

WASHINGTON SEA GRANT PROJECT COMPLETION SUMMARY REPORT

INSTRUCTIONS: Please provide a lay summary for your completed project that includes the following project elements:

- objectives
- methodology
- rationale
- major findings
- significance of results

If relevant, also include:

- students supported (number and degree level)
- partnerships
- outreach activities

Please note that this summary will be submitted in the Washington Sea Grant annual report to the National Sea Grant Office and will be available to the public via the NIMS database and the Washington Sea Grant website.

WSG Project Number: **R/B-46**
Project Title: Genome Enhanced Studies of Low Temperature Enzymes

Project period: 2/1/2004 – 1/31/2008

Principal Investigator(s) and Affiliation:
Jody Deming School of Oceanography, University of Washington

PROJECT COMPLETION SUMMARY REPORT

(Please provide your summary here. Character limit: 5,000 characters, including spaces):

The overall goal of this project was to evaluate how cold-active enzymes, produced by marine bacteria and released into their liquid environments, may interact with exopolymers, the large chemically complex sugar-based compounds that are also released with the enzymes. We were particularly interested to learn how such exopolymers may influence the performance of enzymes at low temperatures, building on past discoveries of cold-active proteases or enzymes that degrade proteins. On the long term, we want to better understand microbial survival strategies in extreme habitats and

contribute new compounds and insights for biotechnological, medical and engineering applications.

We worked primarily with a well-known marine bacterium named *Colwellia psychrerythraea* strain 34H, taking advantage of information in its recently available whole genome sequence to develop defined growth media and optimize the production of exopolymers and enzymes. Our overall experimental approach relied upon established methodologies and instrumentation to obtain and evaluate exopolymers and enzyme activity at low temperatures. We used standard separation and extraction techniques to obtain exopolymers, fluorescent substrate analogs and fluorometry to measure enzyme activity during the course of incubations, and a Thermal Gradient Block to enable sample incubations down to -20°C .

Our rationale was that, given the whole genome sequence for strain 34H, we could make more informed choices for our experiments, while colleagues using recombinant gene technology (also benefiting from the whole-genome sequence) could provide us with test enzymes beyond what we have available in the laboratory. We expected to be able to identify some practical means to stabilize, enhance or (if desired) prevent enzyme activity in the cold and thus better inform solutions to various societal needs; e.g., cold-weather bioremediation of unwanted organic matter and effective frozen storage and shipment of sensitive biomedical and aquacultural materials.

Among our scientific accomplishments, we confirmed that proteases are produced by strain 34H down to -20°C , that exopolymers also produced by 34H stabilize the proteases, and that larger exopolymer compounds (large and more complex sugars) make better enzyme stabilizers than conventional small sugars like glycerol. We also found that complex exopolymers from 34H make superior cryoprotectants for cell storage at -80°C than do conventional compounds like glycerol. The over-production of exopolymers and, in some cases, biofilm formation was stimulated by physiological stress due to subzero temperature, elevated hydrostatic pressure and nutrient deprivation (but not salt). We discovered that viruses infect 34H at subzero temperature, implying the existence of cold-active viral enzymes that penetrate the host's cellular coating of exopolymers produced under stress. Among our educational accomplishments, graduate students Colleen Evans Kellogg and Eric Collins were partially supported to obtain Masters degrees; Llyd Wells, his PhD degree; postdoctoral associate Joe Marx, a new position; and staff member Shelly Carpenter, improved skills in biochemistry and student training. A prototype "cold finger" apparatus, designed to allow for the chemically benign recovery of marine bacterial products based on selective partitioning into ice, was established in the laboratory by rotation student Zach Adams from the College of Engineering; it is now the basis for thesis work by new student Marcela Ewert Sarmiento.

During this award, those supported or affiliated with it participated in numerous outreach activities, including contributions to the annual Polar Science Weekend at the Pacific Science Center in Seattle, the bi-annual Oceanography Open House on campus, and local K-12 classrooms. Typically, we used freshwater and sea-ice demonstrations to teach the general public and school children how salt, exopolymers and microbes change the physical nature of ice, as well as the concept of cryopreservation. The PI also took advantage of opportunities during fieldwork in the Arctic to be interviewed for a front-page article in the Washington Post and for Canada's widely heard radio show "Quirks

and Quarks” to highlight the biotechnological potential of the exopolymers that microbes produce to survive a cold winter in sea ice.

As a result of this award, we have advanced fundamental understanding of production, activity and interactions between extracellular enzymes and exopolymers of marine bacteria at low temperatures, as evidenced by several important research papers. These include publication of the genome sequence of *Colwellia psychrerythraea* strain 34H (Méthé et al., 2005; the PI’s role was partially supported by this award), which helped to initiate the study of cold-active products by marine bacteria from a genomics and proteomics perspective. Publication of viral infections of marine bacteria at -12°C (Wells and Deming, 2006) set a record low temperature for such activity, also revealing the existence of potentially novel cold-active enzymes. Work on stress-related exopolymer production led to improved cryoprotectants for cell storage at -80°C (Marx et al., in preparation), which may bring societal benefits if developed further. We have also interacted with the local business ProFISHent, Inc., interested in cryoprotectants for aquacultural products (<http://www.profishent.com/associates.htm>) and generated software to assist in gene-based analyses of marine bacteria (Collins and Rocap, 2007) available online at <http://staff.washington.edu/rec3141/repk>.