Vol. 24(1):47-53 | Ocean and Polar Research | March 2002

Article

Preliminary Studies on the Relationship between Reed and Bacterial Communities in the Salt Marsh Environment of Namyang Bay, Korea

Kae Kyoung Kwon*[1] and Jong-Geel Je[2]

[1]*Marine Resources Laboratory, KORDI*
Ansan P.O. Box 29, Seoul 425-600, Korea
[2]*Marine Environment and Climate Change Laboratory, KORDI*
Ansan P.O. Box 29, Seoul 425-600, Korea

Abstract : To evaluate the effect of reed population on the distribution and activities of microorganisms, vertical distribution of heterotrophic bacteria, degradation rate of cellulose, extracellular aminopeptidase activity (APA) and metabolic diversity based on GN2 Microlog plate were measured at two salt marsh stations in Hogok-ri, Namyang Bay, west coast of Korea. The number of heterotrophic bacteria at station 1 (reed population inhabited area) showed 2 to 6 times higher than that of station 2 (exposed area) with exception in the surface layer. Cellulose degradation rates in station 1 showed more than 50 % · $month^{-1}$ and higher than that of station 2 (10.2 to 38.4 % · $month^{-1}$). Yet the APA at two stations did not show difference except surface layer and suggested that APA might not be a significant factor in degrading marsh plant debris. Lipid class compounds, cell wall polymers and L-alanine were widely used by microorganisms. The number and activities of bacterial populations especially concerned in plant debris degradation seemed to be stimulated by the reed communities.

Key words : salt marsh, enzyme activity, metabolic diversity, heterotrophic bacteria.

1. Introduction

Temperate salt marshes are one of the most productive ecosystems on Earth with carbon fixation rates exceeding 1,000 g-C · $m^{-2}year^{-1}$, whose productivity is dominated by macrophytes (Schubauer *et al.* 1984). This high productivity supports an active microbial community both on standing dead shoots and in salt marsh sediments (Boschker *et al.* 1999). Large portion of this organic matter is decomposed by the microorganisms within marsh sediments. Thus, the distribution and activities of microorganisms and the interaction with plants in salt marsh are of great interest.

In terrestrial systems, many studies have shown that both bacterial biomass and metabolic activity are generally higher in rhizosphere environments than in bulk soil due to release of root exudates (Schwaner and Kroer 2001). There was evidence that salt marsh plants also has close relationship with microorganisms in rhizosphere (Whiting *et al.* 1986; Blaabjerg *et al.* 1998; Kroer *et al.* 1998). The presence of dense root or rhizome system in salt marsh sediments and the ability of this root system to deliver organic materials and oxidants below ground produce dynamic subsurface redox potentials capable of supporting steep chemical gradients and a diverse microflora (Hines 1991). The marsh is a habitat with high rates of microbial activity that are strongly affected by plant activities. Hence, the rhizosphere is an ideal microhabitat for bacterial proliferations and interactions between marsh plants and rhizosphere microorganisms and it is a key part of plant health and ecosystem function (Giovanni *et al.* 1999; Hines *et al.* 1999).

Most of the works done in coastal wetlands in Korea were on the diversity and distribution of macroorganisms, and little things were known about the distribution and activities of microorganisms and relationship with other organisms (Kwon *et al.* 1998; Baik *et al.* 2000; Lee *et al.*

*Corresponding author. E-mail : kkkwon@kordi.re.kr

2001).

In this report, we compare the number, activities and metabolic diversity of microorganisms with and without reed community for understanding the relationship between marsh plant and microorganisms. We use GN2 Microlog™ plate (Biolog) to characterize the microbial communities of two different samples by typing metabolic diversity (Giovanni *et al.* 1999). Garland and Mills (1991) introduced the Biolog system (Biolog Inc., CA, USA) as a rapid community-level tool to characterize and classify heterotrophic microbial communities based on sole carbon-source utilization patterns. This system is based on 95 individual carbon-source oxidation tests, for which the color change of the redox dye tetrazolium violet is used as an indicator of consumption of the sole carbon source. Because of its simplicity and commercial availability of the microtiter plates, this system has been widely used to characterize microbial communities (Vershuere *et al.* 1997). Degradation rate of cellulose, the major components of marsh plants, was also measured to get the information on the decomposition of dead plant.

2. Materials and method

Research area

The study area is tidal flat located in Hogok-ri, Namyang Bay, west coast of Korea. Due to the military importance, this area has been protected from civilian and consequently maintained typical shoreline of west coastal area of Korea. There is fresh water inflow *via* small stream. Salt marsh plant habitats prolonged to about 100 m from shoreline. *Carex scabrifolia*, *Phragmites communis*, *Suaeda japonica*, *Triglochin maritimum*, and *Zoysia sinica* are major components of plant populations.

Two stations were selected in upper tidal zone (Fig. 1). Station 1 located in the central area of reed populations. The distance from shoreline was about 60 m. Station 2 located in the exposed area of tidal flat, which is approximately 40 m away from the margin of plant populations. The sediments at station 1 contained 64.9 to 83.6 % of mud fraction and showed 4.51-8.09 ϕ of mean grain size. Mud content of station 2 ranged from 92.2 to 98.0 % and mean grain size ranged between 7.08 and

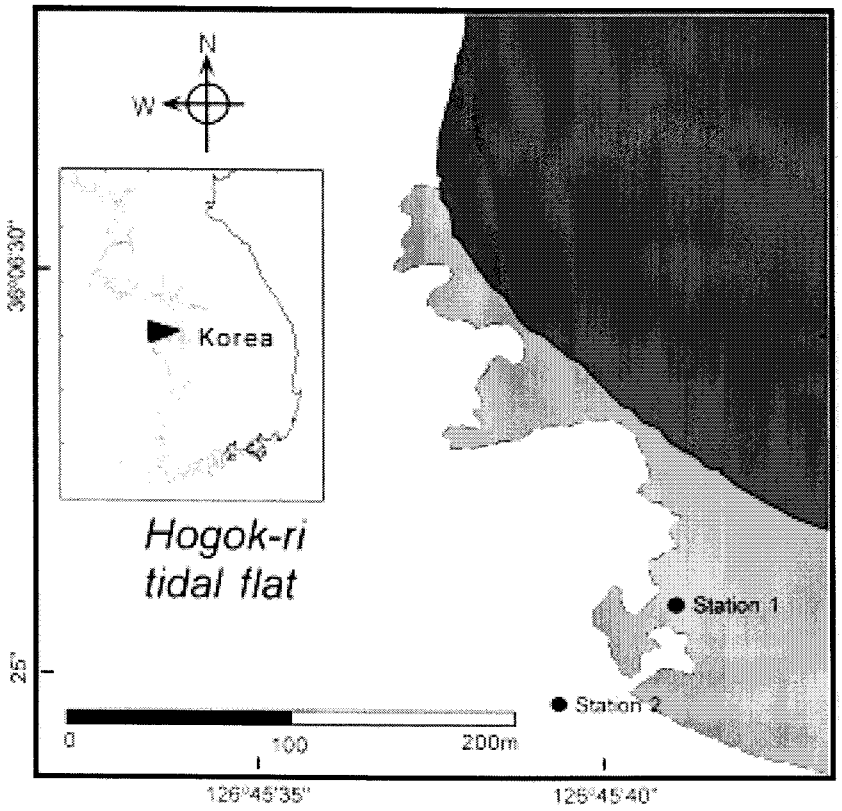

Fig. 1. Location of the salt marsh study sites in Hogok-ri tidal flat in Namyang Bay, west coast of Korea. Gray color represents the marsh plant inhabited area.

8.00 ϕ. Organic matter contents at station 1 ranged from 4.84 (9 to 12 cm) to 6.23 % (3 to 6 cm) and from 4.29 (9 to 15 cm) to 5.41 % (25 to 35 cm) at station 2. Bulk density ranged from 2.5 to 2.6 g · cm^{-3} and was relatively constant with depth (Data offered by Dr. Han Jun Woo, KORDI).

Sampling and pre-treatment

Samples collected with PVC Core (I.D. 10 cm × L. 60 cm) were transported to laboratory and sectioned by appropriate intervals. Then each 3 cm^3 of sectioned sample was slurried with 27 m*l* of sterilized seawater for further treatment.

Distribution of heterotrophic bacteria

One m*l* of serially diluted slurry was inoculated on the Petrifilm™ (3 M Co.) media. Colonies formed on Petrifilm were counted after 2 weeks of incubation at 25 °C.

Activities of extracellular aminopeptidase

Each one m*l* of subsample of slurry was inoculated into 8 microtubes. Leu-MCA was added to give a final concentration of 100 μmol for 3 tubes and the other 3 were added to be a final conc. of 2.5 μmol. Remained two tubes receive 50 *n*mol MCA for measuring recovery rate of MCA. Tubes were incubated for 40 minutes, centrifuged (10,000 rpm for 1 min) to remove solid matters, adjust pH to 10 with glycine-ammonia buffer and measure fluorescence with fluorescence microplate reader (Fluoroscan Ascent, Labsystems). The activity was calculated from the fluorescence difference before and after incubation (Hoppe 1993; Kwon *et al.* 1998).

Degradation rate of cellulose

Samples (diluted with two fold of pre-sterilized aged seawater) from the depth of 0-3, 12-15 and 24-29 cm at station 1 and 0-3, 9-5 and 25-30 cm at station 2 incubated with two small pieces of cellulose dialysis tube (Sigma) in 20 m*l* sample cup for 15 days. Weight loss of cellulose pieces calculated during incubation.

Metabolic diversity of microbial community

GN2 Microlog plate (Biolog) was used to measure metabolic diversity of microbial community (Verschuere *et al.* 1997). Experiments were performed at the same depth where cellulose degradation rate was tested. Slurries diluted to 10^{-4} were used for inoculant. Each well received 100 μl of diluted slurry and was incubated for one week at room temperature. Cluster analysis was done with MVSP-PC+ program.

3. Results

Distribution of heterotrophic bacteria

The number of heterotrophic bacteria ranged between 8.15×10^5 and 3.10×10^7 CFU · cm^{-3} at station 1 (reed area) and between 1.87×10^5 and 7.00×10^7 CFU · cm^{-3} at station 2 (exposed area). It decreased rapidly with depth (Fig. 2). The average number in sediments deeper than 15 cm was approximately one to two order lower than that of surface area. The bacterial numbers at station 1 were 2 to 6 times higher than station 2 except surface layer where the number was more than two times higher at station 2. The average number of heterotrophic bacteria within the depth of 3 cm was 2.21×10^7 CFU · cm^{-3} which included typical range of mud flat and higher than that of sand flat in Korea (Lee 1987; Choi and Lee 1996; Kwon *et al.* 1998; Baik *et al.* 2000).

Extracellular aminopeptidase activity (APA)

The depth distribution of APA ranged between 1.28 and 89.15 *n*mol · cm^{-3} · hr^{-1} at station 1 and between 1.29 and 121.01 *n*mol · cm^{-3} · hr^{-1} at station 2. About 68 % (st. 1) and 64 % (st. 2) of APA existed within the depth of 3 cm and 80 % and 72 % existed within 6 cm depth. The APA showed similar depth distribution pattern with that of heterotrophic bacterial number. Hydrolysis rate of aminopeptide ranged between 0.80 and 9.24 % · hr^{-1} at station 1 and between 0.41 and 3.09 % · hr^{-1} at station 2 (Fig. 3). The value in the surface layer of station 1 was exceptionally higher (9.24 % · hr^{-1}) than that in other samples (0.41 to 3.57 · hr^{-1}). But variation range of hydrolysis rates was not as much as variation of activity.

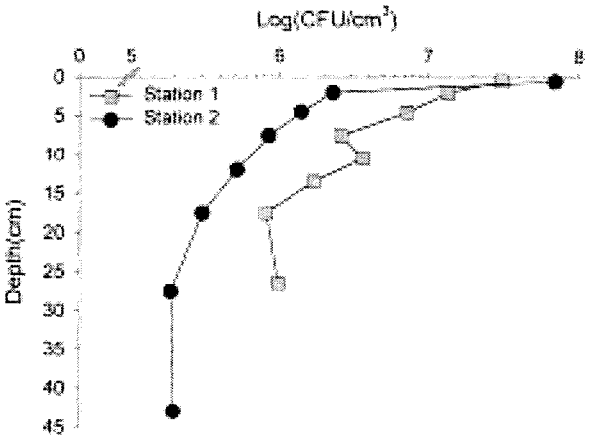

Fig. 2. Vertical distribution of heterotrophic bacteria at reed-inhabited (station 1) and exposed (station 2) area of tidal flat in Hogok-ri, Korea.

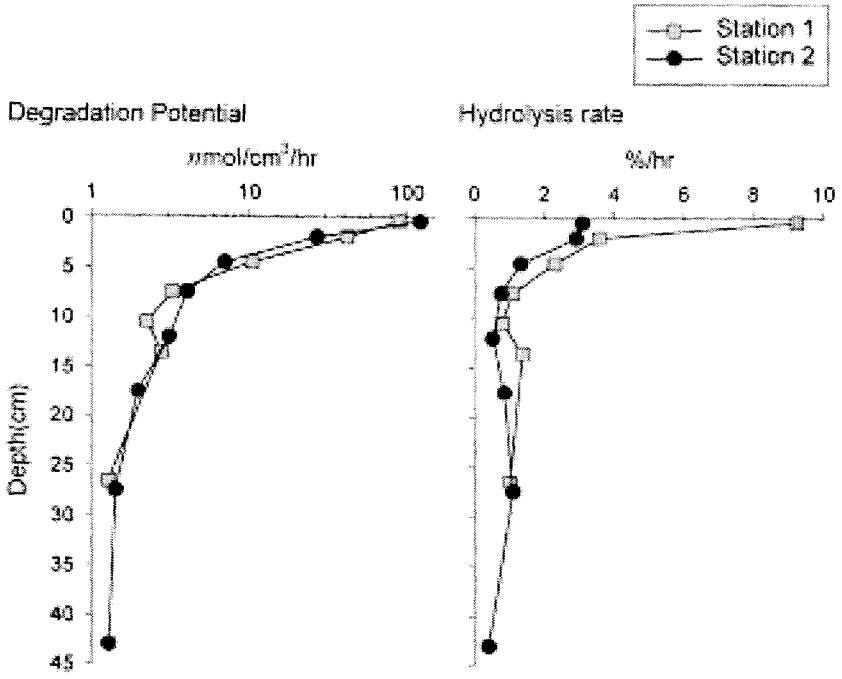

Fig. 3. Vertical distribution of extracellular aminopeptidase activities at reed-inhabited (station 1) and exposed (station 2) area of tidal flat in Hogok-ri, Korea.

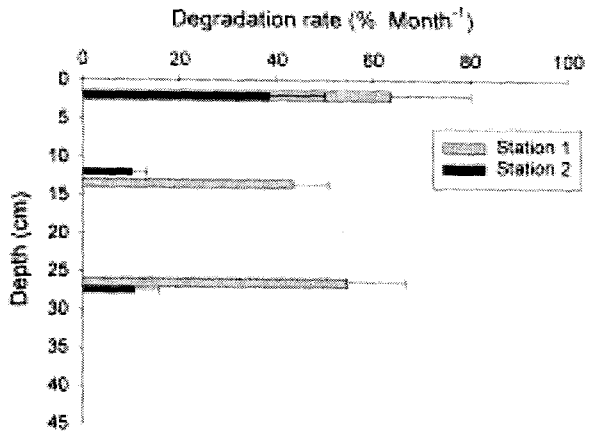

Fig. 4. Vertical distribution of cellulose degradation rates at reed-inhabited (station 1) and exposed (station 2) area of tidal flat in Hogok-ri, Korea.

Degradation rate of cellulose

The monthly degradation rate of cellulose measured at the depth of 0-3 cm, 12-15 cm and 24-29 cm at station 1 were 63.7±16.4 %, 43.3±7.3 % and 54.7±12.0 %, respectively and 38.4±11.5 %, 10.2±2.9 % and 10.6± 5.1 % at the depth of 0-3 cm, 9-15 cm and 25-30 cm at station 2, respectively (Fig. 4). Although the degradation rate at the surface layer was slightly higher, the degradation rate at each depth at station 1 showed similar range. To the contrary, the degradation rates at 9-15 cm and 25-30 cm at station 2 were approximately four times lower than that of 0-3 cm.

Metabolic diversity measured with GN2 Microlog plate

Number of compounds used as sole carbon source at each sample ranged from 2 to 46 (Table 1). Macro molecular compounds such as dextrin and tween 80 were widely used. Carbohydrate and aromatic compounds were also used well. L-alanine was the most widely used amino acid. At the surface layer at station 2, numbers of sugar monomers, amino acids and organic acids also used. There was higher metabolic diversity shown at the surface layer of station 2. Tested 6 samples were divided into 2 clusters, 0-3 cm at station 2 and others, based on the metabolic diversity.

4. Discussion

The hydrolysis rate of oligopeptides represents the pool size of monomeric substances (Chróst 1989). Relatively higher hydrolysis rate in the surface layer of station 1 represented the limitation of monomeric substances or

Table 1. Number of tested substrates in GN2 Microlog plate utilized as sole carbon source for the microbial communities.

Site (Depth, cm) / Substrates (Number)	Station 1			Station 2		
	0-3	12-15	24-29	0-3	9-15	25-30
Polymers (5)	2	1	3	4	1	2
Carbohydrates and derivatives (28)	4	0	3	16	2	2
Methylesters (2)	1	0	1	1	0	0
Carboxylic acids (24)	1	0	1	7	0	1
Brominated chemicals (1)	0	0	0	1	1	0
Amides (3)	0	0	0	1	0	0
Amino acids and derivatives (20)	1	1	1	9	0	0
Aromatic chemicals (4)	0	0	3	4	0	1
Amines (3)	0	0	0	1	0	0
Alcohols (2)	0	0	0	1	0	0
Phosphorylated chemicals (3)	0	0	0	1	0	0
Total (95)	9	2	12	46	4	6

limitation of nitrogen sources, because aminopeptidase participate in regeneration of inorganic nitrogen from organic matter. When compared with other results, the APA in investigation area was higher than that of Adriatic coastal sediment (Danovaro *et al.* 2001), North Sea tidal flat (Mayer 1989), deep sea sediment (Poremba 1995) and sand flat of Korea (Kwon *et al.* 1998).

Although distribution of heterotrophic bacteria was higher at station 1, there was not significant difference in extracellular aminopeptidase activity (Figs. 2, 3). Blaabjerg *et al.* (1998) reported that sulfate reducing rate near the rhizosphere of *Zostera marina* was regulated daily by the photosynthesis *via* supplementation of photosynthetic products and oxygen. Consequently the community inhabited in the *Zostera* bed showed higher activity than communities in no *Zostera* bed. Kroer *et al.* (1998) also reported that addition of root exudates stimulated the metabolic activity of microorganisms. Here, we need to consider the composition of organic components in salt marsh environments. Macrophyte litters are mainly made up of lignin, cellulose and hemicelluloses and are low in protein contents (Boschker and Cappenberg 1998). And the cellulose, the major components of plants breakdown to β-linked disaccharide (e.g. cellobiose) and further decomposed by the action of β-D-glucosidase, which specifically catalyze the hydrolysis of β-linked disaccharide of glucose (Chróst 1989). It means that aminopeptidase may not perform major role in marsh environments. Boschker and Cappenberg (1998) reported that the glucosidase activities inside of the reed bed were always higher than endo-peptidase activity, contrary to the outside of the reed bed in Lake Gooimeer. These results indicated that reed populations were closely associated with enzyme activity in degrading carbohydrate polymers.

Although the samples were divided into two major clusters according to the utilization of sole carbon source utilization pattern, clusters were divided based on not by difference of used substrates but by numbers of used substrate, because less than 10 substrates of tested 95 substrates were used in most tested samples. It might be the result of failure to regulate appropriate inoculum size. Since Biolog GN metabolic fingerprinting is a selective culture assay, some variability or noise is inevitable. Each well on a Biolog microplate may be considered as a sole substrate enrichment culture. The assay may be affected by inoculum homogeneity and density. Verschuere *et al.* (1997) mentioned that some strains did not give a Biolog pattern, especially at low cell densities (near 10^4 CFU $\cdot$ ml^{-1}), while at higher densities a clear Biolog pattern was observed. On the other hand, it has been reported that less than 10 CFU per well was enough to obtain positive reaction (Ellis *et al.* 1995). Sometimes slowly growing strains can be masked by rapid growing strains, so that proper choice of the dilution fold (i.e. cell density) and incubation time is important. On the basis of our experience, about 10^3 to 10^4 CFU $\cdot$ ml^{-1} (10^2 to 10^3 CFU $\cdot$ $well^{-1}$) of inoculum size and about one week of incubation time was reasonable for reliable data production (data not shown). Vershuere *et al.* (1997) also reported that in case of *Cytophaga marinoflava* on L-arabinose reached maximum OD after one week of incubation. If the inoculum size and incubation time can be carefully controlled, GN Microlog plate assay will be an attractive method for metabolic fingerprinting.

Because, most of the work on the cellulose degradation were done at water column, there was not enough data about the degradation rate of cellulose in the tidal flat of Korea (Baik *et al.* 2000). Baik *et al.* (2000) reported that

degradation rate of cellulose in Suncheon Bay ranged from 3.0 to 87.9 % · $month^{-1}$ and showed high rate (more than 80 % · $month^{-1}$ at the surface layer and 60 % · $month^{-1}$ at the 20-cm depth layer) during May-October period. The rates were highly correlated with temperature and extracellular enzyme activities (Baik *et al.* 2000). Comparing with this result, degradation rates in Hogok-ri at the same period were lower than that of Suncheon Bay (approximately 20 % lower at the surface area of station 1 than that of Suncheon Bay at the same period). The degradation rates were affected by many environmental factors like inorganic nutrients, water content, porosity, and existence of macrofauna, etc. As an example, Lillebø *et al.* (1999) reported that the presence of meiofauna and macrofauna significantly enhanced the bacterial activity for the *Spartina maritima* degradation. Our experiment in the laboratory could restrict the supplement of inorganic nutrients and oxygen or activities of meio- or macrobenthic organisms. Difference of conditions from natural environments can reduce the degradation rate.

Contrary to the result of Baik *et al.* (2000), there was significantly different degradation rate with depth at station 2. In Suncheon Bay, Baik *et al.* (2000) used composite sample and did not consider the existence of reed populations, and they couldn't differentiate the effect of reed populations on cellulose degradation activity.

In the study area, organic matter content and sediment characteristics were not responsible to the enzyme activity, rather the number of heterotrophic bacteria was responsible. In case of the surface area, organic content was higher at station 1 than station 2. But the APA and number of heterotrophic bacteria were higher at station 2. It seemed to be the reason of the difference of major component and source of the organic matter (Boschker and Cappenberg 1998). In marsh environments, not only organic matter from macrophyte but large amount of organic matter input from land, microalgae and benthic diatom, etc. (Boschker *et al.* 1999). These matters may accelerate the activity of microorganisms in the surface layer of exposed sediments. This may be the reason why in the surface layer of station 2 showed higher level of the bacterial activity and number. This area can receive organic matters from both macrophyte inhabited in the marsh and from shallow water. But in deeper layer, organic matter from other sources could not influence and the activity of microorganisms mainly depended on root exudates.

In conclusion, the number and activities especially concerned in plant debris degradation in salt marsh seemed to be stimulated by the reed communities.

Acknowledgements

This research was supported by G7 project (PN00423). Thanks to the Ms. SuJin Park for her assistance of lab experiment and Dr. Han Jun Woo for kindly offer the sediment character data. Special thanks to the two reviewers for their kind and constructive comments.

References

Baik, K.S., J.H. Choi, and C.N. Seong. 2000. Cellulose degradation and extracellular enzymatic activity of the mud flat in Sunchon Bay. *Kor. J. Microbiol.*, 36(2), 130-135 (In Korean).

Blaabjerg, V., K.N. Mouritsen, and K. Finster. 1998. Diel cycles of sulphate reduction rates in sediments of a *Zostera marina* bed (Denmark). *Aquat. Microb. Ecol.*, 15, 97-102.

Boschker, H.T.S., J.F.C. de Brouwer, and T.E. Cappenberg. 1999. The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: stable carbon isotope analysis of microbial biomarkers. *Limnol. Oceanogr.*, 44, 309-319.

Boschker, H.T.S. and T.E. Cappenberg. 1998. Patterns of extracellular enzyme activities in littoral sediments of Lake Gooimeer, The Netherlands. *FEMS Microbiol. Ecol.*, 25, 79-86.

Choi, G.G. and G.-H. Lee. 1996. Interaction between saprophytic bacterial distribution and their extracellular enzyme activities in the sediment of the Yellow Sea near Seochon. *The Microbiology and Industry*, 22, 119-126 (In Korean).

Chróst, R.J. 1989. Characterization and significance of glucosidase activity in lake water. *Limnol. Oceanogr.*, 34, 660-672.

Danovaro, R., M. Armeni, A. Dell'Anno, M, Fabiano, E. Manini, D. Marrale, A. Pusceddu, and S. Vanucci. 2001. Small-scale distribution of bacteria, enzymatic activities, and organic matter in coastal sediments. *Microb. Ecol.*, 42, 177-185.

Ellis, R.J., I.P. Thompson, and M.J. Bailey. 1995. Metabolic profiling as a means of characterizing plant-associated microbial communities. *FEMS Microbiol. Ecol.*, 16, 9-18.

Garland, J.L. and A.L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.*, 57, 2351-2359.

Giovanni, G.D., L.S. Watrud, R.J. Seidler, and F. Widmer. 1999. Comparison of parental and transgenic alfalfa rhizosphere bacterial communities using Biolog GN metabolic fingerprinting and enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR). *Microb.*

Ecol., 37, 129-139.

Hines, M.E. 1991. The role of certain infauna and vascular plants in the mediation of redox reactions in marine sediments. p. 275-286. In: *Diversity of Environmental Biogeochemistry Vol. 6.*, ed. by J. Berthelin. Elservier, Amsterdam, The Netherlands.

Hines, M.E., R.S. Evans, B.R. Sharak Genthner, S.G. Willis, S. Friedman, J.N. Rooney-Varga, and R. Devereux. 1999. Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of *Spartina alterniflora*. *App. Environ. Microbiol.*, 65, 2209-2216.

Hoppe, H.G. 1993. Use of fluorogenic model substrates for Extracellular Ezyme Ativity (EEA) measurement of bacteria. p. 423-431. In: *Handbook of Methods in Aquatic Microbial Ecology*, eds. by P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole. Lewis Pub. Boca Raton, Florida, USA.

Kroer, N., T. Barkey, S. Sørensen, and D. Weber. 1998. Effect of root exudates and bacterial metabolic activity on conjugal gene transfer in the rhizosphere of a marsh plant. *FEMS Microbiol. Ecol.*, 25, 375-384.

Kwon, K.K., H.Y. Cho, and H.K. Lee. 1998. Vertical distribution of heterotrophic bacteria, sulfate reducing bacteria and aminopeptidase activity in the tidal flat of Taebudo. *Ocean Res.*, 19(2S), 73-80 (In Korean).

Lee, G.-H. 1987. Environmental factors affecting seasonal distribution of heterotrophic bacteria in Kum River Estuarine sediments. *Kor. J. Microbiol.*, 25, 137-143 (In Korean).

Lee, M.-S., S.G. Hong, D.-H. Lee, C.-K. Kim, and K.S. Bae. 2001. Bacterial diversity in the mud flat of Suncheon Bay, Chunnam Province, by 16S rRNA gene analysis. *Kor. J. Microbiol.*, 37, 137-144 (In Korean).

Lillebø, A.I., M.R. Flindt, M.Â. Pardal, and J.C. Marques. 1999. The effect of macrofauna, meiofauna and microfauna on the degradation of *Spartina maritima* detritus from a salt marsh area. *Acta Oecologica*, 20, 249-258.

Mayer, M.M. 1989. Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. *Limnol. Oceanogr.*, 34, 973-981.

Poremba, K. 1995. Hydrolytic enzyme activity in deep-sea sediments. *FEMS Microbiol. Ecol.*, 16, 213-222.

Schubauer, J.P. and C.S. Hopkinson. 1984. Above- and belowground emergent macrophyte production and turnover in a coastal marsh ecosystem, Georgia. *Limnol. Oceanogr.*, 29, 1052-1065.

Schwaner, N.E. and N. Kroer. 2001. Effect of plant species on the kinetics of conjugal transfer in the rhizosphere and relation to bacterial metabolic activity. *Microb. Ecol.*, 42(3), 458-465.

Verschuere L., V. Fievez, I. Van Vooren, and W. Verstraete. 1997. The contribution of individual populations to the Biolog pattern of model microbial communities. *FEMS Microbiol. Ecol.*, 24, 353-362.

Whiting, G.J., E.L. Gandy, and D.C. Yoch. 1986. Tight coupling of root-associated nitrogen fixation and plant photosynthesis in the salt marsh grass *Spartina alterniflora* and carbon dioxide enhancement of nitrogenase activity. *Appl. Environ. Microbiol.*, 52, 108-113.

Received Sep. 25, 2001
Revised Nov. 1, 2001
Accepted Feb. 14, 2002