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13. ABSTRACT (Maximum 200 words) For many years researchers have tried to identify and monitor PCB dechlorinating bacteria. Strains o-17 and DF-1 are the first PCB dechlorinating bacteria to be identified. These organisms have proven to be very difficult to grow in pure culture thus far. However, for the first time molecular approaches (16S rDNA analysis) has been combined with enrichment culture technique to identify these organisms. This is a significant breakthrough that will advance the application of bioremediation and it proves that such an approach can be used to identify difficult to culture environmental microorganisms, which is to say most microorganisms. Now for the first time these organisms can be monitored in order to better understand their physiology and to track the organisms in situ, before, during and after active and passive remediation attempts.				
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FINAL PROGRESS REPORT

GRANT NUMBERS: N000014-99-1-0101 (KS)/ N00014-99-1-0078 (HM)

PRINCIPAL INVESTIGATORS: Kevin R. Sowers, Ph.D. (e-mail: Sowers@umbi.umd.edu), Harold D. May, Ph.D. (e-mail: mayh@musc.edu)

PI INSTITUTIONS: (KS) University of Maryland Biotechnology Institute, Center of Marine Biotechnology, 701 E. Pratt St., Baltimore, MD 21202.
(HM) Department of Microbiology & Immunology, Medical University of South Carolina, 173 Ashley Avenue, POB #250504, Charleston, SC 29425

GRANT TITLE: Collaborative Research: Microbial Reductive Dechlorination of Polychlorinated Biphenyls (PCBs) in Estuarine and Marine Coastal Sediments

AWARD PERIOD: 1 October 1998 - 30 September 2001 (36 months)

OBJECTIVE: The overall objective of this research is to identify and characterize microbes that catalyze polychlorinated biphenyl (PCB)-dechlorinating processes in the environment. The specific objectives include characterizing the biocatalytic processes that transform PCBs and to developing molecular (DNA) probes that can be used to monitor and assess the effectiveness of natural or bioaugmented PCB-dechlorination *in situ*.

APPROACH: The approach is as follows: (i) use selective enrichment and molecular probes to further define microbial populations associated with specific PCB dechlorination pathways; (ii) isolate and characterize PCB-dechlorinating microorganisms which can be used for mechanistic studies and bioaugmentation of contaminated sites; (iii) develop screening methods for rapid molecular monitoring of PCB dechlorinating potential and activity in the laboratory and *in situ*. Dr. Kevin Sowers (PI) and Dr. Joy Watts (postdoc), both of UMBI, developed much of the molecular screening tools used in this study. Dr. Harold May (PI), Dr. Leah Cutter (Ph.D. student - graduated in 2001) and Dr. Qingzhong Wu, all of MUSC, have carried out much of the enrichment and isolation studies and have contributed to the identification of PCB dechlorinating bacteria.

ACCOMPLISHMENTS: Research accomplishments for the duration of the grant include: i) characterization of microbial community that specifically removes chlorines that are doubly flanked by other chlorine on a PCB molecule, ii) a determination of the carbon isotope selection by PCB dechlorinating bacteria, iii) development and comparison of PCR-ARDRA, PCR-tRFLP and PCR-DGGE for the comparative analysis of PCB dechlorinating microbial communities, iv) determination of the roles of acetate and hydrogen in *ortho* PCB dechlorination, v) demonstration for the first time that the growth of a single microorganism is linked to PCB dechlorination, and vi) the first identification of two distinct PCB dechlorinating bacteria (one *ortho* dechlorinating, bacterium o-17, and the other restricted to double flanked chlorines of PCBs, bacterium DF-1).

CONCLUSIONS: For many years researchers have tried to identify and monitor PCB dechlorinating bacteria. Strains o-17 and DF-1 are the first PCB dechlorinating bacteria to be identified. These organisms have proven to be very difficult to grow in pure culture thus far. However, for the first time molecular approaches (16S rDNA analysis) has been combined with enrichment culture technique to identify these organisms. This is a significant breakthrough that will advance the application of bioremediation and it

proves that such an approach can be used to identify difficult to culture environmental microorganisms, which is to say most microorganisms. Now for the first time these organisms can be monitored in order to better understand their physiology and to track the organisms in situ, before, during and after active and passive remediation attempts.

SIGNIFICANCE: The results from this proposal, the first comprehensive study of microbial dechlorination processes in the marine environment, have provided a basic understanding of the dehalogenating processes extant in coastal sediments using PCBs as a model system. The work has: 1) provided information on the identity of microbes that catalyze the process in marine sediments, 2) provided preliminary information on factors that enhance and limit the process, 3) demonstrated the feasibility of screening for the PCB-dechlorinating potential in sediments using species-specific probes. The combined results provide tools to begin to assess which portion(s) of the degradative process are potentially amenable to biotechnological enhancement. Ultimately, the information and research tools developed in this research with further refinement will facilitate Navy management decisions concerning both remedial site prioritization and appropriate remedial strategies, e.g. assessment of natural attenuation.

PATENT INFORMATION: {Disclosures filed}

1. Stimulation of Microbial Dechlorination of Polychlorinated Biphenyls with Halogenated Ethenes. Harold D. May and Kevin R. Sowers. Filed December 14, 2001.
2. Microbial Catalysts that Reductively Dechlorinate Polychlorinated Biphenyls (PCBs). Harold D. May and Kevin R. Sowers. Provisional disclosure filed May 19, 2000; filed May 19, 2001.

AWARD INFORMATION: HM was promoted to Assoc. Professor in 2000.

PUBLICATIONS AND ABSTRACTS (for total period of grant):

JOURNAL ARTICLES

1. **Wu Q, Watts JEM, Sowers KR, and May HD.** Identification of a bacterium that specifically catalyzes the reductive dechlorination of PCBs with doubly flanked chlorines. *Appl. Environ. Microbiol.* **2002**, 68:807-812.
2. **Cutter LA, Watts JEM, Sowers KR, and May HD.** Identification of a microorganism that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl. *Environ. Microbiol.* **2001**, 3:699-709.
3. **Watts JEM, Wu Q, Schreier SB, May HD and Sowers KR.** Comparative analysis of PCB-dechlorinating communities in enrichment cultures using three different molecular screening techniques. *Environ. Microbiol.* **2001**, 3:710-719.
4. **Drenzek NJ, Eglinton TI, Wirsen CO, May HD, Wu Q, Sowers KR, and Reddy CM.** Stable carbon isotopic fractionation during reductive dechlorination of polychlorinated biphenyls. *Environ. Sci. Technol.* **2001**, 35:3310-3313.
5. **Wu Q, Sowers KR, and May HD.** Establishment of a PCB-dechlorinating consortium, specific for double-flanked chlorines, in a sediment-free medium. *Appl. Environ. Microbiol.* **2000**, 66:49-53.

ABSTRACTS

1. **May HD, Wu Q, Cutter LA, Watts JEM, Sowers KR and Meier GP.** Polychlorinated biphenyls (PCBs) and fungal organochlorides as electron acceptors for the growth of PCB-dechlorinating bacteria. Abstracts of the 9th International Symposium on Microbial Ecology, **2001**.

2. **Watts JEM, Scheier SB, Cutter LA, Wu Q, May HD and Sowers KR.** Molecular analysis and identification of a phylogenetically distinct PCB dechlorinating group from three anaerobic PCB degrading communities. Abstracts of the 9th International Symposium on Microbial Ecology, 2001.
3. **Wu Q, Watts JEM, Sowers KR, and May HD.** Identification of a bacterium responsible for doubly flanked PCB dechlorination. Abstracts of the 101st Annual Meeting of the American Society for Microbiology, 2001.
4. **Reddy CM, Drenzek NJ, Eglinton TI, Wirsen CO, Sowers KR, Wu Q, and May HD.** The absence of carbon isotopic fractionation from microbial dechlorination of polychlorinated biphenyls: potential application. 20th International Meeting on Organic Geochemistry. 2001.
5. **May HD, Cutter LA, Wu Q, Watts JEM, and Sowers KR.** Molecular Identification of Anaerobic Microorganisms Associated with PCB Dechlorination. Proceedings of the 16th Annual International Conference on Contaminated Soils, Sediments and Water. 2000.
6. **May HD, Cutter LA, Watts JEM, and Sowers KR.** Molecular Identification of an Anaerobic Microorganism Whose Growth is Linked to PCB Dechlorination. Proceedings of the 16th Annual International Conference on Contaminated Soils, Sediments and Water. 2000.
7. **Cutter LA, Sowers KR, and May HD.** Acetate vs. hydrogen supported anaerobic *ortho* PCB dechlorination. Abstracts of the 100th Annual Meeting of the American Society for Microbiology, 2000.
8. **Watts JEM, Schreier SB, Wu Q, May HD, and Sowers KR.** A comparison of different molecular techniques to examine microbial diversity in anaerobic polychlorinated biphenyl (PCB) dechlorinating enrichment cultures. Abstracts of the 100th Annual Meeting of the American Society for Microbiology, 2000.
9. **Watts JEM, Schreier SB, Wu Q, May HD, and Sowers KR.** An examination of microbial diversity in polychlorinated biphenyl (PCB) polluted Baltimore Harbour sediment. Int. Mar. Biotechnol. Conf., Townsville, Australia, 2000.
10. **Norman RS, Schreier S, Watts J, Sowers KR, and May HD.** Molecular assessment of the effect of PCBs on the microbial community structure within an enrichment culture. American Society for Microbiology Conference on Microbial Diversity, 1999.
11. **Wu Q, Pulliam Holoman TR, Schreier S, Sowers KR, and May HD.** A 2,3,4,5-tetrachlorobiphenyl-dechlorinating enrichment culture sustained in a defined, sediment-free medium. Abstracts of the 99th Annual Meeting of the American Society for Microbiology, 1999.
12. **Cutter LA, Sowers KR, May HD.** Acetate-dependent *ortho* PCB dechlorination. Joint Mtg. Southeastern Branches of the ASM. October 28-30, Jekyll Island, GA, 1999.
13. **Cutter L, Pulliam Holoman TR, Elberson MA, Sowers KR, and May HD.** Application of selective enrichment and molecular monitoring (SEMM) to characterize 2,3,5,6-tetrachlorobiphenyl dechlorinating microbial enrichments. Student Research Day, Medical University of South Carolina, November 6, 1998.