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INTERACTIONS BETWEEN BACTERIA AND POLYCHAETES IN THE SEDIMENT AND THE ROLE OF TEMPERATURE: A PRELIMINARY EXPERIMENT

S. CORTI[1], G. MASCIANDARO[2], B. CECCANTI[2], C. LARDICCI[1]

[1]Dep. Scienze dell'Uomo e dell'Ambiente, University of Pisa, via Volta 6, 56126, Pisa, Italy
[2]Istituto per gli Ecosistemi (ISE), sezione di Chimica del Suolo, Area della Ricerca, Via Moruzzi 1, 56124, Pisa, Italy
Corresponding author: cortisere@discau.unipi.it

BACTERIA
BIOTURBATION
HEDISTE DIVERSICOLOR
INT
POLYCHAETES

ABSTRACT. – Interactions between benthic macrofauna and bacterial communities in the sediment are very complex. The most conspicuous animal effects on sediment decay processes are caused by burrowing and ventilation activity and by direct feeding on detritus and associated microorganisms. Microbial activity can be stimulated by animals due to fractioning of particles. The direct assimilation of detritus components by macrobenthic organisms can however competitively remove substrates that are otherwise available to microbial decomposers. The aim of this work was to study the possible interactions between macrofauna and bacterial community by a laboratory experiment using microcosms and INT to quantify active bacterial populations with two different temperature values (10ºC and 21ºC). *Hediste diversicolor* (Polychaeta: Nereididae) – a detritivore polychaete – was considered in this study. This species is known as an important bioturbator of shallow coastal sediments. Results showed that the presence of polychaetes in the sediment (muddy and sandy) reduced bacterial activity at 21ºC while no differences were detected at 10ºC. The temperature seemed to play a fundamental role in determining the type of interactions between polychaetes and bacterial community in sediment. The results were similar in both type of sediments.

INTRODUCTION

Some important factors like bioturbation, quali-quantitative composition of sediment and temperature could influence distribution, abundance and bacteria activities in the sediment. The bioturbation processes caused a strong disturbance of sedimentary column by burrowing and/or feeding activities of macrobenthic animals (Commito 1982, Commito & Shrader 1985, Kristensen *et al.* 1991, Gilbert *et al* 1998, 2003). In the bioturbated sediment, bacterial processes, such as aerobic degradation of organic matter, sulfide oxidation are generally enhanced due to introduction of oxygen-rich overlying water via the burrows into otherwise hypoxic sediment (Banta *et al.* 1999, Ferro *et al.* 2003, Heilskov & Holmer 2003). Localized enhancement of bacterial growth and activity in bioturbated sediments and especially around the sediment structures created by macrofauna has also been documented (Aller 1982, Kristensen *et al.* 1985, Kristensen 2000, Heilskov & Holmer 2001). Interactions between benthic macrofauna and microbial communities in sediments are generally complex. Microbial activity can be stimulated by animals due to fractioning of particles (Kemp 1987) and ventilation activity. The direct assimilation of detritus components by animals, however, can competitively remove substrates that are otherwise available to microbial decomposers (Tenore *et al.* 1982, Lucas & Bertru 1997). Another possible and conspicuous animal effect on sediment processes is caused by direct feeding on detritus and associated microorganisms (Hanson & Tenore 1981). These interactions may thus, in addition to direct physical impact, be mutualistic, competitive or predatory, in nature (Kristensen *et al*. 1992). The temperature could play a fundamental role in the determining interactions between bacteria and polychaetes because regulated directly animals activity and metabolism in the sediment (Kristensen *et al*. 1992, Ouellette *et al.* 2004, Deschênes *et al.* 2003). Heterotrophic processes in aquatic environments are usually increased by a factor of 2-3 for a temperature increase of 10ºC (Westrich & Berner 1984), and the relative importance of various sediment processes may change according to the temperature regime. Seasonal variations in benthic metabolism predominantly reflect the temperature dependence of the particular reactions, transport mechanisms involved, and changes in the composition of organic source material (Mackin & Swider 1989, Danovaro & Fabiano 1997).

The aim of this study was to investigate the interaction between bacterial community and polychaetes in two different sediments (sandy and muddy) and the role of temperature in the determining the type of interaction. The polychaete species considered in this work is *Hediste diversicolor* (Polychaeta: Nereididae). The interaction was studied by measuring the bacteria activity levels in a control sediment (without *H. diversicolor*) and a treated sediment (with *H. diversicolor*). A histochemical approach using INT (2-(*p*-iodophenyl)-3-(*p*-nitophenyl)-5-phenyl tetrazolium chloride) for dehydrogenase activity detection has been used frequently to determine the activity levels of bacteria (Yu *et al*. 1995, Wu *et al*. 2003). INT is easily reduced by the bacterial or mitochondrial respiratory dehydrogenase system to form red, water-insoluble crystals (INT-formazan), whose size and optical density correspond to the respiratory activity intensity. In contrast to the lower permeability of INT into the mitochondria of intact eukaryotic cells, respiring bacteria and cyanobacteria accumulate INT-formazan as optically dense, dark red, intracellular spots. Because of the simplicity and specificity of the biological tetrazolium reduction, INT has been used to stain "respiratory active" prokaryotes from various aquatic environments (Zimmermann *et al*. 1978, Tyler & May 2000).

METHOD

The experiments were conducted in laboratory with two sediments type (sandy and muddy) to obtain a major range of situations. Sandy samples exhibited 80% of sand, 15% of silt and 5% of clay and muddy samples showed 54% of silt, 26% of clay and 20% of sand. For each sediment type two temperature levels (10°C and 21°C) were considered. Sediment samples were collected in the Orbetello lagoon (South Tuscany, Italian West Coast) (Fig. 1) and were taken at 2 different dates in May 2003 (10 May 2003 and 20 May 2003 respectively) with a little grab at a depth of 5 cm. Sediments were immediately put at – 4°C for few hours before experiment; this treatment was not expected to reduce the bacterial population severely (Kristensen & Blackburn 1987). After, sediments were stored in laboratory for one week to acclimate at 10°C and 21°C before start the experiment. In our study 16 glass jars (9.5 cm in diameter; 10 cm in height) were used as microcosms; in each jar 200 ml of filtered natural sea water and 200 g of two types of sediment were added. Sea water was only filtered with Millipore filters (0.45 µm) and sediment was homogenized to reduce the natural heterogeneity and to obtain equal starting conditions. The 16 jars were divided in two sets: 8 with sandy sediment and 8 with muddy sediment. For each jar we added 3 *H. diversicolor* with the same size (2-3 cm). This individual number corresponds to a natural density (400 ind/m^2). We put these organisms into each of four boxes (treated sediments) and four boxes without polychaetes were used as control (control

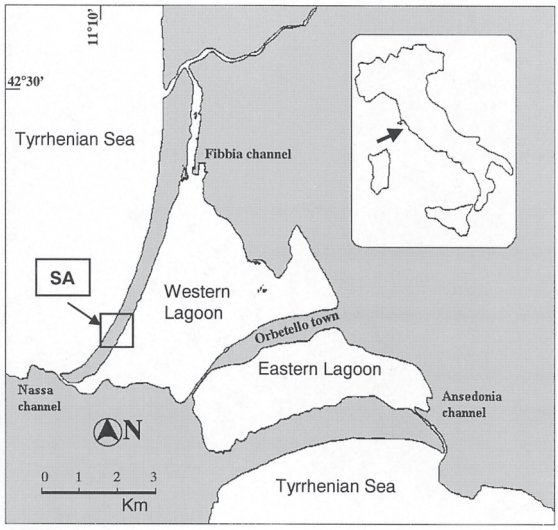

Fig. 1. – Sampling area (SA).

sediments). Polychaetes were collected in a small stream from S. Rossore Park (Pisa, Italy). A few polychaetes that appeared dead on the sediment surface were replaced during the experiment. Experiments were conducted for 1 month. Jars were continously aerated and a 12 h light: 12 h dark cycle was maintained throughout the experiment. At the beginning and at the end of the experiments dehydrogenase activity was measured. To determine the bacterial activity in sediment 0.5 g of dry sediment were added to 2 ml of INT for muddy sediment and to 1.2 ml of INT for sandy sediment, since previous studies (Ceccanti, personal communication) showed that available substrates concentration was reduced in silty-clay samples as muddy sediment. This is probably due to chemico-physical interactions. Samples were mantained for 20 h in the dark at room temperature (about 20°C) and then 5 ml of a solution of tetrachloroethylene and acetone (1:1.5) was added to the samples, according to Masciandaro *et al*. (2000). The sediment suspension was centrifuged for 10 min at 2000 * g, and the supernatant was used for analysis. Absorbance of the supernatant at 490 nm was determined spectrophotometrically, and was converted to formazan concentration by using a calibration curve obtained with chemically reduced INT (Sigma Chemical Co.). Data were analysed by one-way analysis of variance and a posteriori SNK test was used when significant differences were detected. Homogeneity of variances was tested by Cochran's test; when necessary data were transformed to remove heterogeneity (Winer 1971).

RESULTS

After one month control and treated sediments appeared very different; *H. diversicolor* created burrow structures from the first day which exhibited a higher oxidized conditions than defaunated control sediments. The initial level of dehydrogenase activity appeared much higher in muddy sediment than in sandy sediment (in both 10°C and 21°C experiments). Moreover samples

utilized in the experiment conducted at 10°C presented higher levels of dehydrogenase activity than sediments utilized for experiment at 21°C. In the experiment conducted at 21°C bacterial activity after one month presented higher levels in control sediments than in treated sediments, in both sandy (Fig. 2 a) and muddy (Fig. 2 b) samples; these results were confirmed by analysis of variance that showed significant differences between sediments with polychaetes and sediments without polychaetes ($p<0.05$) (Table I a-b). Results at the end of the experiment conducted at 10°C were reported in Fig. 2 c-d; the one-way analysis of variance didn't show any significant differences between control sediments and treated sediments ($p<0.05$) (Table I c-d).

DISCUSSION

Some authors observed that the macrofauna in the sediment had significant effects on chemical fluxes and microbial activity (Aller 1982, Kemp 1987, Kristensen 1992, Banta *et al.* 1999, Mermillod-Blondin *et al.* 2004, 2005). Generally the presence of benthic fauna, especially burrows-dwelling polychaetes, stimulated bacterial activity, as demostrated by Mermillod-Blondin *et al.* (2004, 2005). These stimulatory effects were explained as an enhancement of sediment oxidation in the presence of fauna. Moreover another important factor considered to affect bioturbation processes is the temperature (Kristensen *et al.* 1992, Deschênes *et al* 2003, Corti 2004, Oullette *et al.* 2004). These studies have shown that the reduction of temperature strongly influence the polychaetes metabolism with the decrease of consumption of organic matter loaded in the sediment, foraging activity and grazing. In our experiment the results showed a negative effect of the presence of *H. diversicolor* on bacterial activity, in the experiment conducted at 21°C. This type of results was probably due to these reasons: *a)* In the experiment little microcosms were used and so the competition for food between polychaetes and bacteria could be very high in a reduced space as previously reported by Carlèn & Olafsson (2002) for gastropods and bacteria. *b)* At 21°C *H. diversicolor* showed the maximum of metabolic processes and the foraging activity and grazing of polychaetes on bacteria could be strong, in according with Hymel & Plante (2000). *c)* The quali-quantitative composition of sediment is very important to determine the type of interaction between polychaetes and bacteria (Hymel & Plante 2000). Mutualism is predicted if a high-carbohydrate, fibrous food is consumed whereas a competitive interaction should be found if food-resources rich in protein or easily digested carbohydrates is eaten (Bianchi & Levinton 1981, Plante *et al.* 1990). The two types of sediment utilized in these experiments presented high levels of protein in respect to carbon compounds with low levels of C:N ratio (10-12). This type of composition of organic matter probably determined a competitive interaction between polychaetes and bacterial community.

In the experiment at 10°C no significant differences between control and treated sediments in both type of substrates were detected. This temperature correspond to winter conditions in the water column and in the cold regime polychaetes metabolism and activity were lower compared to warm regimes (in our experiment 21°C), in according with Oullette *et al.* (2004). The reduction of polychaetes metabolism with the decrease of foraging activity and grazing probably determined an important effect on microbial community with an enhancement of bacterial activity.

In this study the use of INT appeared a good approach to evaluate bacterial activity in the sediment, especially in heterogeneous medium. This method is very sensitive and it is possible to measure bacterial activity in aerobic conditions because INT compete with oxygen (Trevors 1984, Benefield *et al.*1977). Recently the *in situ* application of INT has been performed by Wu *et al.* (2003). *In situ* application of INT to the bioturbated sediment provided a unique opportunity to visualize the effects of the biological activities of the worm on the sediment microbial population and this is another advantage of this method. Other methods have been established for enumeration of active bacteria (Dufour & Colon 1992, Yu *et al.* 1995) as CTC technique (5-cyano-2,3-ditolyl tetrazolium chloride). CTC has been used as a vital stain of actively respiring bacteria for several years (Rodriguez *et al.* 1992, Yu *et al.* 1995) but some authors demonstrated that this method can suppressed bacterial metabolism and underestimated the portion of active bacteria in natural aquatic environment (Ullrich *et al.* 1996, 1999).

On the basis of our results we can conclude that the temperature seemed to play a fundamental role in determining the type of the relationship between polychaetes and bacterial community of the sediment especially in temperate areas. This suggests that interactions between deposit feeders and sedimentary bacteria vary seasonally. Nevertheless this model of experiment and the use of INT to measure the level of bacterial activity could be very important tools to study the possible interactions, generally very difficult to identify, among different organisms in the sediment.

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