BACTERIAL EXTRACELLULAR ENZYMATIC ACTIVITIES IN A TYRRHENIAN ECOSYSTEM (GULF OF MILAZZO)

Caruso G., * Decembrini F., Azzaro F., Raffa F., Galletta M.G.

Istituto per l'Ambiente Marino Costiero (IAMC) - Section of Messina, C.N.R., Messina, Italy - * caruso@ist.me.cnr.it

Abstract

Leucine aminopeptidase, β-glucosidase and alkaline phosphatase activities were estimated by fluorogenic substrates in a Tyrrhenian ecosystem; two different periods were studied, as indicative of different hydrographic structures (i.e., water column stratification and homogeneity). The measured values of metabolic activities sometimes followed a general decreasing coastal-offshore gradient; during water homogeneity, enhanced leucine aminopeptidase activity rates, heterotrophic and autotrophic biomasses occurred, while during water stratification high levels of metabolic activity were detected.

Key-words: Enzymes, bacteria, phytoplankton biomass, Mediterranean Sea

Introduction

Heterotrophic bacteria, which play a major role in the particulate to dissolved matter transformation, are known to promptly respond to environmental changes, modifying their metabolic patterns according to available organic compounds. Microbial activity rates are enhanced in highly enriched environments (1, 2). The Gulf of Milazzo ecosystem is located along the north-eastern coast of Sicily; in its more western part it receives organic matter inputs coming from stream and urban and industrial settlements. A tendency of this area towards eutrophication has been observed in the past (3). Unlike the hydrological and general biological parameters, bacterial dynamics and metabolism in this ecosystem remained poorly understood. As part of the Cluster 10 - MIUR Project, aimed at monitoring coastal Sicilian areas, the distribution and variation in extracellular enzymatic activity and bacterial abundance together with the phytoplankton biomass, were investigated. Focus was put on evaluating both the autotrophic and heterotrophic communities in relation to spatial (i.e., effect of continental input) and temporal scale (i.e., trophic status of waters due to seasonal organic enrichment).

Materials and Methods

Two oceanographic cruises (December 2002 and February 2003) have been performed by the R/V *L. Sanzo* of the IAMC-CNR, in the Gulf of Milazzo during late fall and winter, as representative of different hydrological conditions (water stratification and homogeneity, respectively). The ecological characteristics of the area are described in the same issue (4). Seawater samples (n=15 for each cruise) were collected by a rosette sampler above, within and below the Deep Chlorophyll Maximum (DCM) along a coastal-offshore transect located in the central-western section of the Gulf (latitude 38°12'N, longitude 15°14'E to latitude 38°17'N, longitude 15°22'E). The following parameters were measured: temperature, salinity, dissolved oxygen, fluorescence, nutrients (ammonia, nitrate, orthophosphate), autotrophic biomass expressed as chlorophyll-*a* (Chl-*a*), extracellular enzymatic activities (leucine aminopeptidase, AMP, β-glucosidase, β-Glu, alkaline phosphatase, AP) and heterotrophic bacterial abundance (MA) by Marine agar plate counts. Bacterial extracellular enzyme activities (EEA), expressed as the maximum velocity of hydrolysis (V_{max}), were determined using the specific fluorogenic substrates L-leucine-7-amido-4-methyl-coumarin hydrochloride (LEU-MCA), 4-methylumbelliferyl (MUF)-ß-glucoside and MUF-phosphate for the ectoenzymatic activities AMP, β–Glu and AP (2).

Results

Enzyme patterns displayed a general decreasing trend with increasing distance from the coast in both the periods examined. In December 2002, AMP values ranged from 0.284 to 15.36 μM/h, while β-GLU and AP values were between 0.0545 and 3.562 nM/h and between 0.14 and 867.62 nM/h, respectively. Heterotrophic bacteria ranged from 2 to 57 CFU/100ml. Auto- and heterotrophic biomasses, calculated from all the data, were significantly reciprocally correlated (r= 0.758, P<0.01, n=13) and correlated with temperature (r= 0.734 and 0.941, P<0.01, n=13, for MA and Chl-*a* respectively). In the stratified surface layer, the highest AMP and AP levels, as well as Chl-*a* and MA concentrations were found. Here, the metabolic activities correlated positively with temperature (r= 0.997, P<0.01; 0.954, P<0.05; 0.993, P<0.01, n=4 for AMP, β-Glu and AP respectively).

The stratified water structure was also reflected in the highest coefficient of variations (C.V.= standard deviation/mean *100) measured for heterotrophic (94.96) and autotrophic (50.53) biomasses. Within the DCM layer, phytoplankton contributed to the release of AP, as suggested by the close relationship (r= 0.938, P<0.05, n=4) between AP and Chl-*a*. Below this layer, the heterotrophic growth correlated with phytoplankton biomass, as shown by the MA-Chl-*a* relationship (r= 0.976, P<0.05, n=4). In February 2003, when water column became mixed, values of about 2 orders of magnitude higher than those previously observed were found for AMP (12.31-73089.26 nM/h) and bacterial heterotrophic density (70-1880 CFU/100ml). A similar increasing pattern was displayed by Chl-*a*, with values (from 0.065 to 0.45 μg/l) higher than in December (0.015 to 0.238 μg/l). The phytoplankton growth was supported by high ammonia (0.10-7.02 μM) and inorganic phosphate (0.09-1.01 μM) amounts available in this period. In contrast, AP and β-Glu activity values were lower than in December, ranging from 0.256 to 463.96 nM/h and from 0.00107 to 0.01505 nM/h, respectively. Peaks of AP and Chl-*a* shifted from surface towards intermediate depths, following the slight increase (0.37 °C) of temperature towards the bottom, while AMP activity and MA did not seem affected by the hydrological condition, being always higher in surface layers. Within the DCM layer, all the bacterial enzyme activities were significantly reciprocally correlated (r= 0.989, P<0.01; 0.952 and 0.909, P<0.05, n=4, for AMP-AP, AMP-β-Glu and β-Glu-AP, respectively).

Discussion

The bacterial activity values measured in our study ranged in the same order of magnitude as those reported for other pelagic Mediterranean waters (5). AMP was the prevalent enzyme, as observed in other temperate environments (6). Although the supply of terrigenous organic matter determined a spatial decreasing pattern of bacterial activity towards offshore stations, some enzyme peaks were also observed offshore in relation to the particular (anticyclonic) mesoscale water column structure (4). Temporal variations in organic matter decomposition rates were also found. In the well-mixed water column, the availability of nutrients supported both autotrophic and heterotrophic growth. When the water column becomes stratified, enhanced activity rates towards more refractory compounds were found. This preliminary study contributes to the knowledge of mean-term changes in microbial processes in Mediterranean ecosystems in relation to environmental conditions.

References

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