EXTRACELLULAR ENZYMATIC ACTIVITIES IN WATER AND SEDIMENT: FIRST EVALUATION IN THREE SITES DESIGNATED FOR MARICULTURE

Caruso G.

Istituto Sperimentale Talassografico, C.N.R., Messina, Italy - caruso@ist.me.cnr.it

Abstract

Extracellular enzymatic activities (leucine aminopeptidase, β-glucosidase and phosphatase) and heterotrophic bacterial density were investigated both in water and sediment samples of three marine sites of the Mediterranean Sea, in order to point out any variations related to mariculture activity. The preliminary data obtained before the beginning of fish farming experiments showed significant variation patterns among the sites for all the parameters examined even in undisturbed conditions.

Key-words: enzymes, bacteria, aquaculture

Introduction

Microbial hydrolysis of natural polymers through extracellular enzymes is considered a fundamental step in the degradation of the organic matter in both pelagic and benthic environments (1). Enzyme activities are known to be highly sensitive to environmental changes and respond differently to the organic matter supply (2, 3). Microbial abundance and metabolic activities may undergo substantial alterations, i.e. such as in intensive aquaculture farms (4) or in mariculture sites (5).

In the framework of a multidisciplinary research program aimed at evaluating the impact of fish-farming plants on the environment, extracellular enzyme activities and heterotrophic bacterial abundance were studied in order to evaluate changes in their distribution induced by mariculture activities. The preliminary data, reported here, outline a preliminary picture of the metabolic potentialities of bacterial communities present in natural undisturbed conditions, before the beginning of fish-farming experiments.

Material and methods

Surface water (0.5m) and sediment (0-1.5cm) samples were collected from two areas located along the Tyrrhenian coast, Castellammare Gulf (A) and Capo d'Orlando (B), in both spring and summer (area A: late March and June 2000; area B: May and July 2000). A third area, Portopalo of Menfi (C), in the Mediterranean Sea, was sampled only in summer (July 2000). Three extracellular enzymes (leucine aminopeptidase, LAP, b-glucosidase, glu, and phosphatase, phos), involved in the hydrolysis of proteins, mucopolysaccharides and organic phosphates respectively, were estimated using specific fluorogenic substrates (L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl(MUF)β-D-glucopyranoside and MUF-phosphate) (2, 6). Measurements were performed after 3 hours at the *in situ* temperature in a TD-700 Turner fluorimeter, at 380/440 excitation/emission wavelengths, for LAP, and at 365/455 for glucosidase and phosphatase. Enzyme levels in sediments were also normalised to grams of dry weight. Bacterial heterotrophic density was evaluated on Marine agar 2216 (Difco) plates incubated at 22°C for 7 days. A three-way Anova test (F-test) (areas X months X stations) was used to assess statistical significance of variations in the variables measured.

Results

The mean values of enzyme levels measured in water samples showed a strong variability from site to site; bacterial density values were more homogeneous (range 1.4-1.9x10^3CFU/ml). In area A, aminopeptidase (68.4±7.9-541.3±9.1nM/h/l in spring and summer respectively) and phosphatase (9.2±1.8-99.3±1.7nM/h/l in the two sampling periods) predominated; glucosidase was detected only at low concentrations (3.6±1.7-17.0±5.1nM/l/h in spring and summer respectively). Area B displayed high phosphatase and glucosidase (peak value 91.8±6.5nM/h/l) values. In Area C phosphatase levels reached their maximum (1.8±0.3μM/h/l). Within each area temporal variations were also observed, mainly in summer for LAP and phosphatase values in area A.

In sediments, the mean values of enzyme activities were 2-3 orders of magnitude higher than those reported for water. Phosphatase activity prevailed over the other enzymes in the three sampled areas (range 1.5±0.05-98.1±0.1μM/l/h). High aminopeptidase levels were also recorded in areas C (16.8±1.6μM/l/h) and B (14.6±3.2μM/h/l). This latter was characterised by the highest values (4.9±0.3μM/l/h) of glucosidase activity. Enzyme activities, in particular phosphatase, were consistently higher in summer. Bacterial densities were on average one order of magnitude higher than in waters (range 7.8x10^3-2.9x10^4CFU/ml).

The ANOVA test of data showed highly statistical significant differences, due to the variables "areas", "months" and "stations", for phosphatase (F=7.77, 4.61, 15.48 respectively) and density (F=8.44, 9.72, 8.91 respectively) data. In sediments, variations in glucosidase and density levels were ascribable to variable "stations" (F = 4.63 and 4.70, respectively). Pearson's correlation coefficients revealed significant ($P<0.05$) relations between aminopeptidase and glucosidase only in summer in the waters of area A (r=0.84) and C (r=0.99) and in the sediments of area C (r=0.98). Significant relationships between bacterial abundance and both LAP and phos (r=0.99, $P<0.05$) were evidenced in summer in the sediments of area B only.

Discussion

Enzyme values obtained in this study are the first available in the areas examined and may add some useful information to knowledge of the metabolic capabilities of microorganisms involved in organic matter decomposition. This preliminary study points out how the distribution of bacterial abundance and enzyme activities in water and in sediments did not follow a clear pattern; changes in activity levels were apparently independent from changes in bacterial abundance. The summer increase in heterotrophic bacterial concentration is probably a consequence of higher temperature and of a seasonal nutrient supply both in water and sediment. The occurrence of enzyme activity in surface sediments with remarkably higher values than in water suggests the high catabolic potentiality of heterotrophic bacteria and a faster organic matter turnover due to the presence of a higher fraction of active bacterial cells (7). Further studies carried out at intermediate and final step of the establishment of productive activities will allow to follow the course of metabolic processes and verify it or identify microbial functional alterations related to mariculture activity. In particular, heterotrophic bacterial communities present in marine sediments, able to respond to short term environmental changes more rapidly than those present in water column (8), could potentially be used as early warning indicators of environmental disturbance.

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