RESOURCE AVAILABILITY AND THE GROWTH OF DIFFERENT BACTERIOPLANKTON

Jarone Pinhassi *, Laura Alonso and Josep M. Gasol
Institut de Ciències del Mar-CMIMA (CSIC), Barcelona, Catalunya, Spain - * jarone@cmima.csic.es

Abstract
The simultaneous use of nutrient limitation and dissolved organic carbon utilization bioassays during different seasons provided insights into the role of variable supply of resources for the growth of marine bacterioplankton. Furthermore, phylogenetic analyses of bacteria responding to different treatments in bioassays yield important novel clues to the adaptation of different bacterial groups and species to particular levels of nutrient availability in the sea. These findings suggest that variable patterns of resource availability and utilization may aid in explaining the abundance and activity of specific marine bacteria.

Key words: Marine bacterioplankton, Dissolved organic carbon, Nutrient limitation

Introduction

Present inventories of the composition of bacterioplankton offer a fairly good picture of the overall diversity among bacteria in the sea. Nevertheless, almost no information exists to explain precisely why particular groups or species of bacteria are successful. It is reasonable to assume that several physical, chemical and biological factors act selectively on the bacterioplankton. Considering the plethora of possible combinations between these factors it is intriguing that patterns of preference for particular conditions of some bacteria are emerging. For example, distinct marine bacterial isolates differ substantially in their enzymatic capacities, suggesting they are adapted to utilize different portions of organic matter [1]. In mesocosm experiments, a few *Cytophaga* species had a high growth capacity in the presence of elevated protein concentrations [2]. Using microautoradiography, it was confirmed that marine *Cytophaga* have a preference for complex macromolecules, such as protein [3]. To contribute understanding of how bacterioplankton are affected by resource availability we report results from a study of the growth of bacterioplankton in nutrient limitation and dissolved organic carbon utilization bioassays.

Material and methods

Surface seawater from the NW Mediterranean Sea was collected monthly from 28 January to 16 September 2003 at a coastal site 40 km N of Barcelona, Spain (41°40'N, 2°48'E).

The effect of nutrient addition on the growth of heterotrophic bacteria was examined in samples of 250 ml of unfiltered seawater. Nutrients were added to final concentrations of 20 µM C (as glucose or dimethylsulfoniopropionate, DMSP), 2 µM N (NH_4Cl), and 0.6 µM P (NaH_2PO_4), singly and in all different combinations in duplicates. Control bottles received no nutrients. After incubation for 24 h at in situ temperature in the dark, samples for bacterial production, bacterial abundance and microbial community DNA were collected.

We determined the growth of bacteria combined with measurements of dissolved organic carbon (DOC) consumption in duplicate seawater dilution cultures enriched with ammonium and phosphate as well as in duplicate control cultures without additions. The bacterial assemblage (0.8 µm-pore-size filtered) was inoculated into 0.2 µm-pore-size filtered water used as growth medium. Bacterial growth was monitored until stationary phase (4 d), and subsamples for bacterial abundance and thymidine and leucine incorporation were collected twice daily. Microbial community DNA for phylogenetic analyses was taken at the endpoint. These analyses included PCR-denaturing gradient gel electrophoresis (DGGE) of 16S rDNA and sequencing of bands.

Results and discussion

The nutrient addition experiments with unfiltered seawater showed that bacterioplankton growth was limited by the availability of P during most of the year (Fig. 1). Notable exceptions occurred during winter, when rain storms provoked temporary upwelling of deep water and/or land runoff that provided bacteria with nutrients. Combined addition of C, either as glucose or DMSP, and P consistently yielded higher growth compared to only P (Fig. 1). This suggests that although labile DOC accumulates in this sea area during summer (see [4] and below) it is less easily utilized compared to glucose or DMSP. Our data also indicate that DMSP is a highly preferred source of organic carbon to marine bacteria.

Bacteria in the control dilution cultures ranged between 0.7 to 1.2 x 10^6 cells ml^{-1}, with highest values in winter. During winter, growth of bacteria in the enriched dilution cultures was only slightly higher than in the controls, while in spring and summer enrichment caused up to fourfold higher bacterial growth rates and abundance. These data corroborate that DOC accumulates in surface seawater in regions where bacterioplankton growth is limited by mineral nutrients [4].

Analyses of bacterioplankton composition in our bioassays showed that some bacteria abundant in the initial water also grew in the control dilution cultures - particularly *Roseobacter* and *Cytophaga* relatives. Although these groups are frequently detected as abundant in seawater using DGGE and FISH, it was found that specific members of these groups became dominant in the cultures depending on nutrient status and season. In the enriched cultures, fast-growing gamma-proteobacteria, such as *Alteromonas*, quickly became dominant. Overall, there was a intimate relationship between the amount of DOC consumed and the composition of the bacterial assemblage.

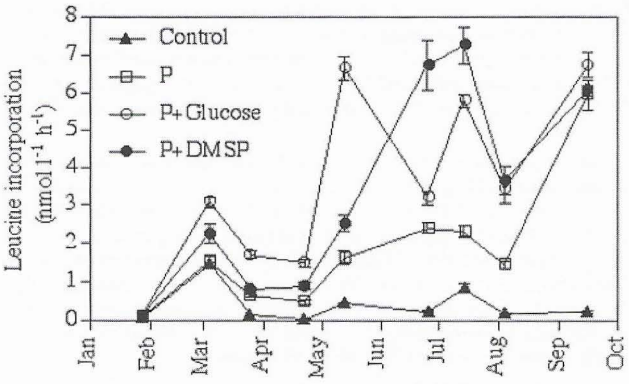

Fig. 1. Temporal changes of the response in bacterial production to the addition of nutrients. Treatments without P behaved as the control.

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