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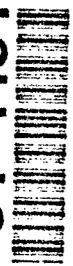
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FIELD	GROUP	Archaeobacteria - Initial Microfouling Community, Anti-fouling Test	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The research program represented a consolidation of three research programs 1) the examination of the lipid metabolism of the Archaeobacteria from the hydrothermal vents, 2) the analysis of the initial microfouling community using ultrasensitive signature lipid biomarker techniques, and 3) the development of an assessment program for the detection of microbial biofouling on painted surfaces for the Antifouling Coatings Development Program. Significant progress was made in detecting and monitoring the hydrothermal vent microbiota from in situ samples and in bioreactors. New methodologies for significantly reducing chemical noise were developed for assessing the initial microfouling film and a test system for detecting biofilm formation and sublethal toxicity on the test surfaces with the initial microfouling organism <u>Pseudomonas atlantica</u> .			
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Contract #: N0014-89-J-3095

Principal Investigators: David C. White, James B. Guckert

Institute: Institute for Applied Microbiology, University of Tennessee

Contract Title: Ecology of Archaeobacteria, initial microfouling community, and antifouling coatings development program.

Period of Performance: 05-01-89 through 06-01-91

Objectives:

1. To develop and analyze the ether lipid components of the Archaeobacteria from the hydrothermal vents.
2. To develop sensitive methods for analysis of signature biomarkers for the initial microfouling community.
3. To develop an assay system for the formation and toxicology of the microbial fouling community on the surfaces of developed in the antifouling coatings program.

Accomplishments:

1. We were able to develop a chromatographic method for the separation and quantitative analysis of the ether lipids from Archaeobacteria. These lipids which consist of diphytanyl- and bidiphytanyl-glycerol ether derivatives are too high molecular weight to be made volatile at the temperatures that are possible for gas chromatography. D. B. Hedrick developed a method based on supercritical fluid chromatography (SFC) with supercritical carbon dioxide with a pressure gradient where he was able to separate the large molecular weight alcohols formed after the acid methanolysis of the lipids. These were then separated by capillary chromatography and detected by flame ionization detection. The analysis allowed us to show that there are diphytanyl glycerol ethers (DPE) and bi-diphytanyl glycerol ethers (BGE) in the neutral lipid, glycolipid, and polar lipid fraction. We were able to account for low levels in some Archaeobacteria by showing that in these organisms the lipids are not extractible until **after** acid methanolysis (1). Using this technique it was possible to develop a rapid method for distinguishing the ether lipids utilizing FT/IR (2).

2. The principal problem with the ultrasensitive analysis of polar lipid biomarkers for very sparse microbial biofilms such as the initial microfouling community is the contamination of the solvent used for extraction with fatty acids. This leads to chemical noise. To decrease this problem we developed a microtechnique where we utilize a very small volume 5 ml total of extract and microcolumns for extraction. This was then further

Research based on the data and techniques developed in this test system have led to the development of an on-line test system utilizing the bioluminescent bacteria.

Publications:

1. Hedrick, D. B., J. B. Guckert, and D. C. White. 1991a. Archaeobacterial ether lipid diversity analyzed by supercritical fluid chromatography: Integration with bacterial lipid protocol. *J. Lipid Research* 32: 659-666.
2. Hedrick, D. B., D. E. Nivens, C. Stafford, and D. C. White. 1991b. Rapid differentiation of Archaeobacteria from eubacteria by diffuse reflectance Fourier-transform infrared spectroscopic analysis of lipid preparations. *J. Microbiol. Methods* 13: 67-73.
3. White, D. C., D. B. Ringelberg, J. B. Guckert, and T. J. Phelps. 1991. Biochemical markers for *in situ* microbial community structure. *In* Proceedings of the First International Symposium on Microbiology of the Deep Subsurface, (C. B. Fliermans and T. C. Hazen, eds.). January 15-19, 1990, Orlando, FL. WSRC Information Services, Aiken, SC. pp. 4-45 to 4-56.
4. Guckert, J. B., D. B. Ringelberg, D. C. White, R. S. Henson, B. J. Bratina. 1991. Membrane fatty acids as phenotypic approach to taxonomy of methylotrophs within the proteobacteria. *J. Gen. Microbiol.* 137: in press.