

20030205003

AD-A216 187

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION (U)			1b. RESTRICTIVE MARKINGS NA		
2a. SECURITY CLASSIFICATION AUTHORITY NA			3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA			5. MONITORING ORGANIZATION REPORT NUMBER(S) NA		
4. PERFORMING ORGANIZATION REPORT NUMBER(S) University of Maryland Baltimore County			7a. NAME OF MONITORING ORGANIZATION Office of Naval Research		
6a. NAME OF PERFORMING ORGANIZATION University of Maryland Baltimore County		6b. OFFICE SYMBOL (If applicable) NA	7b. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		
6c. ADDRESS (City, State, and ZIP Code) Baltimore, Maryland 21228		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0158			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research		8b. OFFICE SYMBOL (If applicable) ONR	10. SOURCE OF FUNDING NUMBERS		
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		PROGRAM ELEMENT NO. 61153N	PROJECT NO. RR04106	TASK NO. 4412-035	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) (U) The Role of Extracellular Slime in Adhesion and Motility of Gliding Bacteria					
12. PERSONAL AUTHOR(S) Burchard, Robert P. & Schwarz, William H.					
13a. TYPE OF REPORT Annual		13b. TIME COVERED FROM 1/89 TO 12/89		14. DATE OF REPORT (Year, Month, Day) 12/20/89	
15. PAGE COUNT					
16. SUPPLEMENTARY NOTATION N.A.					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	Adhesion, <u>Flexibacter</u> , Gliding Bacteria, Hydrophobicity, Motility, Slime		
06	03				
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The goal of this research is to characterize the role(s) of extracellular slime and of the cell surface in adhesion and motility of aquatic gliding bacteria. Initial biochemical and rheological analyses of the slime of <i>Flexibacteria maritimus</i> and several of its adhesion/motility mutants are described. A theoretical analysis of the function of slime in a motility mechanism based on travelling transverse undulating waves is described. Inconsistencies in adhesion of <i>F. maritimus</i> led us to measure the cell surface hydrophobicity of this and selected other gliding bacteria and determine how adhesion and motility are affected by critical surface energy (CSE) of the substratum. Wild-type gliders are relatively hydrophobic; they adhere most tenaciously to low CSE surfaces. For several gliding bacteria and their adhesion-deficient mutants and revertants, cell surface proteins that directly contact glass surfaces have been vectorially radio-iodinated. Distinct, complex labelling patterns are described. We also report the isolation of two marine gliding bacteria, one of which produces a high molecular weight inhibitor of adhesion of the second. (S)					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION (U)		
22a. NAME OF RESPONSIBLE INDIVIDUAL M. Marron			22b. TELEPHONE (Include Area Code) 202 696-4760		22c. OFFICE SYMBOL ONR

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted

All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE (4)

DISTRIBUTION STATEMENT A

Approved for public release
Distribution Unlimited

89 12 28 029

PRINCIPAL

INVESTIGATOR:

Robert P. Burchard, Univ. of Maryland Baltimore
County (UMBC)

CO-P.I.:

William H. Schwarz, Johns Hopkins University

CONTRACTOR:

U.M.B.C.

SUB-CONTRACTOR:

Ian W. Sutherland, University of Edinburgh

CONTRACT TITLE:

The Role of Extracellular Slime in Adhesion and
Motility of Gliding Bacteria

START DATE:

January 1, 1988

REPORT PERIOD:

January 1 - December 31, 1989

PROJECT GOALS:

1. Characterization of the extracellular slime of aquatic gliding bacteria, particularly those isolated from marine biofilms, by physiological, genetic, biochemical, immunological, and rheological approaches.
2. Determination of the mechanism(s) of slime function in adhesion and motility of these gliding bacteria.
3. Characterization of cell envelope adhesins of the aquatic gliding bacteria that produce little or no slime.

Accomplishments for Year 2:

I. Extracellular Slime of Flexibacter maritimus

We have begun biochemical analyses of the extracellular polymers of static and shake cultures of several strains of F. maritimus. Freeze-dried supernatants of static cultures were separated on a Biogel P2 column. Carbohydrate-containing material was eluted in two fractions of molecular weight ~5 and 80 kDa. The higher molecular weight fraction polymer(s) contains D-glucose, D-galactose, D-mannose, L-rhamnose and an unknown sugar (ratio of 2 : 1.9 : 2.9 : 1 : 0.5). Shake culture supernatants contained a single high molecular weight carbohydrate peak. This material is now being analyzed by HPLC.

In contrast to the common wisdom that the bulk of slime is polysaccharide, we have found a carbohydrate:protein ratio of

static culture supernatants of ~1:3; that of shake cultures is ~1:15. SDS-PAGE analysis of the slime reveals a variety of 2 polypeptides ranging in molecular weight from ~26-175 kDa. We are currently determining whether any of these polypeptides may be glycosylated. Some of these polypeptides are likely to be the extracellular hydrolases produced by this bacterium. Others may function in adhesion and/or motility.

Work Plan for Year 3: During the next year, we expect to complete the biochemical and rheological (see section V) analysis of F. maritimus slime, including a comparison of the polysaccharides and polypeptides produced by adhesion and motility mutants grown in suspension and on agar. We will also determine whether our slime preps are contaminated with lipopolysaccharide from the bacterial outer membrane. Additionally, we expect to initiate NMR analysis of the slime in collaboration with Allen Bush of UMBC's Chemistry Dept. Finally, we hope to begin a molecular genetic analysis of F. maritimus slime.

II. Adhesion Studies:

Some of the aquatic gliding bacteria, both marine and fresh water, demonstrate reproducible adhesion and motility on standard microscope slides. This has not been the case for F. maritimus, leading us to examine the influence of substratum properties on behavior of these gliders. In collaboration with J. Bonaventura and D. Rittschof of the Duke University Marine Lab, we have made extensive observations of F. maritimus and several other gliders on microscope slides derivatized with silanes so as to obtain a range of critical surface energies (CSE). At one extreme is TriDecaFluoroSilane (TDF) which has a low CSE (i.e. extremely hydrophobic). Muffled glass slides (baked at 500°C) provide surfaces of high CSE (extremely hydrophilic). The chemical uniformity of the surfaces of these derivatized slides is currently being assessed by J. Wightman (Dept. of Chemistry, Virginia Tech).

Our observations lead to the generalization that adhesion is most tenacious on low CSE surfaces and very tenuous on substrata of high CSE. Cells quickly and irreversibly attached to TDF; adhesion was so tight that the bacteria were able to glide little if at all. In contrast, adhesion to muffled glass was weak, if it occurred at all. In some cases, it appeared that the gliders "skittered" on high CSE slides.

We quantitated adhesion to these derivatized slides by counting the density of adherent cells after submersion in a suspension of fixed bacterial concentration. While adhesion of F. maritimus cells correlated with our behavioral observations (i.e. highest density on lowest CSE), there was no correlation between cell density and behavior for Cytophaga U67, a fresh water glider.

Work Plan for Year 3: We expect to use the results from these derivatized slide studies to quantitate adhesion and motility of

Availability Codes	
Avail and/or	Special
DATE	
A-1	

F. maritimus and its mutants, grown under conditions that result in variations in slime production. These studies will involve a tenacity-of-adhesion assay in which bacteria adherent to a rectangular cross-section capillary are subjected to a centrifugal forces or to regulated flow of medium.

III. Cell Surface Properties of Aquatic Gliding Bacteria

Recognition of the differences in adhesion/motility behaviors on substrata of varying CSE (section II) have lead us to examine the surface hydrophobicity of several species of gliding bacteria and selected non-adherent mutants. We have used several procedures to determine surface hydrophobicity including Rosenberg's bacterial adherence to hydrocarbon (BATH) assay, ammonium sulfate salting out, and hydrophobic interaction chromatography (HIC). In addition, H. Busscher (U. of Groningen) is currently examining some of our strains by Fourier transform IR spectroscopy.

F. maritimus grown in static culture has cell-associated slime that confers relatively hydrophilic properties on the bacteria; they partition into the aqueous phase in the BATH assay and pass through an octyl sepharose column. In contrast, shake culture-grown cells, in which extracellular polymers appear to be sheared off the bacterial surface, behave as though they are relatively hydrophobic. Thus, it appears that the bound slime confers hydrophilic properties on these marine bacteria.

Cytophaga U67, which appears to lack significant levels of extracellular slime, behaves as though it is relatively hydrophobic. Its adhesion-deficient mutants, selected by enrichment for bacteria that partition into the aqueous phase in the BATH procedure, are relatively hydrophilic.

IV. Iodination of Cell Surface Polypeptides that Contact Substrata

In this collaborative project with R.A. Bloodgood of the U. of Virginia School of Medicine, we have used an immobilized iodination catalyst (Iodo-Gen) to label those surface polypeptides of Cytophaga U67 that make contact with glass substrata. One or more of the ≥ 15 (!) polypeptides that are radio-iodinated is likely to be an adhesin in this strain. Some of these polypeptides have been localized to the cell envelope fraction; others may be periplasmic. The fact that integral cell envelope proteins are labelled suggests that what extracellular slime this bacterium may produce does not interfere with direct cell envelope contact with the substratum.

We have also compared the labelling pattern of the wild-type with that of three adhesion-deficient mutants and their revertants. We have found three categories of changes in these mutants:

- 1) appearance of new polypeptide species as detected by Coomassie blue staining and/or appearance of new iodinated polypeptides;
- 2) loss of labelled species that are iodinated in the wild-type;

3) apparent changes in migration of polypeptides in SDS-PAGE (always reflecting lower apparent molecular weight). In the first case, the changes could be due to alterations in targeting of polypeptides to the cell surface and/or to changes in protein conformation resulting in increased accessibility to iodination. In the second case, the loss of labelling may be due to a decrease in the actual amount of polypeptide or to change in its conformation. The third case may reflect alterations in post-translational processing.

We have isolated several revertants of the adhesion-deficient mutants. Some of these revertants demonstrate a labelling pattern like that of the wild-type. However, one class of revertants of strain Adh2 demonstrates a unique radio-iodination pattern, unlike either parent or wild-type.

We are currently using vectorial iodination to label proteins on the surface of F. maritimus cells grown in static and shake culture. Preliminary results demonstrate qualitative and quantitative differences in iodination of cells grown under different culture conditions. It appears that cell-associated slime of static cultures blocks accessibility of some cell envelope proteins to the substratum.

Work Plan for Year 3: We hope to identify those vectorially iodinated proteins that function as adhesins for F. maritimus and Cytophaga U67 and localize them in the cell envelope-slime complex.

V. Modeling of the Role of Slime in Gliding Motility

We have completed two parts of our development of a mathematical model for gliding motility driven by travelling waves of transverse undulations in the cell envelope:

1. by obtaining analytic solutions of the governing differential equations using a perturbation series in a small parameter; and
2. by writing computer codes for numerical computation using the method of finite differences.

The purpose for using two independent techniques for solving the mathematical problem is to establish the validity of the calculations (It is well known that computer codes and complex analytic methods can be wrong!). We are focussing on the numerical method because it can be used for a wider range of conditions.

In the calculations, we have assumed that the rheological properties of the slime can be represented by a model known as a third-order fluid. Presently, we are measuring the actual rheological properties of F. maritimus slime.

Work Plan for Year 3: Data from the rheological analyses will be used to establish representative rheological equations for this mathematical model of gliding motility.

VI. Inter-Specific Inhibition of Adhesion

In the course of seeking additional slime-producing marine gliding bacteria, we have isolated two benthic Cytophagales (not yet speciated) from a bio. m in Buzzard's Bay, Massachusetts. These strains appear to be closely related based on physiology, morphology and % G+C of Chromosomal DNA (based on T_m). However, EcoRI digests of the DNA of each strain provides some differences in banding profiles on agarose gels. And, the two colony types can be distinguished by striking differences in their iridescence that suggest differences in the cell surfaces of the two strains. We have just completed an experiment to identify cell surface proteins with the immobilized iodination protocol described in section IV. The strain that demonstrates red iridescence has a variety of polypeptides that make contact with glass, similar in complexity to Cytophaga U67. In contrast, the green strain has one clearly dominant ~45 kDa polypeptide that is iodinated. The results suggest that one protein dominates the surface of this strain.

Of particular interest to the goals of this research is the fact that the red strain produces a extracellular diffusible material that inhibits adhesion to and thus motility of the green strain on hydrophilic (only) substrata. The material is a heat-labile and protease-resistant macromolecule. Column chromatographic results suggest a molecular weight of > 200 kDa. We are currently fractionating concentrated culture supernatants on Biogel P2 to isolate this adhesion inhibitor.

Work Plan for Year 3: We propose to characterize the adhesion inhibitor and determine the range of bacteria against which it is active. We will also characterize the cell surface of the green strain. An independent source of funds will be sought to support this research.

PUBLICATIONS and PRESENTATIONS

1. Godwin, S.L., M. Fletcher & R.P. Burchard. 1989. Interference reflection microscopic study of sites of association between gliding bacteria and glass substrata. J. Bacteriol. 171:4589-4594.
2. McGrath, C.F., C.W. Moss & R.P. Burchard. 1990. Effect of temperature shifts on gliding motility, adhesion, and fatty acid composition of Cytophaga U67. Accepted by J. Bacteriol.
3. Burchard, R.P. & R.A. Bloodgood. 1990. Surface proteins of the gliding bacterium Cytophaga U67 and its adhesion mutants. Accepted by J. Bacteriol.
4. Burchard, R.P. 1989. Adhesion of Gliding Bacteria. Invited presentation to the 1989 Cordon Conference on The Science of Adhesion.
5. Siddiqui, A., A. Provost and W.H. Schwarz. Analysis of the undulating surface model for the motility of gliding microorganisms. Submitted for presentation to the 11th U.S. National Congress of Applied Mechanics, May 1990.
6. Burchard, R.P., D. Rittschof and J. Bonaventura (in preparation). Adhesion and motility of gliding bacteria on substrata of different surface free energies.

AMZEL, L. Mario
Department of Biophysics
Johns Hopkins School of Medicine
725 North Wolfe Street
Baltimore, MD 21205

ANDERSEN, Niels H.
Department of Chemistry
University of Washington
Seattle, WA 98195

ARNOLD, Frances H.
Dept of Chemical Engineering
California Institute of Technology
Pasadena, CA 91125

AUGUST, J. Thomas
Department of Pharmacology
Johns Hopkins Medical School
725 North Wolfe Street
Baltimore, MD 21205

BEVERIDGE, David L.
Department of Chemistry
Wesleyan University
Hall-Altwater Laboratories
Middletown, CT 06457

BRAMSON, H. Neal
Department of Biochemistry
Univ of Rochester Medical Center
601 Elmwood Avenue
Rochester, NY 14642

BRUCE, Thomas C.
Department of Chemistry
University of California-Santa
Barbara
Santa Barbara, CA 93106

CASE, Steven T.
Department of Biochemistry
Univ of Mississippi Medical Center
2500 North State Street
Jackson, MS 39216-4505

CHANG, Eddie L.
Bio/Molecular Engineering
Naval Research Laboratory
Code 6190
Washington, D.C. 20375-5000

CHRISTIANSON, David W.
Department of Chemistry
University of Pennsylvania
231 South 34th Street
Philadelphia, PA 19104-6323

CORDINGLEY, John S.
Department of Molecular Biology
University of Wyoming
Box 3944 University Station
Laramie, WY 82071

DeGRADO, William F.
E. I. du Pont de Nemours & Co
Central R & D, Experimental Station
P. O. Box 80328
Wilmington, DE 19880-0328

EVANS, David R.
Department of Biochemistry
Wayne State Univ School of Medicine
540 E. Canfield Street
Detroit, Michigan 48201

FEIGON, Juli F.
Department of Chem & Biochemistry
UCLA
405 Hilgard Avenue
Los Angeles, CA 90024-1569

FICHT, Allison R.
Dept of Med Biochem & Genetics
Texas A&M University
College Station, TX 77843

FRAUENFELDER, Hans
Department of Physics
University of Illinois
Urbana, IL 61801

GABER, Bruce
Naval Research Laboratory
Bio/Molecular Engineering Branch
Code 6190
Washington, DC 20375

GETZOFF, Elizabeth D.
Scripps Clinic & Research Foundation
Department of Molecular Biology
10666 North Torrey Pines Road
La Jolla, CA 92037

GOODMAN, Eugene M.
Biomedical Research Institute
University of Wisconsin
P. O. Box 2000
Kenosha, WI 53141

HO, Pui Shing
Department of Biochemistry and
Biophysics
Oregon State University
Corvallis, OR 97331

HOGAN, Michael L.
Baylor Center for Biotechnology
4000 Research Forest Drive
The Woodlands, TX 77381

HONIG, Barry
Columbia University
Dept of Biochem and Molec Biophys
630 West 168th St
New York, NY 10032

HOPKINS, Paul B.
Department of Chemistry
University of Washington
Seattle, WA 98195

KAHNE, Daniel
Department of Chemistry
Princeton University
Princeton, NJ 08544

KEMP, Robert G.
Chicago Medical School
Dept of Biological Chemistry
3333 Green Bay Rd.
North Chicago, IL 60064

KHORANA, Gobind H.
Department of Biology
MIT
77 Massachusetts Ave.
Cambridge, MA 02139

KIM, Sangtae
Chemical Engineering
University of Wisconsin
1415 Johnson Drive
Madison, WI 53706

LANSBURY, Peter T.
Department of Chemistry
MIT
Cambridge, MA 02139

LAURSEN, Richard A.
Chemistry Department
Boston University
590 Commonwealth Avenue
Boston, MA 02215

LENZ, Robert W.
Chemical Engineering Department
University of Massachusetts
Amherst, MA 01003

LEWIS, Randolph V.
Molecular Biology Department
University of Wyoming
University Station Box 3944
Laramie, WY 82071

BELAS, M. Robert
Center of Marine Biotechnology
University of Maryland
500 East Lombard Street
Baltimore, MD 21202

BLAKE, III, Robert C.
Department of Biochemistry
Meharry Medical College
Nashville, TN 37208

BLAKEMORE, R. P.
Department of Microbiology
University of New Hampshire
Durham, New Hampshire 03824

BURCHARD, Robert P.
Department of Biological Sciences
Univ of Maryland-Baltimore County
Catonsville, MD 21228

CHAPMAN, David J.
Department of Biology
UCLA
405 Hilgard Avenue
Los Angeles, CA 90024

CLARK, Douglas S.
Dept of Chemical Engineering
University of California
Berkeley, CA 94720

COLWELL, Rita
Maryland Biotechnology Institute
University of Maryland
Microbiology Building
College Park, MD 20742

COOKSEY, Keith E.
Department of Microbiology
Montana State University
Bozeman, MT 59717

DANIELS, Charles J.
Department of Microbiology
Ohio State University
484 West 12th Avenue
Columbus, OH 43210

DANIELS, Lacy
Department of Microbiology
University of Iowa
College of Medicine
Iowa City, IA 52242

DENNIS, Patrick P.
Department of Biochemistry
University of British Columbia
2146 Health Sciences Mall
Vancouver, B.C. V6T 1W5

DOOLITTLE, W. Ford
Department of Biochemistry
Dalhousie University
Halifax, Nova Scotia
CANADA B3H 4H7

EISENBERG, Henryk
The Weizmann Institute of Science
Dept of Polymer Research
P.O. Box 26
Rehovot 76100, Israel

EPEL, David
Hopkins Marine Station
Stanford University
Pacific Grove, CA 93950

FELBECK, Horst
Marine Biology Research Division
Scripps Institution of Oceanography
University of California - San Diego
La Jolla, CA 92093

FISHER, Charles R.
Marine Science Institute
University of California-Santa
Barbara
Santa Barbara, CA 93106

GIBOR, Aharon
Marine Science Institute
University of California
Santa Barbara, CA 93106

CONZALEZ, Elma
Department of Biology
UCLA
Los Angeles, CA 90024

GREENBERG, Everett P.
Department of Microbiology
University of Iowa
Iowa City, Iowa 52242

GUNSALUS, Robert P.
Department of Microbiology
UCLA
405 Hilgard Avenue
Los Angeles, CA 90024

GUPTA, Ramesh
Southern Illinois University
Dept of Chem and Biochemistry
Carbondale, IL 62901

HASTINGS, J. Woodland
Biological Laboratories
Harvard University
16 Divinity Avenue
Cambridge, MA 02138

HAYGOLD, Marjorie
Marine Biology Research Division
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093

JENSEN, Roy A.
Department of Microbiology
University of Florida
Gainesville, FL 32611

KELLY, Robert M.
Dept of Chemical Engineering
The Johns Hopkins University
Baltimore, MD 21218

KIRCHMAN, David L.
College of Marine Studies
University of Delaware
Robinson Hall
Newark, DE 19716

KONISKY, Jordan
Department of Microbiology
University of Illinois
809 Sout Wright Street
Champaign, IL 61820

LEADBETTER, Edward R.
Dept of Molecular and Cell Biology
University of Connecticut
Box U-131
Storrs, CT 06268

LIAO, Hans H.
Biotechnology Center
University of Wisconsin
1710 University Avenue
Madison, WI 53705

LIDSTROM, Mary E.
Keck Laboratories 138-78
California Institute of Technology
Pasadena, CA 91125

MITCHELL, Ralph
Division of Applied Sciences
Harvard University
125 Pierce Hall
Cambridge, MA 02138

MORSE, Daniel E.
Marine Science Institute
University of California
Santa Barbara, CA 93106

NADATHUR, Govind S.
Marine Science Institute
Univ Cal-Santa Barbara
Santa Barbara, CA 93106

LINDSAY, Stuart M.
Department of Physics
Arizona State University
Temp, AZ 85278

LOEB, George I.
David W. Taylor Research Center
Code 2341
Annapolis, MD 21402-5067

MASILAMANI, Divakar
Biotechnology Department
Allied-Signal Inc.
P. O. Box 1021R
Morristown, NJ 07960

McCONNELL, Harden M.
Stanford University
Department of Chemistry
Stanford, CA 94305

McELROY, William D.
Department of Chemistry
University of California - San Diego
La Jolla, CA 92093-0601

MERTES, Kristin Bowman
University of Kansas
Dept of Chemistry
Lawrence, Kansas 66045

NAGUMO, Mark
Bio/Molecular Engineering Branch
Naval Research Laboratory
Code 6190
Washington, DC 20375-5000

OLIVERA, Baldomero M.
Department of Biology
University of Utah
Salt Lake City, UT 84112

PABO, Carl O.
Department of Biophysics
Johns Hopkins University
School of Medicine
Baltimore, MD 21205

PRENDERGAST, Franklyn G.
Dept of Biochemistry & Molec Biol
Mayo Foundation
200 First St. SW
Rochester, MN 55905

PUGH, Jr., Edward N.
Department of Psychology
University of Pennsylvania
3815 Walnut Street
Philadelphia, PA 19104-6196

RACKOVSKY, Shalom R.
Department of Biophysics
University of Rochester
School of Medicine and Dentistry
Rochester, NY 14642

RAJAN, K. S.
Illinois Institute of Technology
Research Institute
10 W. 35th St.
Chicago, IL 60616

REINISCH, Lou
Laser Biophysics Center
Uniformed Services University
4301 Jones Bridge Road
Bethesda, MD 20814

RICH, Alexander
MIT Department of Biology
Cambridge, MA 02139

RICHARDS, J. H.
California Institute of Technology
Division of Chemistry and Chemical
Engineering
Pasadena, CA 91125

ROTHSCHILD, Kenneth J.
Department of Physics
Boston University
590 Commonwealth Avenue
Boston, MA 02215

SCHULTZ, Peter G.
Department of Chemistry
University of California-Berkeley
Berkeley, CA 94720

SEEMAN, Nadrian
Department of Chemistry
New York University
New York, NY 10003

SELSTED, Michael E.
UCLA
Dept of Medicine
37-055 CHS
Los Angeles, CA 90024

SIGMAN, David S.
UCLA School of Medicine
Dept of Biological Chemistry
Los Angeles, CA 90024
SIKES, Steven C.
Department of Biological Sciences
University of South Alabama
Mobile, AL 36688

SINSKE, Anthony J.
Laboratory of Applied Microbiology
MIT Department of Biology
Cambridge, MA 02139

STEWART, James M.
Department of Chemistry
University of Maryland
College Park, MD 20742

STEWART, John M.
Department of Biochemistry
University of Colorado
Health Science Center
Denver, CO 80262

TURNER, Douglas H.
Department of Chemistry
University of Rochester
Rochester, NY 14627

URRY, Dan W.
Laboratory of Molecular Biophysics
University of Alabama
P. O. Box 311
Birmingham, AL 35294

WAITE, J. Herbert
College of Marine Studies
University of Delaware
Lewes, DE 19958

WARD, Keith B.
Naval Research Laboratory
Code 6030
Washington, DC 20375

WARSHEL, Arie
Department of Chemistry
University of Southern California
University Park
Los Angeles, CA 90089-0482

WATT, Gerald D.
Dept of Chemistry & Biochemistry
University of Colorado
Campus Box 215
Boulder, CO 80309-0215

NEALSON, Kenneth H.
Center for Great Lakes Studies
University of Wisconsin-Milwaukee
600 E. Greenfield Avenue
Milwaukee, WI 53204

OLSEN, Gary J.
Indiana University
Department of Biology
Jordan Hall 138
Bloomington, Indiana 47405

PACE, Norman R.
Department of Biology
Indiana University
Bloomington, IN 47405

PREZELIN, Barbara B.
Marine Science Institute
University of California
Santa Barbara, CA 93106

REEVE, John N.
Department of Microbiology
Ohio State University
484 West 12th Avenue
Columbus, OH 43210-1292

ROSEMAN, Saul
Department of Biology
Johns Hopkins University
Baltimore, MD 21218

SEARCY, Dennis G.
Zoology Department
University of Massachusetts
Amherst, MA 01003

SILVERMAN, Michael
Agouron Institute
505 Coast Blvd. South
La Jolla, CA 92037

SMIT, John
Department of Microbiology
University of British Columbia
#300 - 6174 University Blvd
Vancouver, British Columbia
V6T 1W5 CANADA

SPUDICH, John L.
Dept of Anat and Structural Biolgy
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461

STAHL, David A.
College of Veterinary Medicine
University of Illinois
Urbana, IL 61801

SWIFT, Hewson
Dept of Molec Genetics
and Cell Biology
University of Chicago
1103 East 57th Street
Chicago, IL 60637

TAYLOR, Gordon T.
Hawaii Institute of Geophysics
University of Hawaii
2525 Correa Road
Honolulu, HI 96822

TOSTESON, Thomas R.
Department of Marine Sciences
University of Puerto Rico
Mayaguez, PR 00709

TRENCH, Robert K.
Marine Science Institute
University of California-Santa
Barbara
Santa Barbara, CA 93106

WALEH, Nahid
Molecular Biology Department
SRI International
333 Ravenswood Avenue
Menlo Park, CA 94025

WHITE, David
Institute of Applied Microbiology
University of Tennessee
P. O. Box X, Building 1503/6
Oak Ridge, TN 37831

WCESE, Carl R.
Genetics Department
University of Illinois
515 Morrill Hall
Urbana, IL 61801

YAYANOS, A. Aristides
Physiological Research Laboratory
Scripps Institution of Oceanography
University of California-San Diego
La Jolla, CA 92093

ZINDER, Stephen H.
Department of Microbiology
Cornell University
Stocking Hall
Ithaca, NY 14853