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SEATTLE, WASHINGTON 98195

School of Medicine
Department of Microbiology, SC-42

Defense Technical Information Center
Building 5, Cameron Station
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Dear Sir/Madam:

Enclosed is a copy of the Final Technical Report for my Office of Naval Research grant (Grant No. N00014-91-J-1792) that recently expired. Please contact me if you would like any additional information.

Sincerely,

Russell P. Herwig
Research Assistant Professor

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FINAL TECHNICAL REPORT

Grant#: N00014-91-J-1792 R&T Code: 3412-204

PRINCIPAL INVESTIGATOR: Russell P. Herwig

CO-PRINCIPAL INVESTIGATORS: John F. Ferguson and James T. Staley

INSTITUTION: University of Washington

GRANT TITLE: Biodegradation of Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons in Contaminated Marine Sediments

REPORTING PERIOD: 1 April 1991 - 30 September 1994 (42 months)

AWARD PERIOD: 1 April 1991 - 30 September 1994

OBJECTIVES: Determine the ability of naturally occurring microorganisms in marine sediments to metabolize polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Enrich, isolate, and characterize the microbial species in marine sediment consortia that metabolize these compounds. Determine the ability of anaerobic sediment enrichments to transform PCBs under methanogenic and sulfidogenic conditions.

APPROACH: Our research was a collaborative effort by investigators in the University of Washington's Departments of Microbiology and Civil Engineering. The investigation in Microbiology was lead by Drs. Russell P. Herwig and James T. Staley; in Civil Engineering by Drs. John F. Ferguson and Jaakko A. Puhakka. Dr. Puhakka was a visiting scientist from Finland. To initiate the investigations sediment samples were collected from various sites on Puget Sound. These samples served as the source material for the isolation of aerobic PCB- and PAH-degrading bacteria, and were used to establish anaerobic marine cultures that were exposed to either a defined mixture or a commercial mixture of PCBs. Marine PAH-degraders were also enriched in a continuous-flow fluidized-bed system. During Year 3, biological transformations of partially dechlorinated PCBs under aerobic conditions were examined.

ACCOMPLISHMENTS: Investigators in Microbiology began their investigation by collected marine sediments from three different locations in Puget Sound, Washington. Aerobic enrichments were begun using a mineral salts - synthetic seawater medium and biphenyl as the sole source of carbon. Their investigation continued by examining the taxonomy and phylogeny of isolates retrieved from Puget Sound sediments. Isolates PS-1, PS-2, and PS-3 were examined and were described as a new bacterial species. These isolates are specialists in the degradation of aromatic hydrocarbons, including PAHs, biphenyl, lightly chlorinated PCB congeners, and toluene. They do not utilize carbohydrates and many other organic compounds as sole sources of carbon. The 16S rDNA of these organisms was sequenced and a phylogenetic analysis was performed using a maximum likelihood method. These newly discovered aerobic, polar-flagellated, gram negative rods are members of the γ (gamma) subgroup of the *Proteobacteria* and were named *Cycloclasticus pugetii*.

For a 10 week period during summer 1994, Dr. Herwig worked in the Remediation Laboratory of Dr. Sabine Apitz (NRaD, San Diego,

California). Dr. Herwig collaborated with Dr. Apitz in examining the effect of mineralogy on the degradation of the aliphatic and aromatic component of diesel fuel marine by an enrichment culture of San Diego sediment bacteria, and used ^{14}C-radiolabeled PAHs to examine the mineralization in the presence of different minerals and humic acid.

Investigators in Civil Engineering examined the transformation of PCBs by sulfidogenic (sulfate-reducing) and methanogenic anaerobic cultures enriched from Puget Sound sediments. Initially they examined the ability of the anaerobic cultures to transform a defined mixture of four individual congeners and later examined the transformation of a complex commercial PCB mixture as contained in Aroclor 1254. PCBs were added to the enrichments by coating the PCBs onto chitin particles. No dehalogenation was observed in cultures exposed to the four congeners in concentrations from 6 to 9 mg/L over 17 months. The two other cultures fed Aroclor 1254 demonstrated a reductive dechlorination of Aroclor 1254 of 7-8% after one year under either sulfidogenic and methanogenic conditions. Under both conditions, 15% *meta* and 10% *para* chlorines were removed from the PCB molecules. Aeration of the anaerobic Aroclor 1254 cultures resulted in removal of tri-, tetra-, and some pentachlorinated PCB congeners formed during the reductive dechlorination process. Thus a sequential two-step procedure by the marine sediment enrichments, anaerobic followed by an aerobic process, had limited ability to eliminate the PCB congeners found within the Aroclor 1254 mixture.

An aerobic fluidized-bed system inoculated with supernatant from PAH contaminated marine sediments was continuously fed a mixture of naphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, biphenyl, acenaphthene, fluorene, and phenanthrene. After 11 days of operation, no PAHs were found in the effluent. Biodegradation of each PAH used in the feed was confirmed in batch assays. This culture also degraded 1-methylnaphthalene, 1,2-dimethylnaphthalene, 2-ethylnaphthalene, anthracene, pyrene, and fluoranthene. Isolates from this culture are being characterized.

SIGNIFICANCE: Aerobic isolates capable of growing on PAHs have been enriched and isolated from the marine sediments of Puget Sound, Washington. A group of these organisms are a bacterial species that has not been previously described, and they are not closely related to previously well characterized PAH-degrading strains of the genus *Pseudomonas*. These new organisms have been named *Cycloclasticus pugetii*. Interestingly, however, this newly described species is phylogenetically most closely related to a group of sulfur-oxidizing bacterial symbionts and species of methanotrophic (methane-oxidizing) bacteria. These newly discovered marine bacteria were able to degrade a variety of mono- and polycyclic aromatic hydrocarbons and grew on few other organic compounds.

Reductive dechlorination of Aroclor 1254 was partially complete in sulfidogenic and methanogenic marine cultures. We published the first report of PCB transformation under sulfidogenic conditions. Reductive dechlorination was only partial, and chlorines were removed from *meta* and *para* positions. Some resulting congeners from reductive dechlorination were amenable to aerobic degradation.

Aerobic marine cultures capable of degrading a wide variety of PAHs, including compounds generally considered to be recalcitrant, were easily enriched using a fluidized-bed system. The fluidized-bed system was

found to be a useful means for enriching bacteria capable of degrading multi-ringed PAHs.

FUTURE WORK PLANS: Part of the work initiated and funded by this grant will be continued as part of ONR's supported University of Washington research initiative in marine bioremediation (Dr. Jody Deming, Principal Investigator). A graduate student working with Dr. Staley will continue the taxonomic and phylogenetic evaluation of additional strains of marine PAH-degrading bacteria from Puget Sound and from the Gulf of Mexico. Dr. Herwig will examine Puget Sound isolates for hybridization with *nah* genetic probes. Future efforts as a result of the initial ONR support described in this report have been proposed and include: (1) Collaborative research investigations with Dr. Apitz (NRaD, San Diego) in which bench-scale and *ex situ* bioreactors will be constructed for the remediation of contaminated marine sediments. (2) Sequencing and characterization of the *nah*-like genes from *C. pugetii*. (3) Microbial community analysis of contaminated and "control" sediments using modern molecular and phylogenetic techniques.

THESIS AND DISSERTATIONS:

Dyksterhouse, S.E. 1992. Isolation of polychlorinated biphenyl degrading microorganisms from Puget Sound, Washington. M.S. thesis, University of Washington.

Øfjord, G.D. 1993. Reductive dehalogenation of polychlorinated biphenyls and chlorobenzenes by marine microorganisms. M.S. thesis, University of Washington.

PUBLICATIONS AND RESEARCH ABSTRACTS:

Apitz, S.E., K.J. Meyers-Schulte, and R.P. Herwig. 1995. The biodegradability of fuel components in marine sediments: PAH versus TPH. International Conference on Marine Pollution and Ecotoxicology in Hong Kong.

Clarke, H.R.G., and R.P. Herwig. 1994. Genes for PAH degradation in bacteria from marine sediments. American Society for Microbiology, Annual Meeting.

Dyksterhouse, S.E., R.P. Herwig, and J.T. Staley. 1992. Isolation of biphenyl degrading microorganisms from Puget Sound. American Society for Microbiology, Annual Meeting.

Dyksterhouse, S.E., J.P. Gray, R.P. Herwig, J.C. Lara, and J.T. Staley. 1995. *Cycloclasticus pugetii* gen. nov., sp. nov., an aromatic hydrocarbon degrading bacterium from marine sediments. Int. J. System. Bacteriol. 45: 116-123.

Geiselbrecht, A.D., J.T. Staley, and R.P. Herwig. 1994. Biodiversity of PAH-degrading bacteria in marine sediments. American Society for Microbiology, Annual Meeting.

Gray, J.P., S.E. Dyksterhouse, R.P. Herwig, and J.T. Staley. 1994. Phylogenetic and phenotypic characterization of novel PAH-degrading marine bacteria. American Society for Microbiology, Annual Meeting.

Lee, J.H., J.F. Ferguson, J.A. Puhakka, and R.P. Herwig. 1993. Reductive transformations of chlorophenols and chlorogaiacols by anaerobic marine consortia. American Society for Microbiology, Annual Meeting.

Ofjord, G.D., J. Lee, J.A. Puhakka, R.P. Herwig, J.F. Ferguson. 1993. Dechlorination of pentachlorophenol and polychlorinated biphenyls by marine sediment enrichments. Preprints of the First International Conference on Contaminated Aquatic Sediments, Milwaukee, WI.

Øfjord, G.D., J.A. Puhakka, J.A. Ferguson. 1994. Reductive dechlorination of Aroclor 1254 by anaerobic marine sediment enrichments. Environ. Sci. Technol. 28: 2286-2294.

INVITED TALKS:

Herwig, R.P. 1992. "Microbial oxygenases: catalysts for degradation of environmental pollutants" American Society for Microbiology, Northwest Branch, Annual Meeting.

Herwig, R.P. 1992. "Biodegradation of halogenated compounds by bacteria isolated from bioreactors" Pacific Northwest International Section/Air and Wastewater Management Association, Annual Convention. Bellevue, Washington.

Herwig, R.P. 1993. "Genetic probes for bioremediation and biodegradation Studies" Pacific Northwest International Section/Air and Wastewater Management Association, Annual Convention. Victoria, British Columbia.

MANUSCRIPTS IN PREPARATION:

Park, J.E., J.P. Gray, and R.P. Herwig. Development of a chromatographic procedure and whole-cell fatty-acid database for identifying aerobic marine bacteria.

ABSTRACTS IN FUTURE MEETINGS:

Herwig, R.P., K.J. Meyers-Schulte, and S.E. Apitz. 1995. Effect of mineralogy and humic acid on the biodegradation of diesel fuel marine by San Diego Bay sediment microorganisms. American Society for Microbiology, Annual Meeting (Washington, DC).

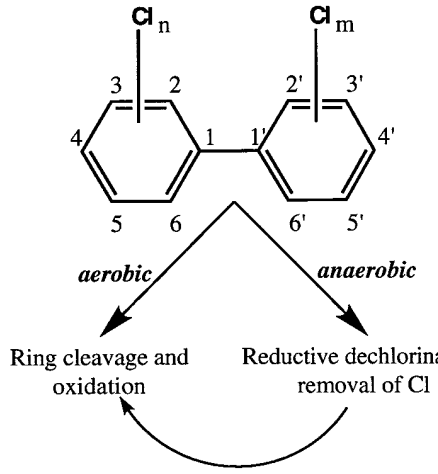
Gray, J.P., and R.P. Herwig. 1995. Extraction and amplification of DNA from creosote contaminated marine sediments. American Society for Microbiology, Annual Meeting (Washington, DC).

Gray, J.P., A. Withington, C. Furlong, and J.T. Staley. 1995. *Rhodococcus* spp., commonly found hydrocarbon degraders from widely distributed crude oil samples. American Society for Microbiology, Annual Meeting (Washington, DC).

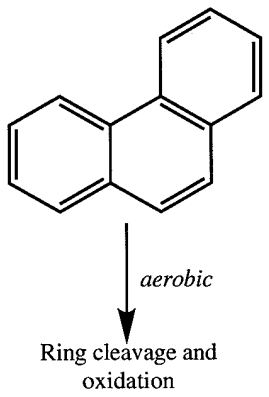
Melin, E.S., J.A. Puhakka, M. Männistö, and J.F. Ferguson. 1995. Degradation and remediation of polyaromatic hydrocarbons by a marine fluidized-bed enrichment. Third In-situ and On-site Bioremediation Symposium (San Diego, CA).

R.P. Herwig, University of Washington

PCB Biodegradation



PAH Biodegradation



Objectives

- Isolate and characterize aerobic PAH- and PCB-degrading microorganisms from marine sediments.
- Enrich and characterize anaerobic and aerobic marine consortia that are capable of degrading PCB and PAH mixtures.
- Develop information for the design of molecular probes for marine PAH-degrading microorganisms.

Accomplishments

- Characterized previously undescribed aerobic PAH and PCB-degrading bacterium from marine sediments, and named newly discovered organism *Cycloclasticus pugetii.*
- Reductive dechlorination of PCBs demonstrated under methanogenic and sulfidogenic conditions. Products of reductive dechlorination of PCBs found to be amenable to aerobic degradation.
- Aerobic fluidized-bed enrichment capable of degrading wide variety of PAHs, and is excellent method for microbial enrichment.

Significance

- New genus of PAH and PCB-degrading marine bacterium discovered, *Cycloclasticus pugetii.* Organism is specialist in degradation of aromatics and may be widely distributed in marine environment.
- Anaerobic consortia from marine sediments found to degrade PCBs. First observation of reductive dechlorination of PCBs in presence of sulfate.
- Anaerobic followed by aerobic degradation of PCBs by marine enrichments successfully removed a wide variety of PCB congeners.

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