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<p>ABSTRACT: Two estuaries on the central California coast, Tomales Bay and Elkhorn Slough, which harbor well developed microbial mats in the intertidal region were chosen as experimental sites. At both sites, we investigated denitrification, benzoate degradation and bacterial production rate processes of the intact community whole cores. At Elkhorn Slough, we used a newly designed flow-through incubator to assess bacterial community acclimation to challenge from xenobiotics, using 2,4-D as a model. The simulated in situ incubation apparatus was used to 1) measure integrated total secondary production within the mats and 2) determine the ability of the consortium to transform xenobiotic compounds and assess the changes which occur during acclimation of the mat to xenobiotic exposure. An immunomagnetic bead separation method was optimized for measuring species specific bacterial production, using a marine denitrifying strain to demonstrate the approach. The diversity of denitrifying bacteria, including several isolated from Tomales Bay mats, was investigated using RFLP analysis and probes for nitrite reductase. Quantitative, non-radioactive, hybridization methods for measurement of gene abundance were developed and used to quantify nitrite reductase and xyle in mm scale depth profiles in the sediments at the study sites.</p>														
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~~FINAL REPORT~~

Grant #: N00014-92-J-1224 R & T Code: 4412115

PRINCIPAL INVESTIGATOR: Bess B. Ward

INSTITUTION: University of California, Santa Cruz

GRANT TITLE: Xenobiotic degradation by denitrifying bacteria in intertidal microbial mats

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OBJECTIVE: To investigate organic matter decomposition by layered bacterial consortia (microbial mats) which occur in intertidal environments; to investigate the role of facultatively anaerobic denitrifying bacteria in total mat metabolism and in decomposition of xenobiotics in particular.

ACCOMPLISHMENTS: In order to assess the in situ activity of the layered microbial community, we modified whole core incubation methods to accommodate measurements of potential denitrification rates using acetylene block and benzoate degradation using a ^{14}C -tracer. Both sets of rate measurements were made at both experimental sites. Some of the data were presented in a student poster at a regional meeting (Smalheer and Ward, abstract, 1994) and the remaining data are being analyzed for inclusion in the Masters thesis which will result from this portion of the research. Significant differences were observed between the two sites: at the time of sampling, denitrification rates and benzoate degradation rates were both higher in the Elkhorn Slough cores than in Tomales Bay cores. This is consistent with the more eutrophic, more anthropologically impacted state of Elkhorn Slough compared to Tomales Bay.

Development of the flow through incubation chamber constituted a second master's thesis arising from this project. The time and space scales of flow and sediment penetration by dissolved components in the overlying water of the flow-through incubation system were studied to determine an apparent diffusion coefficient, which was used to design the acclimation and exposure experiments. Methods for measurement of total bacterial productivity and 2,4-D degradation (using radiotracers) in mat sections were optimized. Mat sections were exposed to 2,4-D in the overlying water on a schedule that simulated the naturally occurring tide. Pre- and post-exposure measurements detected a slight, but significant, increase in the degradation capability of the mat exposed to 2,4-D vs. a control mat exposed only to seawater. The main accomplishments of this project were the perfection of the incubation chamber to allow long term simulated exposure experiments and the illustration of its usefulness in the 2,4-D experiment (Hogan and Ward, in preparation).

In both of these projects, radiotracer methods for evaluation of xenobiotic degradation were developed and applied to complex

samples successfully. The sediment/soil matrix is sometimes difficult to work with, and development of suitable controls for abiotic absorption required some effort.

In a third project, immunoglobulin coated magnetic beads were used to determine the proportion of the total substrate transformation (incorporation) due to a specific denitrifying strain. Initial work in sediments showed that the interference of sediment particles with the bead separation method and the inhomogeneity of the sediment matrix in regard to antiserum access and nonspecific interactions would be problematic. Therefore, we chose to optimize the method in a water column situation first. Laboratory experiments in which target strain was added to natural seawater demonstrated the capability to assay target cells specifically. While non specific interactions occur, they are reproducible and can be accommodated with suitable controls. Various aspects of the immunomagnetic separation method were optimized in laboratory and field experiments and a protocol devised for future application of the method. These results form the basis of a third Masters thesis on this project and a manuscript in preparation for submission in the next few weeks (Bard and Ward, in preparation).

Molecular and immunological probes for the denitrifying organisms of the mats were also investigated (Ward et al., 1993; Ward and Cockcroft 1993). Probes developed for nitrite reductase (NiR) from marine strains were tested against scores of denitrifying isolates obtained from the mats. A few of these isolates were also included in a larger study of diversity of denitrifying bacteria by RFLP analysis of rRNA genes (Ward, 1995). Many of the mat denitrifying isolates were shown to possess NiR genes and proteins with homology for the probes, suggesting that if these isolates are significant members of the natural community, then the natural communities may be usefully studied using those probes. The diversity study showed that the mat denitrifiers clustered together and were probably not closely related to *Pseudomonas stutzeri*, the organism from which the probes were developed. Quantitative hybridization methods were optimized for quantification of the NiR gene in sediments. These results correlated well with the measured distribution of potential denitrification (see above).

The quantitative hybridization method was also used to measure the vertical distribution of the xylE gene, the gene responsible for the ring breaking step in the TOL pathway, in the mat sediments. The results are consistent with the xylE gene being present on a plasmid that may be very common at certain depth horizons in the sediment. A component of this project was accomplished as part of senior thesis projects by two undergraduate students in my lab and is in preparation for submission (Francis et al., in preparation).

SIGNIFICANCE: Two model compounds were used in this study: benzoate represented an intermediate in the TOL pathway for breakdown of toluenes and xylenes and 2,4-D represented chlorinated pesticides, both of which are common contaminants in coastal environments of California. The capacity for degradation of both test compounds was found to exist in unperturbed samples from the intertidal environment. Optimization of these activities

in circulating flow reactors might form the basis of bioreactors which retain the diversity of natural systems. Quantitative hybridization, performed here for the *NiR* gene and the *xylE* gene, can be used in conjunction with in situ rate measurements to interpret regulation and environmental control of biogeochemical transformations. Optimization of the immunobead approach provides the basis from which to develop this approach for different strains and different substrates.

B. PUBLICATIONS, ABSTRACTS and PAPERS in PREPARATION:

Ward, B. B., A. R. Cockcroft and K. A. Kilpatrick. 1993. Antibody and DNA probes for detection of nitrite reductase in seawater. *Journal of General Microbiology*, 139: 2285-2293.

Ward, B. B. and A. R. Cockcroft. 1993. Immunofluorescence detection of the denitrifying bacterium, *Pseudomonas stutzeri* (ATCC 14405), in seawater and intertidal sediment environments. *Microbial Ecology*, 25: 233-246

Smalheer, D. L. and B. B. Ward. 1994. Fine scale distribution of denitrification and denitrifying bacteria in intertidal microbial mats. ABSTRACT. Monterey Bay Research Symposium, poster, February 1994.

Ward, B. B. 1994. Microbial ecology in estuarine systems. ABSTRACT. Monterey Bay Research Symposium, invited lecture, February 1994.

Ward B. B. 1995. Diversity in denitrifying bacteria: Limits of rDNA RFLP analysis and probes for the functional gene, nitrite reductase. *Archives of Microbiology*, 163: 167-175.

C. Francis, A. Kirk and B. B. Ward. Detection and quantification of the *XylE* gene in intertidal sediments. (in preparation)

Bard, D. G. and B. B. Ward. An immunomagnetic bead method to assess the in situ productivity of the marine bacterium, *Pseudomonas stutzeri*. (in preparation for *Microbiological Methods*)

Hogan, M. A. and B. B. Ward. Degradation of 2,4-D and acclimation by a microbial mat community to 2,4-D exposure. (in preparation for *Applied and Environmental Microbiology*)

C. Patents filed or pending: None