HIGHLY ACTIVE BACTERIA IN THE SURFACE WATERS OF THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA)

A. Karuza *, P. Del Negro, A. Paoli, S. Comisso, S. Fonda Umani
Laboratory of Marine Biology, Via Auguste Piccard 54, 34010 Trieste, Italy

Abstract
In this study we examined the metabolically active fraction of bacterial community in surface waters of the Gulf of Trieste using the CTC incubation method technique. The results suggested that the CTC+ cells are not responsible for the bulk of bacterial activity. The method seems to be adequate to detect only the cells with very active metabolism but not the cells in a transitory metabolic state. Therefore the CTC reduction assay should be viewed and interpreted as an efficient method for identifying the most highly active cells in bacterioplankton populations or assemblages.

Keywords: CTC, active bacteria, Gulf of Trieste

In the bulk of bacterial community there are at least three categories of cells that should be of biogeochemical relevance: i) actively growing cells which contribute to production and biomass ii) living but inactive cells and iii) dead and inactive cells. Although discrimination among these three cellular categories remains unclear (1), some methods have been suggested to determine the fraction of actively growing cells in complex assemblages.

For the purpose of our study we choose the CTC incubation method, a simple and fast technique to determine the number of bacteria that have measurable rates of electron transport system and therefore an active respiration.

Surface (0.5 m) water samples were bimonthly collected in a coastal station of the Gulf of Trieste from June 2002 to April 2003. Total bacteria abundances were determined using DAPI staining method (2), while metabolically active cells were detected using a CTC incubation technique (3). Bacterial Carbon Production (BCP) was determined by ^{3}H-leucine and ^{3}H-thymidine incorporation (4). Dissolved Organic Carbon (DOC) concentration was assessed by high temperature catalytic oxidation (5). Rates of oxygen utilization were calculated from changes in dissolved oxygen concentration, using the Winkler method, over a 24 h period in samples incubated in the dark and *in situ* temperature. Temperature data were obtained by a Idronaut Ocean Seven (Model 316) multiparametric probe.

Bacterial abundances ranged between 2.51 x 10^8 and 4.78 x 10^9 cells L^{-1} whereas the number of active cells (CTC+) fluctuated from 1.72 x 10^6 to 9.92 x 10^7 cells L^{-1}. The percentage of CTC+ bacteria ranged between 0.03 and 7.41%. The abundance of CTC+ cells was strongly correlated to total bacterial numbers and, better than with total bacteria, it showed strict relationships with temperature and substrate availability, evaluated as DOC concentration. On the contrary, bacterial production, measured as ^{3}H-leucine and ^{3}H-thymidine incorporation, were correlated to total number of bacteria only (Tab. 1). Respiration rate within the plankton community resulted strongly correlated to total number of bacteria where the active fraction only partially support the respiration process.

Setting aside methodological differences, our results, like those of many other authors (e.g. 6), show that not all bacteria are metabolically active and that the water temperature appears to have had a profound effect on the pattern of induction of respiration activity. The statistical dependence between CTC+ bacteria and DOC, could have been caused solely by the increase in temperature which is also usually the controlling factor in the phenomena involved in the production of autochthonous organic matter readily assimilable by bacteria.

Although it could seem surprisingly because of the principle of the CTC method based on detecting cell respiratory activity characteristic for growing cells, oxygen consume rate and BCP had better statistical relationships with total bacteria abundances rather than active cells, contrarily to the results of Smith (7). This could concern a limit of the method that detects only the cells with very active metabolism but not the cells in a transitory state, between CTC+ and CTC-, that contribute to the total respiration measured and therefore the CTC reduction assay should be viewed and interpreted as identifying the most highly active cells in bacterioplankton populations or assemblages. Indeed, the level of cell activity is what determines the detectability of respiring cells, since even bacteria in a starvation survival state must sustain certain functions.

Table 1. Parameters of linear regression analysis.

x	y_1	r_1	p_1	y_2	r_2	p_2	n
temperature	CTC+	0.91	<0.001	total bacteria	0.79	<0.001	n=17
^{3}H-TdR	CTC+	0.21	n.s.	total bacteria	0.7	<0.01	n=15
^{3}H-Leu	CTC+	0.24	n.s.	total bacteria	0.77	<0.001	n=17
respiration	CTC+	0.67	<0.01	total bacteria	0.87	<0.001	n=17
DOC	CTC+	0.63	<0.01	total bacteria	0.5	<0.05	n=18

References

1 - Gasol J.M., Zweifel U.L., Peters F., Fuhrman J.A. and Hagstrom A., 1999. Significance of size and nucleic acid content heterogeneity as measured by flow citometry in natural plankton bacteria. *Appl. Environ. Microbiol.*, 65: 4475-4483.
2 - Porter K.G. and Feig Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, 25: 943-948.
3 - Rodriguez G.G., Phipps D., Ishiguro K. and Ridgeway H.F., 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl. Environ. Microbiol.*, 58: 1801-1808.
4 - Smith D.C. and Azam F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in sea water using 3H-leucine. *Mar. Microb. Food Webs*, 6: 107-114.
5 - Sugimura Y. and Suzuki Y., 1988. A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Mar. Chem.*, 24: 105-131.
6 - Jugnia L.B., Debroas R.D., Sime-Ngando T. and Devaux J., 2000. Variations in the number of active bacteria in the euphotic zone of a recently flooded reservoir. *Aquat. Microb. Ecol.*, 22: 251-259.
7 - Smith E.M., 1998. Coherence of microbial respiration rate and cell-specific bacterial activity in a coastal planktonic community. *Aquat. Microb. Ecol.*, 16: 27-35.