciliates themselves and hydrodynamic perturbations can explain the relatively low, but more or less constant number of the ciliate fauna at such locations.

Introduction

Bacteria are well known as a food source for protozoa (GAST 1985, FENCHEL 1987). Trophic interactions between microorganisms are characterized in the pelagial (SHERR and SHERR 1984, SHERR and SHERR 1987), but little is known from the benthic (LIGHTHART 1969, KEMP 1988). In marine sediments generally high numbers of bacteria, i.e. 10^9 cells per gram dry weight of sediment (MEYER-REIL 1986) are present. The number of benthic ciliates seems to be relatively low (SCHARF and SCHNESE 1984) in comparison to such an amount of a food source. Besides many other physico-chemical and biological factors in sandy sediments (HARTWIG 1973), available food has a very important impact on the expansion of the ciliate fauna (FENCHEL 1987). In order to explain the apparent inconsistency between low numbers of ciliates and high numbers of bacteria investigations of natural communities of sediment-microorganisms were made.

Material and methods

The sampling area was located at 'Gabelsflach', Western Kiel Bight (Baltic Sea, Germany) between 54° 31.70' N, 10° 20.00' E and 54° 32.26' N, 10° 18.20' E. The water depth was 10 to 12 m and the surface of the bottom was covered with middle sand. Sediment samples were collected with plastic cores of 5.4 cm diameter from the center of a Van Veen grab. Samples were taken from February 1986 to September 1988 once a month. The first 5 cm of the sandy sediment were used for each analysis. Bacterial counts were determined according to MEYER-REIL (1983). The biomass of the bacteria was calculated volumetrically by measuring in minimum 230 cells per sample with an ocular micrometer and converting biovolume in biomass by using the factor of 10^{-7} (Gast 1985). Benthic ciliates were counted alive, in minimum $9 \times 6.5 \text{ cm}^3$ sediment each time per sampling day. For their extraction out of the sediment Uhlig's seawater ice treatment (UHLIG et al. 1973) was chosen. The ciliates were counted, and sorted into three size classes: 1. $>0-30 \mu\text{m}$, 2. $>30-200 \mu\text{m}$, 3. $>200-1000 \mu\text{m}$. Their biomass was calculated via body-volume for each class referring similar to SCHARF and SCHNESE 1984. All ciliates were taxonomically brief classified in genera and their food source was determined from the literature (FENCHEL 1968, KAHL 1935).

For the feeding experiments, freshly extracted ciliates from about 30 cm^3 were concentrated by low power centrifugation. They were incubated together with the food source, either fluorescently labelled, suspended bacteria or sediment grains, colonized with stained bacteria on the surface. The suspended bacteria originated from an enrichment of sediment bacteria. They were collected from the sediment of the sampling location after centrifugation, stained with DTAF, 5-(4,6 Dichlorotriazin-2-yl) Aminofluorescein, and prepared as described in SHERR et al. 1987. To follow the uptake of bacteria by ciliates, the extracted ciliate community, 30 - 200 individuals, was incubated together with the suspended, DTAF-stained bacteria in a final concentration of $4 \times 10^6 - 3 \times 10^7$ bacteria ml^{-1} . Subsamples were taken from the beginning of the experiment up to several days. This experiment was repeated 5 times.

For the other food source, the sediment grains colonized with bacteria, natural sediment from the sampling location was washed several times with particle free, filtered seawater, to remove free, in the interstitial pore-water floating bacteria. One cm^3 of sediment was incubated 12 h with a DTAF-solution in a ten times higher concentration as described in SHERR et al. 1987, to get a bright stain of bacteria. To remove the unnecessary, suspended dye, the stained sediment was washed again several times with particle free seawater, splitted in portions of 0.1 - 0.8 gram dry weight and deep-frozen at $-18 \text{ }^\circ\text{C}$. The final concentration of bacteria attached to the sand grain surface was 2×10^8 cells g^{-1} dry weight of sediment. This experiment was repeated 3 times.

During the run of each feeding experiment several times subsamples of $20 \mu\text{l}$ water were fixed with OsO_4 -vapour, deep-frozen at $-18 \text{ }^\circ\text{C}$ and analyzed with a fluorescence microscope under $1250 \times$ magnification. All ciliates were searched, whether stained bacteria could be seen within their food vacuoles. The number of ciliates in a subsample varied between 2 and 16 individuals, with a mean of 9 ciliates per $20 \mu\text{l}$.

Results and discussion

The distribution of total bacterial number showed a mean of $9 \pm 1.3 \times 10^8$ cells g^{-1} dry weight of sediment (95 % confidence level, $n=36$). The counts varied between 4×10^8 and 2.4×10^9 bacteria g^{-1} , with minima at the end of April and maxima at the end of May and during August. The total bacterial biomass ranged from 3.8 to $54 \mu g$ bacterial carbon g^{-1} , with a mean of $12.4 \pm 5.5 \mu g C g^{-1}$ (95 % confidence level, $n=19$). These results are in the range of values known for sediment bacteria from the Western Baltic (MEYER-REIL 1986).

The mean number of the benthic ciliate community was 21 ± 2 cells g^{-1} dry weight of sediment (99 % confidence level, $n=91$). The counts of ciliates varied from 1 to $87 g^{-1}$, with minima during February/March and maxima in May, August, October and the beginning of December. They showed seasonal variations with a factor of 2. Similar quantitative results are known from other investigations (cf. FENCHEL 1987, SCHARF and SCHNESE 1984). In general, at every sampling day, 80 % (range: 52-100 %) of the ciliates were small forms of about $30 \mu m$ length. During the year, every time, the overwhelming portion of the ciliate community, in mean 54 % (range: 33-67 %), was predominantly bacterivorous. So these group of protozoa seemed to be significant in the investigation area.

The biomass of the total ciliate community varied from 2 to $70 ng$ ciliate carbon g^{-1} , with a mean of $18.5 \pm 3 ng C g^{-1}$ (99 % confidence level, $n=91$).

In comparison with each other, the total number of bacteria is in mean 7 orders of magnitude higher than the number of ciliates; in terms of biomass it is about 3 orders of magnitude. Thus, bacteria in the sediment should be - potentially - an unlimited food source for bacterivorous ciliates.

During the feeding experiments well stained bacteria could be seen about 30 minutes later within the food vacuoles of the small ciliates. This was only the case, if the added bacteria were free, suspended cells. Adding stained bacteria, which were attached to the surface of the sand grains, ciliates - mainly of the genus *Euplotes* - could be counted certainly for several days, but no uptake of bacteria into their bodies was visible. The presence and survival of ciliates without sufficient food by starvation was investigated by JACKSON and BERGER (1984). SHERR (1988) showed the direct use of dissolved polysaccharids by heterotrophic flagellates. Accordingly the survival of the investigated, benthic ciliates during the experiments can be explainable. During the experiments, the ciliates therefore were not able to incorporate bacteria, which were attached to surfaces. This may be indirectly a protection for bacteria against ciliate suspension feeding. In contrast other protozoa, like some flagellates, can use this food source (CARON 1987).

If benthic ciliates cannot use the whole sediment-bacteria population as food, it has to be distinguished between the total bacteria amount and the bacteria utilizable as food. Ciliates are minor vectors of total bacterial carbon in the sediment (KEMP 1988). But for the free, floating portion of bacteria, which lives in the interstitial pore-water, they can be very important. These unattached bacteria can supply up to 50 % of the total bacterial number (MEYER-REIL 1986). But taking into account, that protozoa can choose distinct size classes of food-organisms (REPAK 1983), have species specific grazing rates (LESSARD and SWIFT 1985) and use distinct strains of bacteria (BERK et al. 1976), may be a very small fraction of the total bacterial number in sandy sediments will be available as food. Besides this reflection the permanent reduction of benthic

ciliates by metazoa-feeding (RIEPER and FLOTOW 1981), ciliate-ciliate feeding (HEWETT 1980) and hydrodynamic perturbations (HARTWIG 1973) can cause the detectable, relatively small amount of ciliates in the sediments. The present numbers may reflect also an equilibrium stage of protists at such locations (PRATT and CAIRNS 1985). Nevertheless the microbiological food interactions in the sediment are far from an intensive understanding.

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