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SECURITY CLASSIFICATION OF THIS

(2)

TATION PAGE

Form Approved
OMB No 0704-0188

1a REPC AD-A208 975

1b RESTRICTIVE MARKINGS
N/A

2a SECU
N

3 DISTRIBUTION/AVAILABILITY OF REPORT
Distribution unlimited

2b DECLASSIFICATION/DOWNGRADING SCHEDULE
N/A

4 PERFORMING ORGANIZATION REPORT NUMBER(S)
N/A

5 MONITORING ORGANIZATION REPORT NUMBER(S)
N/A

DTIC ELECTE JUN 12 1989 S D

6a NAME OF PERFORMING ORGANIZATION
Univ. of British Columbia

6b OFFICE SYMBOL (If applicable)
N/A

7a NAME OF MONITORING ORGANIZATION
Office of Naval Research

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Arlington, VA 22217-5000

8a NAME OF FUNDING/SPONSORING ORGANIZATION
Office of Naval Research

8b OFFICE SYMBOL (If applicable)
ONR

9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
N00014-89-J-1749

8c ADDRESS (City, State, and ZIP Code)
800 N. Quincy St.
Arlington, VA 22217-5000

10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO.
61153 N	RR04106	441h-006	-

11 TITLE (Include Security Classification)
Chemical, structural & genetic analysis of the adhesive holdfast of biofouling caulobacters

12 PERSONAL AUTHOR(S)
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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 3-1-89 TO 6-5-89	1989, June 5	3

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Biofouling, holdfast, Caulobacters, Adhesives Biofilms

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

We are primarily engaged in an analysis of the adhesives that enable the stalked Caulobacter bacteria to attach and remain adherent to surfaces in the environment for long periods of time. The specific goals are: 1) Determine the chemical composition and structural arrangement of monosaccharides and other substituents within the holdfasts of selected marine and freshwater Caulobacters.
2) Characterize the types of surfaces to which holdfasts will adhere.
3) Clone and analyze the genes specifying the holdfasts of selected marine and freshwater Caulobacters.
4) Develop the capabilities of selected marine Caulobacters for molecular genetic experimentation.
5) To evaluate the occurrence, stability and behavior of Caulobacters on surfaces and in complex biofilms, in collaboration with other ONR-funded researchers.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	U

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DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

89 6 12 122

Annual Report on grant N00014-89-J-1749
Tuesday, June 6, 1989

Principle Investigator: John Smit

Contractor University of British Columbia

Contact Title: Chemical, structural and genetic analysis of the adhesive holdfast of biofouling Caulobacters

Start Date: 1 March 1989

Research Objectives:
1) Determine the chemical composition and structural arrangement of monosaccharides and other substituents within the holdfasts of selected marine and freshwater Caulobacters. This also includes determining the periodicity and regulation of synthesis during the cell cycle.
2) Further characterize the types of surfaces to which holdfasts will adhere.
3) Clone and analyze the genes specifying the holdfasts of selected marine and freshwater Caulobacters.
4) Continue developing and evaluating the capabilities of selected marine Caulobacters for molecular genetic experimentation.
5) To evaluate the occurrence, stability and behavior of Caulobacters on surfaces and in complex biofilms, in collaboration with other ONR-funded researchers.

Progress and Planned Activities
Chemical analysis of the adhesive holdfast produced by Caulobacters
Based on our studies using lectins to determine the composition of holdfasts, we have selected 2 marine Caulobacters (MCS6 and MCS24) and 2 freshwater Caulobacters (Caulobacter crescentus CB2A and Caulobacter subvibrioides) for in-depth composition analysis. This is also done in concert with decisions regarding the strains selected for gene isolation (MCS6 and Caulobacter crescentus CB2A). As we generate mutants with altered holdfasts, the composition of these will be determined as well.

Our first efforts have been directed to CB2A. We have been concentrating on an isolation procedure based on the observation that holdfasts bind very tightly to colloidal gold particles. Once bound, the holdfast is no longer adhesive and the complex can be isolated readily by CsCl density centrifugation, relying on the high density imparted by the gold binding. However, the method did not work adequately well with wild type cells. We attempted to dissociate holdfast from the main cell body by extensive sonication. This proved to be inadequate; antisera raised against the holdfast-colloidal gold complexes produced sizable activities to the lipopolysaccharide coat of Caulobacters.

We are now using a mutant we isolated after UV mutagenesis that sheds its holdfast into the medium. The holdfast can be collected by attachment to the colloidal gold particles and appears to be free of all cell constituents. As discussed below, we believe we can introduce this shedding phenotype into strains with altered holdfast composition, to enable chemical analysis of the alterations.

With holdfast attached to the colloidal gold we shall be doing a composition chemical analysis, raising specific antisera for the development of an immunoassay and acquire surface-enhanced Raman spectroscopy "fingerprints" for normal and altered holdfast, as a collaboration with both

David White (U. of Tennessee) and Gordon Taylor (U. of Hawaii).

On the adhesive properties of the holdfast.
As part of studies aimed at discerning what types of surfaces to which the Caulobacters will attach, glass surfaces were covalently modified with a variety of chemical substituents (provided by Dan Rittschoff, Duke University Marine Labs), resulting in surfaces ranging from highly charged to very hydrophobic. From a quantitative static flow attachment assay we learned was that Caulobacters will attach to virtually all surfaces at some frequency, but appear to prefer substrates that are moderately hydrophobic. Freshwater Caulobacters attach better to very hydrophobic surfaces than do marine Caulobacters. By growing marine strains that tolerate low ionic strength media in a freshwater medium, we learned that the salts in seawater are apparently responsible for lowered adhesiveness to hydrophobic surfaces. Of practical significance was the finding that dimethyldichlorosilane treated glass (ie classical "silanizing") was reasonably effective in discouraging attachment, a convenience for many future experiments, especially holdfast isolation procedures. We are presently using the same assay to determine the preference of the Caulobacter adhesive for various metal surfaces, by evaporating metals onto glass surfaces. We know the holdfast binds tightly to metals such as gold and silver , but wish to learn if there is a preference for these metals over other types of surfaces.

On the genes that specify the holdfast structure
We prepared a library of 16000 independent transposon Tn5 insertions in Caulobacter crescentus CB2A, isolated 78 holdfast-defective mutants and began genetic analysis of the mutants to learn where the transposon was inserted. We have learned that the mutants cluster in 4 regions throughout the genome and have a number of phenotypes. Among them is the "shedder" phenotype discussed above. We also have cloned a segment of DNA from each of the 4 regions. Our results are summarized in this table:

Cluster	Mutant Group	# Detected	Phenotype	Size of Cloned Region (Restriction Enzyme)
A	g5	6	Reduced level or altered(?) holdfast	9.2 Kb (Eco R1)
B	g7	2	Holdfast shedder	12 Kb (SstI/EcoR1)
	g8	2	" " " "	
	g9	2	" " " "	
C	g1	6	No Holdfast	14.3 Kb (SstI)
	g2	16	"" " "	
D	g3	30	No Holdfast	12.2 Kb (ClaI/KpnI)
	g4	4	"" " "	
	g10	1	"" " "	
	g6	9	Curved or weak stalk, Reduced level or altered(?) holdfast	

We are in the process now of a wide variety of experiments aimed at further characterization of the genetic regions involved with the holdfast production and are still searching for additional genes since we believe there was a degree of non-randomness in the transposon mutagenesis.

We are also repeating the process in the marine Caulobacter MCS6. In addition we will learn whether there is significant homology between the freshwater and marine holdfast genes. If

true, we will be able to use the cloned segments from the freshwater Caulobacter to speed up identification of marine holdfast genes.

On the development of molecular genetic capabilities of marine Caulobacters.
A very important development during the last several months was to determine the conditions for electroporation of both marine and freshwater Caulobacters. This is a method for introducing plasmids into bacteria without the necessity of transformation or conjugation techniques. The marine Caulobacters were particularly troublesome, since the technique tolerates very little ionic strength. We were able to adapt strain MCS6 (chosen for genetic studies) for growth in very low ionic strength medium and have successfully electroporated in plasmids at a frequency of $2X10^3/\mu g$. This frequency is low compared to our results with freshwater Caulobacters ($5x10^8/\mu g$) and we are working to improve the efficiency. But it is a very usable efficiency for many experiments.

Publications

Manuscripts currently in preparation

•Merker, R. M., D. Rittschoff and J. Smit. Analysis of the attachment of marine and freshwater Caulobacters to surfaces using chemically defined substrates.

•Nivens, D.E. , A. Tunlid, M.J. Franklin, J. Smit, and D. White. Infrared monitoring of interactions between Caulobacter species and solid surfaces.

•Ong, C and J. Smit. The adhesive holdfast of Caulobacters: Isolation and analysis of mutants defective in the attachment of the organelle to the cell.

•Gilchrist, A., W. Bingle and J. Smit. Introduction of plasmids into freshwater and marine Caulobacters by electroporation.

•Mitchell, D. and J. Smit. Identification and cloning of the genes affecting production of the adhesion organelle of Caulobacter crescentus CB2.

Training Activities

One graduate student (Canadian, Caucasian) and one postdoctoral (South African, Caucasian) are currently supported by this contract. This will rise to 2 of each by 1 September 1989.

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