Large Differences in the Fraction of Active Bacteria in Plankton, Sediments, and Biofilm

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Abstract

Generally, only a small fraction of free-living pelagic bacteria are metabolically active, while particle-associated bacteria usually exhibit a larger proportion of active bacteria. Most previous studies on the active fraction of bacteria focus on planktonic communities, and there are only a few studies on sediment and epiphytic biofilm bacteria. We compared the active fraction of the total number of bacteria in three different habitats of the littoral zone of Lake Erken, Sweden, including the sediments, the epiphytic biofilm of the submerged macrophyte Ranunculus circinatus, and the water column. Active bacteria were detected as those with an active electron transport system, identified by the capacity to reduce the tetrazolium salt CTC (5-cyano-2,3-ditolyltetrazolium chloride) into its fluorescent, water insoluble state. There were large differences between habitats. The active fraction of the total number of bacteria detected by fluorescence microscopy (annual mean ± SD) in the sediments was 46 ± 10%, on R. circinatus 37 ± 18%, and in the water column 4 ± 4%. The abundance of CTC-reducing cells was correlated with total bacterial abundance, and the fraction of CTC-reducing bacteria generally increased with total bacterial abundance, for all the habitats. Consequently, the difference in the fraction of CTC-reducing bacteria between the habitats could be attributed to different densities of bacteria, with a larger proportion of active bacteria at higher bacterial densities.

Introduction

There is increasing evidence that all cells in natural bacterial communities are not metabolically active [e.g., 10, 44]. Most assessments of the active fraction of aquatic bacteria have focused on free-living bacteria [reviewed in 10 and 35]. The reported fractions of active bacteria range from a few percent to almost 100%, very much depending on the method used to estimate the abundance of active cells. Studies on the active fraction of bacteria in sediments and biofilms are comparatively few. The low total bacterial activity of sediments in combination with high numbers of bacteria implies long doubling times or a small active fraction. This has led to the assumption that the proportion of active cells in the sediment is low [24]. The

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generally high bacterial activity and short average doubling times of bacteria in epiphytic biofilms [e.g., 20], on the other hand, indicate a high fraction of metabolically active bacteria.

During the past 20 years several methods for estimating the active proportion of bacteria in aquatic habitats have evolved, e.g. microautoradiography [e.g., 34, 40] and epifluorescence microscopy using universal 16S rRNA probes [e.g., 19]. The latter method is not direct proof of metabolic activity but rather indicates potential viability of a cell. Nalidixic acid has been used to specifically inhibit the DNA synthesis, whereby active cells become elongated and microscopically recognizable [29]. Zweifel and Hagström [47] developed a procedure to remove excess dye from bacterioplankton stained with DAPI, to show the fraction of bacteria that contain nucleoids. Artificial electron acceptors, tetrazolium salts, are common indicators of electron transport system (ETS) activity. In bacteria with an active ETS, the tetrazolium salt is reduced to an insoluble colored or fluorescent product, which can be visualized with microscopy. Zimmerman et al. [46] introduced the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) and Rodriguez et al. [30] developed a method using CTC, 5-cyano-2,3-ditolytetrazolium chloride. CTC is more directly coupled to respiratory activity than is INT [38]. The CTC method often yields lower fractions of active bacteria than, e.g., microautoradiography or universal 16S rRNA probes [e.g., 19]. Not all bacterial strains are capable of reducing CTC [38], and CTC can be poisonous to the bacteria [33, 42]. Sherr et al. [35] showed that a number of taxonomically diverse bacterial strains are capable of reducing CTC. They suggested that cells that are able to reduce detectable amounts of CTC constitute the most active cells in the bacterial community, and thus are responsible for the bulk of bacterial metabolism. Despite uncertainties on how widely applicable the CTC method is, it has been used to detect respiring bacteria in several different habitats over the past decade. In addition to bacterioplankton studies [reviewed in 10 and 35], CTC has also been used in drinking water supply systems [6, 31] and in studies of the formation of biofilm on different substrates in drinking water [17, 31, 32]. Sherr et al. [35] included particle-associated bacteria in their study on CTC-reducing pelagic bacteria. Data on CTC-reducing bacteria in sediments are scarce. Miskin et al. [23] used CTC to assess the viability of prokaryote communities in post-glacial profundal freshwater sediments, and there are also some studies on soil [44]. Based on these references, it appears that particle-associated bacterial communities generally have a higher proportion of metabolically active cells than free-living communities.

Most previous studies on bacteria with an active ETS in freshwater have focused on pelagic waters. Only a few include particle-associated or sediment bacteria. We report a comparison of the proportion of CTC-reducing bacteria in the sediment, in the epiphytic biofilm on the submerged macrophyte Ranunculus circinatus, and of planktonic bacteria in the littoral zone of a lake. We found large differences in the fraction of CTC-reducing bacteria between these habitats. By a comparison across these different habitats, we demonstrate that the abundance of active (CTC-reducing) bacteria was correlated to the total bacterial counts, and that the fraction of active bacteria increased with the total abundance, independent of habitat.

**Materials and Methods**

**Study Site and Sampling**

The study was carried out from early June 1999 to May 2000 in the littoral zone of Lake Erken, a relatively large, mesotrophic lake situated 60 km north of Stockholm, Sweden (59° 51’N, 18° 35’E). The sampling was performed in a wind-sheltered bay with soft sediments every third or fourth week at sampling stations at 1.5 and 4 m depths. At each station an integrated water sample was taken from the entire water column. In addition, three sediment cores were retrieved and sectioned to retain the top 0.5 cm layer for following analyses (sediment density 1.0 g/cm³). In addition, leaves from a stand of the submerged macrophyte Ranunculus circinatus were collected at 1.5 m depth. Three leaves were taken at different locations on three plants (nine independent replicates) for bacterial abundance. For bacterial production nine independent replicates from three other plants were used.

**Abundance, Biomass, and Proportion of Bacterial Cells with Active ETS**

The fluorogenic tetrazolium salt 5-cyano-2,3-ditolyltetrazolium chloride (CTC, Molecular Probes) was used to detect bacterial cells with an active ETS [30]. In order to determine optimal final concentrations of CTC and incubation times, experiments were performed according to Choi et al. [5], using CTC concentrations ranging from 1 to 10 mM and incubation times from 1 to 10 h. The optimal CTC concentration was 6 mM for all three habitats, and optimal incubation time was 6 h for bacterioplankton and epiphytic bacteria and 8 h for sediment bacteria.

For the CTC incubations, approximately 0.5 g of wet sediment from the surface layer of each core (three independent replicates