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Grazing of particle-associated bacteria—an elimination of the non-viable fraction

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ABSTRACT

Quantification of bacteria being grazed by microzooplankton is gaining importance since they serve as energy subsidies for higher trophic levels which consequently influence fish production. Hence, grazing pressure on viable and non-viable fraction of free and particle-associated bacteria in a tropical estuary controlled mainly by protist grazers was estimated using the seawater dilution technique. In vitro incubations over a period of 42 h showed that at the end of 24 h, growth coefficient (k) of particle-associated bacteria was 9 times higher at 0.546 than that of free forms. Further, 'k' value of viable cells on particles was double that of free forms at 0.016 and 0.007, respectively. While bacteria associated with particles were grazed (coefficient of removal (g) = 0.564), the free forms were relatively less grazed indicating that particle-associated bacteria were exposed to grazers in these waters. Among the viable and non-viable forms, 'g' of non-viable fraction (particle-associated bacteria = 0.615, Free = 0.0086) was much greater than the viable fraction (particle-associated bacteria = 0.056, Free = 0.068). Thus, grazing on viable cells was relatively low in both the free and attached states. These observations suggest that non-viable forms of particle-associated bacteria were more prone to grazing and were weeded out leaving the viable cells to replenish the bacterial standing stock. Particle colonization could thus be a temporary refuge for the "persistent variants" where the viable fraction multiply and release their progeny.

Introduction

Bacteria are capable of consuming more than 50% of primary production in the form of dissolved organic matter.[1,2] Thus, they play an important role as energetic subsidies for higher trophic levels. In recent years, studies focusing on grazing of bacteria by bacteriophagous microfauna have gained importance. Predation by microzooplankton in aquatic habitats is known to decrease bacterial abundance and stimulate mineralization of nutrients.[3] The process could influence the morphological structure, taxonomic composition[4] and physiological status of bacterial communities.[5] The grazers (microzooplankton) consist of holoplanktonic (protozoa, ciliates, flagellates, copepod nauplii, etc.) and meroplanktonic organisms (larval stages of benthic invertebrates:

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trochophores, veligers, etc.). Protozoans are known to play a major ecological role in the aquatic environment due to their effectiveness in consuming a wide range of prey size classes and types.[6] They are capable of consuming other protozoa,[7] phytoplankton[8] and bacteria.[9–11] Their grazing ability depends on the concentration of bacteria and digestion capacity of the grazer. Some ubiquitously distributed protists like ciliates occasionally form a major component of the microzooplankton community[12,13] and have a key position in plankton food webs as major consumers of pico- and nano-plankton.[14,15] Availability and concentration of the prey size and type, attached vs. unattached cells and viable vs. non-viable cells are some of the factors affecting ciliate grazing rates.

Among the planktonic bacterial community, particle-associated bacteria (PAB) have received more emphasis than free-living forms because of their relatively easier accessibility to filter feeders[16,17] and their ability to improve the nutritional quality of the particles.[18] The PAB can be directly grazed by larger metazoans, bypassing consumption by protozoan grazers and short-circuiting the microbial loop.[16] Reports on pelagic bacterial community have demonstrated that it is unwarranted to club particle-attached or unattached bacteria as a single unit.[19] To understand grazing preferences on the bacterial community in a tropical estuary dominated by protists, we conducted a seawater dilution experiment to measure the coefficients of increase '*k*' and grazing '*g*'.[20] The following queries were put forth: (1) do grazers exhibit selective preference for free or particle-associated bacteria (PAB) and (2) which physiological state (viable or non-viable) of free or particle-associated bacteria of the bacterioplankton will be eliminated by the grazers. Though previous work has highlighted the importance of PAB as food for grazers like microzooplankton[16,17,21] for the first time we have demonstrated elevated grazing pressure on non-viable cells of PAB and conservation of their viable forms for stock replenishment.

Materials and methods

Study area and sampling

A Niskin sampler was used to collect water samples at 3 m depth in Dona Paula Bay (15°27′ N, 73°48′ E; Fig. S1) which is located at the terminus of Zuari estuary in west coast of India. This semi-enclosed bay is undisturbed by seaport and riverine traffic. Details on the climatic conditions, annual variation in hydrographic parameters and biotic variables in the study area have been described elsewhere.[22] The temperature, salinity and pH of seawater during sampling were 24 °C, 21 psu and 6.3, respectively.

Grazing studies

The dilution technique[23] was used for grazing studies in the present work. The technique has been widely used to reduce the number of predators by creating a gradient.[24] The method has also been used to estimate growth rates of phytoplankton/bacterioplankton[25,26] as well as grazing pressure exerted by microzooplankton on these forms.[26,27] We set up incubations with whole and diluted seawater to determine bacterial abundance (total bacterial cells, PAB and free-living). A schematic representation of the experimental design is shown in Fig. S2.

For preparing particle-free water (used for diluting whole seawater), the filtration assembly was washed with 50% HCl and rinsed with Milli-Q water. Two litres of seawater was then filtered through a 0.22 μm filter (pressure < 100 mm Hg). Thus, to prepare 'diluted' samples for incubation, untreated 'whole' sample water was added to particle-free water in the ratio 1:4.

The PAB were considered as those that would be retained on a 3 μm pore-size filter (Fig. S2). Bacteria that passed through these filters were considered as free-living bacteria. An estimate of the total and free-living bacterial population in the whole and diluted seawater was carried out. The abundance of PAB was derived by subtracting the free-living from the total bacterial population. Controls were maintained for bottle effect and these were used to subtract from the experimental values. Diluted and whole water samples were incubated for up to 42 h at 27 ± 2 °C under static conditions. Sub-samples were removed from the whole and diluted seawater bottles at 6 h intervals for the estimation of TC (total bacterial cells), the total, viable and non-viable fraction of PAB and free-living forms. For the enumeration of total bacterial cells (TC), a 5 mL aliquot of water sample was fixed using 250 μL of buffered formalin (2% final concentration) as described by Hobbie et al.[28] For enumeration of viable cells (VC), 5 mL of seawater samples were amended with 0.001% final concentration of yeast extract and 0.0016% final concentration of antibiotic cocktail solution containing piromedic, pipemedic and nalidixic acid which were in the ratio of 1:1:1.[29] Samples were incubated statically in dark for 6 h. At the end of the incubation, the aliquots were fixed with 2% of buffered formalin. For TC and VC, 1 mL of sub-sample was filtered over a 0.2 μm black Isopore polycarbonate filter paper (Millipore Corp., MA, USA) and stained with acridine orange (final concentration 0.01%, w/v). The samples were incubated for 2 min and then filtered. Bacterial cells retained on the filter papers were counted using Nikon 50i epifluorescence microscope equipped with a 100× oil immersion objective. Viable cells refer to those which enlarge using the direct viable count (DVC) procedure.[30] These enlarged cells comprise of a mixture of cells which have increased in size and actively dividing cells. Thus, in this study, only cells that enlarge or replicate are counted as viable while the non-viable forms are considered as the ones which did not enlarge/replicate. Bacterial abundance has been expressed as cells L^{-1}.

The specific rate of increase in bacterial cells '*k*' and mortality/decrease '*g*' of bacterioplankton were estimated. The constants *k* and *g* are coefficients of population increase and grazing mortality, respectively.[23] These constants also refer to increase and removal not necessarily linked to growth and death. Therefore, it would mean an increase in one pool i.e., particle association by colonization, and decrease in another pool i.e., free-living forms. Both these coefficients may vary with time of day without affecting our comparisons of increase in bacterial numbers in the dilution over a fixed period of incubation. Thus, the abundance and grazing mortality of bacterioplankton was inferred from observed changes in population.