REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT DATE	3. DATES COVERED *(From - To)*
30-11-2000	Final Technical Report	Feb 1996 - Sept 1999

4. TITLE AND SUBTITLE Degradation of aliphatic and aromatic hydrocarbons by the microbiota of animal burrow wall sediments

5a. CONTRACT NUMBER N00014-96-1-592

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Gary M. King

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Darling Marine Center
University of Maine
Walpole, ME 04547

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
Ballston Centre Tower One
800 North Quincy Street
Arlington, VA 22217-5660

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT

Available for public distribution

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of our research was to document the extent to which burrowing macrofauna can be used to enhance biodegradation of aromatic hydrocarbon pollutants in marine sediments by stimulating aerobic microbial metabolism in burrow walls. Our specific objectives included characterizing degradation potentials for a variety of macrofauna. We also enriched and isolated relevant novel PAH-degrading bacteria and conducted manipulative experiments with them to enhance degradation in sediments. In a parallel effort, we have explored the anaerobic degradation of polar PAH metabolites that often persist in environments with significant PAH loading.

15. SUBJECT TERMS

polyaromatic hydrocarbons marine macrofauna PAH-degrading bacteria

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Gary M. King
U	U	U	UU	3	19b. TELEPONE NUMBER *(Include area code)* (207) 563-3146

Final Report

Grant Number: N00014-96-1-0592

Principle Investigator: Gary M. King

Institution: University of Maine

Grant Title: "Degradation of Aliphatic and Aromatic Hydrocarbons by the Microbiota of Animal Burrow Wall Sediments"

Award Period: 1 March 1996 - 30 September 1999

Objective: The overall objective of our research were to document the extent to which burrowing macrofauna can be used to enhance biodegradation of aliphatic and aromatic hydrocarbon pollutants in marine sediments by stimulating aerobic microbial hydrocarbon metabolism in burrow walls.

Research Accomplishments: Our specific objectives included characterizing degradation potentials for burrow wall sediments from a variety of common macrofauna. In addition, we enriched and isolated relevant PAH-degrading bacteria and conducted manipulative experiments with them to enhance degradation in sediments. In a parallel effort, we have explored the anaerobic degradation of polar PAH metabolites that often persist in environments with significant PAH loading. We used burrow wall sediments from polychaetes, molluscs and hemichordates in a variety of laboratory assays to determine patterns and potentials of PAH degradation. These assays identified significant degradation potentials for a range of aliphatic and aromatic hydrocarbons relative to non-burrow sediments. PAH degradation potentials of burrow sediments from two polychaetes (*Nereis virens* and *Clymenella torquata*), a mollusc (*Mya arenaria*) and an enteropneust (*Saccoglossus bromophenolosus*) were generally greater than potentials for non-burrow sediments; relative rates of degradation varied among the burrow wall sediments depending on the PAH assayed. Comparisons of the effects of available electron acceptors (oxygen, nitrate, ferric iron, sulfate) indicated that significant degradation of benzene, hexadecane and PAH occurred only in the presence of molecular oxygen. However, the capacity for oxic phenanthrene degradation was stable during incubations with alternating oxic and anoxic

conditions, suggesting significant anoxia tolerance for the PAH degraders. Although burrow wall sediments were biogeochemically distinct with respect to rates of sulfate reduction, potential denitrification and potential ammonia oxidation, these patterns were not related to those of PAH degradation.

We have isolated two pure cultures and currently have several mixed cultures of PAH degraders from various burrow wall sediment enrichments. The pure cultures, LC8 and M4-6 originated from different burrow types. Both readily degrade naphthalene, phenanthrene and anthracene. In addition, LC-8 cometabolized a variety of larger or more complex PAH including dibenzothio-phene, pyrene and benz(a)anthracene. A phylogenetic analysis of the two isolates (based on full length 16S rRNA gene sequences) indicates that LC-8 is a novel organism related to *Sphingomonas*, but mostly likely in a new genus. The second isolate, M4-6 has a similar degradation spectrum as LC-8, but is a novel spirillum within the genus *Cycloclasticus*. Based on its distinct phylogeny, morphology and biochemical characteristics, we have proposed a new species name for it, *spirillensus*. We have also isolated a third novel PAH degrader from a naturally hydrocarbon-enriched freshwater system; phylogenetic and microbiological analyses indicate that it is closely related to *Sphingomonas aromaticovorans*, a known PAH degrader Our isolate grows rapidly on a range of PAH and appears more robust in this capacity than many previously reported PAH degraders. Finally, a long-term assay for anaerobic 4-HNA degradation has revealed a slow but significant degradation under sulfate reducing conditions from some but not all sediments examined.

Conclusions: We have isolated novel marine PAH-degrading bacteria from animal burrow walls. These bacteria can be used in controlled additions to contaminated sediments or as sources for genetic engineering of improved PAH degradation. We have shown that when added to sediments, these isolates enhance PAH degradation.

Significance: Collectively, the results of this study provide a basis for designing systems for bioremediation of coastal sediments contaminated by Navy fuels.

Publications and Abstracts:

Chung, W.K. and G.M. King. 1999. Potential polyaromatic hydrocarbon degradation and biogeochemical transformations

in macrofaunal burrow sediments. Aquat. Microb. Ecol. 19:285-295.

King, G.M. and M.A. Garey. 1999. Ferric iron reduction by bacteria associated with the roots of freshwater and marine macrophytes. Appl. Environ. Microbiol. 65:4393-4398.

Chung, W.K. and G.M. King. Isolation and characterization of novel marine PAH-degrading bacteria from burrow wall sediments. In prep. for Appl. Environ. Microbiol.

Chung, W.K. and G.M. King. Enhanced degradation of PAH in marine sediments by two novel PAH-degrading bacteria. In prep. for Mar. Ecol. Prog. Ser.

Chung, W.K. and G.M. King. Isolation of a novel PAH-degrading Sphingomonas from a Pitch Lake, Trinidad, a naturally hydrocarbon-rich ecosystem. In prep. for Appl. Environ. Microbiol.

Chung, W.K. and G.M. King. 1997. Polyaromatic hydrocarbon degradation in the burrow wall sediment of marine macrofauna. Annual Meetings of the American Society for Microbiology, Miami, FL.

Chung, W.-K. and G.M. King. 1998. Controls of PAH degradation in the burrow wall sediments of marine macrofauna. Annual Meetings of the American Society for Microbiology, Atlanta, GA.

Chung, W.-K. and G.M. King. 1999. Characterization of novel marine PAH-degrading isolates from animal burrows. NEMPET, Blue Mountain Lake, NY.

Chung, W.-K. and G.M. King. 2000. Isolation and characterization of novel PAH degraders from macrofaunal burrow sediments. Annual Meetings of the American Society for Microbiology, Los Angeles, CA.