AD-A250 167

ON PAGE

Form Approved
OMB No. 0704-0188

Public gather collecti Davis H

hour per response, including the time for reviewing instructions, searching existing data sources, ollection of information. Send comments regarding this burden estimate or any other aspect of this ington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson gement and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AI (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	5/31/92	Final (1/1/88 -- 6/30/91)

4. TITLE AND SUBTITLE
Inter-species Inhibition of Adhesion Between Gliding Bacteria from Marine

5. FUNDING NUMBERS
N00014-88-K-0158

6. AUTHOR(S)
Dr. Robert P. Burchard

DTIC ELECTE APR 1 4 1992 S D C

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Maryland Baltimore County
Baltimore, MD 21228

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
ONR, U.S. Navy, Arlington, VA

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Several cell surface properties of gliding bacteria isolated from marine and fresh water biofilms have been characterized. No generalization about the relationship of surface hydrophobicity and adhesion can be made. An immobilized iodination protocol allowed the identification of cell surface-exposed proteins of some of these gliding bacteria. Adhesion-defective mutants demonstrated differences in their iodination patterns. Extracellular polymers, thought to mediate adhesion in these bacteria, have also been partially characterized. One species produces two polysaccharides, one of which is a glucose homopolymer; the second is a heteropolysaccharide. A second species produces a viscous slime, the rheological properties of which are due to the presence of polypeptide(s). A high molecular weight inhibitor of adhesion of a number of aquatic bacteria is produced by another marine biofilm glider. This has been partially characterized and has been tentatively identified as a protein.

92 4 09 053

14. SUBJECT TERMS	15. NUMBER OF PAGES
Marine gliding bacteria, biofilm, adhesion, slime	one
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	

ANNUAL PROGRESS REPORT

GRANT #: N00014-88-K-0158 **R&T CODE:** 4412035

PRINCIPAL INVESTIGATOR: Robert P. Burchard

INSTITUTE: University of Maryland Baltimore County

GRANT TITLE: Inter-species Inhibition of Adhesion Between Gliding Bacteria from Marine

PERIOD OF PERFORMANCE: 7/1/90 - 6/30/91

AWARD PERIOD: 1/1/88 - 6/30/91

OBJECTIVES:

1. To characterize the extracellular slime of selected marine gliding bacteria;
2. To determine the function(s) of extracellular slime in adhesion and motility of these gliding bacteria; and
3. To characterize the cell surface adhesins of selected marine gliding bacteria and how they interact with inhibitors of adhesion.

ACCOMPLISHMENTS of the past 12 months:

1. The polysaccharidic component of the extracellular slime of *Flexibacter maritimus* is predominantly a glucose polymer. In collaboration with Ian Sutherland (Edinburgh), linkage and branching is being determined. A second heteropolysaccharide (D-glucose, D-galactose, D-mannose and L-rhamnose) has also been identified.

2. The slime of *Cytophaga* sp. Strain 1327B has proven more amenable to analysis than that of *F. maritimus* since it is released into the extracellular medium. It has been partially purified by gel filtration. Preliminary evidence suggests that the viscous properties of the slime are due to the presence of polypeptide(s), not polysaccharide as predicted. W.H. Schwarz (Johns Hopkins) has performed rheological analysis of this slime using a Weissenberg rheogoniometer. Specifically, values of the apparent shear viscosity versus the rate-of-strain, and the complex modulus versus the frequency of oscillation were obtained. Preliminary data indicate that the slime is non-Newtonian and viscoelastic. These results will be applied to a mathematical model to describe motility and adhesion of some gliding bacteria.

3. We have extended our studies of the surface properties of a variety of gliding bacteria, some grown under different conditions. The intent has been to relate cell surface hydrophobicity to adhesion. Hydrophobicity has been assayed by bacterial adherence to hydrocarbons (BATH), hydrophobic interaction chromatography (HIC)

and salt aggregation (SAT). No generalization about the surfaces of these bacteria can be reached nor is there any one measure of hydrophobicity that is predictive of adhesion capability.

4. The bacterial adhesion inhibitory factor (AIF) produced by a marine biofilm gliding bacterium has been at least partially purified by gel exclusion and anion exchange chromatography. It appears to be a high molecular polysaccharide which is sensitive to glucuronidase from *E. coli*. The material inhibits adhesion of a variety of aquatic of bacteria, both gliders and non-gliders from natural and industrial biofilms, to a variety of substrata differing widely in critical surface energy (CSE). It is most effective on high CSE substrata. We are currently working to improve the efficiency of recovery of AIF in order to generate enough material to permit its identification.

5. Identification of cell surface-exposed polypeptides of several gliding bacteria in collaboration with R.A. Bloodgood (U.Va.) are ongoing. This research utilizes a surface-immobilized iodination catalyst (Iodo-Gen).

   (A) We are determining whether extracellular polymers mask the bacterial surface, preventing cell envelope proteins from contacting substrata.
   (B) We are determining whether AIF, the adhesion inhibitor described above, interferes with labelling of cell surface proteins.
   (C) We previously identified one predominant ~42 kDa polypeptide exposed on the surface of *Cytophaga* RB1058, the marine biofilm isolate that led to the AIF research. We hypothesize that this polypeptide is the adhesin of this bacterium. Several adhesion-defective mutants and revertants have been isolated. We are currently comparing the surface-exposed polypeptide(s) of these strains with those of the wild-type.
   (D) We are completing studies that demonstrate that arrays of surface-exposed proteins can be used for bacterial taxonomy (ie. closely related bacteria have similar arrays of surface polypeptides).

SIGNIFICANCE:

* Characterization of the production, structure and rheology of the extracellular slime of gliding bacteria should lead to an understanding of its role in their adhesion and motility.

* Studies of the hydrophobicity of various gliding bacteria and of their adhesion to and motility on substrata differing in critical surface energy should lead to an understanding of the mechanism(s) of adhesion of these bacteria.

* An immobilized radiolabelling technique has provided a tool for the identification of surface proteins, including putative adhesins, that make contact with substrata. Application of this technique permits molecular characterization of the cell surfaces of adhesion-defective mutants and of bacteria grown under conditions resulting in differences in cell surface polymer production. It also provides a powerful taxonomic tool for the gliding bacteria.

* A unique type of inter-specific inhibition of bacterial adhesion within biofilms may have been discovered. The inhibitor, tentatively identified as an exopolysaccaride, may represent a new class of anti-fouling agent.

WORK PLAN: ONR funding terminates on 6/3/91. Work in progress as described above, will be supported until 8/30/91 by the University of Maryland.

PUBLICATIONS:

Burchard, R.P., D. Rittschof and J. Bonaventura. 1990. Adhesion and motility of gliding bacteria on substrata with different surface free energies. Appl. Environ. Microbiol. 56:2529-2534.

MANUSCRIPTS IN PRESS or IN PREPARATION:

Accepted (with revisions)

Sorongon, M., R.A. Bloodgood and R.P. Burchard. Hydrophobicity, adhesion and surface-exposed proteins of gliding bacteria. Appl. Environ. Microbiol.

In Preparation

Burchard, R.P. Inter-species inhibition of adhesion between gliding bacteria from marine biofilms.

Burchard, R.P. and R.A. Bloodgood. Heterogeneity of cell surface proteins classified as *Flexibacter columnaris* and *Cytophaga* strains.

Dull, C.L. and R.P. Burchard. The role of extracellular polymers in adhesion of *Flexibacter maritimus*.