UNIVERSITY OF CALIFORNIA, SAN DIEGO

# Microscopic, Genetic, and Biochemical Characterization of Non-Flagellar Swimming Motility in Marine Cyanobacteria

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Marine Biology

by

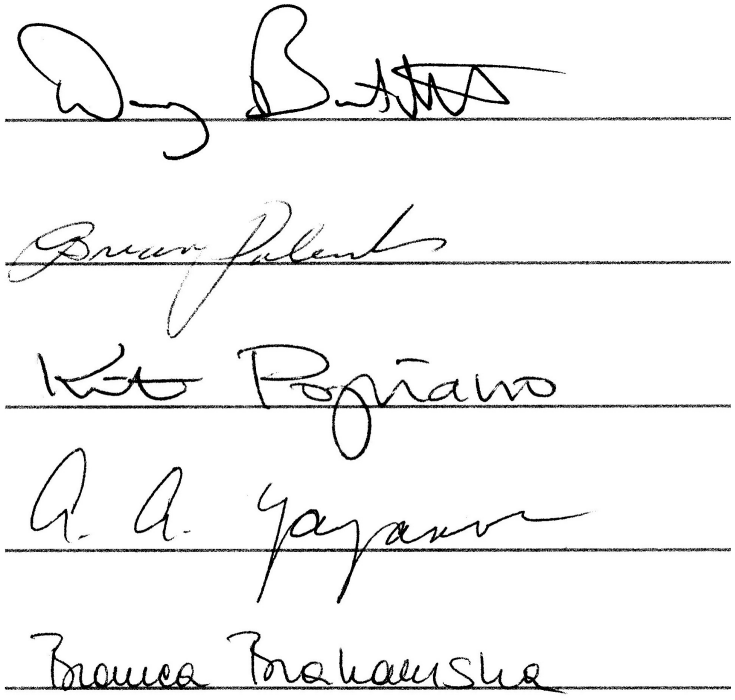
Jay William McCarren

Committee in charge:

Bianca Brahamsha, Chair
Douglas Bartlett
Brian Palenik
Kit Pogliano
Aristides Yayanos

2005

The dissertation of Jay William McCarren is approved, and is acceptable in quality and form for publication on microfilm:



Chair

University of California, San Diego

2005

DEDICATION

To Alex

## TABLE OF CONTENTS

## ABBREVIATIONS

| | |
|---|---|
| ABC transporter | ATP binding cassette transporter |
| ATP | Adenosine triphosphate |
| BLOTTO | Bovine lacto-transfer technique optimizer |
| CM | Cytoplasmic membrane |
| CMi | Cytoplasmic membrane inner face |
| EDTA | Ethylenediaminetetraacetic acid |
| EL | External layer |
| FITC | Fluorescein isothiocyante |
| FL | Fibrillar layer |
| HSP | High-speed pellet containing insoluble OM proteins |
| HSS | High-speed supernatant containing soluble OM proteins |
| IgG | Immunoglobulin G |
| MFP | Membrane fusion protein |
| MSCRAMMS | Microbial surface components recognizing adhesive matrix molecules |
| MWCO | Molecular weight cut-off |
| OM | Outer membrane |
| OMP | Outer membrane protein |
| ORF | Open reading frame |
| PAGE | Polyacrylamide gel electrophoresis |
| PAS stain | Periodic acid-Schiff stain |

| | |
|---|---|
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PPIase | Peptidyl-prolyl isomerase |
| Prot1E family | Protein-1 exporter family |
| RSCU | Relative synonymous codon usage |
| RTX | Repeats in toxin |
| S-layer | Surface layer |
| SAPS | Statistical analysis of protein sequences |
| SN | Natural seawater based medium |
| SOW | Synthetic ocean water |
| TOF | Time of flight |
| TEM | Transmission electron microscopy |
| WH8102 | *Synechococcus* sp. strain WH8102 |

## LIST OF FIGURES

CHAPTER II Page



CHAPTER III



CHAPTER IV

CHAPTER V



CHAPTER VI

## LIST OF TABLES

CHAPTER IV Page



CHAPTER V



CHAPTER VI

## ACKNOWLEDGEMENTS

This thesis would not have been possible without the guidance I have received from my advisor Bianca Brahamsha. Bianca has been invaluable to my development as a scientist. She exemplifies what every graduate student wants in an advisor. She has provided advice, criticism, and encouragement, while allowing me the freedom to grow and develop independently. Her advice on everything, down to the tiniest detail of a protocol, has been impeccable. Many thanks to my thesis committee members: Brian Palenik, Doug Bartlett, Art Yayanos, and Kit Pogliano who have helped to guide this research. Thanks also to Maryann Martone, Naoko Yamada, John Heuser, Robyn Roth, and Kit Pogliano. They have all helped me immensely, provided expert advice on different microscopic techniques, and given me a chance to work in their laboratories.

In addition to the formal guidance I have received, there are innumerable people who have helped me to get to this point, especially labgroup members past and present: Sonya Dyhrman, Gerardo Toledo, Aubrey Davis, Dori Landrey, Eric Allen, Chris Dupont, and Vera Tai. They have all been a great source of advice and suggestions. Thanks also to the entire Hubbs Hall community, which always came through in a pinch whether I needed an enzyme, some test tubes, or a helping hand.

Friends and family have been an essential part of this endeavor, having made the time here so enjoyable. I am grateful for their encouragement and inspiration. Special thanks to my parents for fostering my love for all things aquatic. Foremost I

want to acknowledge my wife, Alexandra, for encouraging me to follow my goal and her seemingly endless faith in me.

Funding for this research was generously provided by the Scripps Institution of Oceanography Graduate Department, as well as by the National Science Foundation (NSF grant MCB 97-27759) and the Department of Energy (DOE grant DE-FG03-03ER63148).

The text of Chapter III, in full, is a reprint of the material as it appears in McCarren, J., J. Heuser, R. Roth, N. Yamada, M. Martone, and B. Brahamsha. 2005. Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J. Bacteriol. 187: 224-230. The dissertation author was the primary author, and co-author B. Brahamsha directed and supervised the research, which forms the basis for this chapter. Cryofixation, freeze-substitution, and TEM work was performed at the National Center for Microscopy and Imaging Research, University of California, San Diego, by the dissertation author and N. Yamada under the direction of M. Martone. Freeze-fracturing and etching EM work was performed at Washington University, St. Louis, by R. Roth under the direction of J. Heuser.

The text of Chapter IV, in full, is a reprint of the material as it appears in McCarren, J., and B. Brahamsha. 2005. Transposon mutagenesis in a marine *Synechococcus*: isolation of swimming motility mutants. J. Bacteriol. 187:4457-4462. The dissertation author was the primary author, and co-author B. Brahamsha directed and supervised the research, which forms the basis for this chapter.

The text of Chapter V, in full, is being prepared for publication. The dissertation author was the primary author, and co-author B. Brahamsha directed and supervised the research, which forms the basis for this chapter.

The text of Chapter VI, in full, is being prepared for publication. The dissertation author was the primary author, and co-author B. Brahamsha directed and supervised the research, which forms the basis for this chapter.

## VITA

| | |
|---|---|
| 1997 | B.A., Biochemistry, Colorado College, Colorado Springs, CO |
| 1997-1999 | Research Associate, AMC Cancer Research Center, Denver CO |
| 1999-2005 | Research Assistant, Scripps Institution of Oceanography, University of California, San Diego, CA |
| 2003, 2005 | Teaching Assistant, Department of Biology, University of California, San Diego, CA |
| 2005 | Ph.D. University of California, San Diego, CA |

### Publications

McCarren, J., and B. Brahamsha. 2005. Transposon mutagenesis in a marine *Synechococcus*: isolation of swimming motility mutants. J. Bacteriol. 187:4457-4462.

McCarren, J., J. Heuser, R. Roth, N. Yamada, M. Martone, and B. Brahamsha. 2005. Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J. Bacteriol. 187: 224-230.

Palenik, B. B. Brahamsha, F. W. Larimer, M. Land, L. Hauser, P. Chain, J. Lamerdin, W. Regala, E. E. Allen, J. McCarren, I. Paulsen, A. Dufresne, F. Partensky, E. A. Webb, and J. Waterbury. 2003. The genome of a motile marine *Synechococcus.* Nature 424:1037-1042.

### Conference Participation and Awards

Wenner-Gren Foundations International Symposium "Marine cyanobacteria: evolution, function and genomes". Poster titled: Identification and characterization of SwmB, an unusual protein that is required for non-flagellar swimming in marine *Synechococcus* (2005)

ASM 104$^{th}$ General Meeting. Poster titled: Identification and characterization of SwmB, a 1.1 MDa protein that is required for non-flagellar swimming in marine *Synechococcus* (2004)

ASLO Aquatic Sciences Meeting. Poster titled: Gene clusters required for swimming motility in marine *Synechococcus* (2003) *Winner of Student Poster Award*

ASM 102$^{nd}$ General Meeting. Poster titled: Identifying components involved in marine *Synechococcus* swimming motility by transposon mutagenesis (2002)

San Diego Microbiology Group Annual Symposia. Talk titled: Ultrastructural and genetic investigation of swimming motility in marine *Synechococcus* (2002)

VIIth Cyanobacterial Workshop. Poster titled: SwmA forms an additional envelope layer in motile marine *Synechococcus* (2001)

**ABSTRACT OF THE DISSERTATION**

# Microscopic, Genetic, and Biochemical Characterization of Non-Flagellar Swimming Motility in Marine Cyanobacteria

by

Jay William McCarren

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2005

Bianca Brahamsha, Chair

The mechanism of motility in marine *Synechococcus*, which swim without any apparent extracellular appendages, remains a mystery 20 years after its discovery. A multifaceted investigation including direct microscopic visualization, genetic analyses, and biochemical approaches was carried out in order to better understand the physiology of this globally important primary producer. Ultrastructural analyses provided a detailed view of the cell envelope layers and aided in the identification of a structure important for motility. Electron microscope tomographic reconstructions

revealed the even distribution of SwmA, a protein required for motility, across the cell surface. Various cryo-fixation techniques were required for the preservation and visualization of a para-crystalline S-layer formed by this protein.

As complete genomic sequence information failed to identify genes involved in motility, a transposon mutagenesis technique was developed to identify components of the motility apparatus. Utilizing this genetic tool, 17 independent transposon insertions that abolish motility were localized to clusters in three separate chromosomal regions. Included within these clusters are several multicomponent transport systems, as well as a number of glycosyltranferases. One cluster is characterized by DNA with an exceptionally low % G+C content relative to the genome average. Additionally, inter-genome comparisons reveal the absence of this stretch of DNA in two non-motile strains of *Synechococcus*, suggesting acquisition of this genetic information by horizontal gene transfer. Contained within this region of low % G+C content is an extremely large gene called *swmB*, which is required for motility in these cells. The sequence of SwmB is highly repetitive, with 4 domains of tandem repeats comprising over 60% of the protein. Analyses confirm that this gene is indeed translated into a megadalton-size protein, which is localized on the cell surface. Cellular localization of the two motility proteins SwmA and SwmB revealed that all motility mutants in culture have a defect in the localization of either SwmA or SwmB and in some instances both of these proteins. Additionally, two outer membrane polypeptides of 70 kDa and 80 kDa are absent in some of these mutants, suggestive of a role in motility.