Physiological diversity of an attached marine bacterial population
by Ace Melton Baty III

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Microbiology
Montana State University

Abstract:
This research characterizes chitinolytic gene expression and the partitioning of bacterial biomass between the surface and bulk aqueous phase on a spatial and temporal scale, at the single cell level during chitin degradation. The relationship between chitinolytic gene expression and extracellular chitinase activity was evaluated in individual cells of the marine bacterium Pseudoalteromonas sp. strain S91 attached to solid chitin. Green Fluorescent Protein (GFP) reporter genes under the control of the chiA/chiB and chiA promoters were used to evaluate chitinase gene expression. Furthermore, this work describes the synthesis of a new ELF®-97 enzyme substrate for chitinase activity that permitted the identification of single cells participating in the production of extracellular enzyme during chitin degradation.

Upon initial attachment to a pure chitin surface, the chiA/chiB genes of the marine bacterium, Pseudoalteromonas sp. strain S91, became highly up-expressed in discrete clusters that represented less than 20% of the total colonized surface area. During bacterial reproduction, 96.8% of the total bacterial production (BP) generated on the surface of pure chitin detached to form free living bacterial biomass. The resulting high detachment rates suggest detachment may play a major role in the dispersion and survival strategies of marine bacteria. Furthermore, 96% of the bacterial biomass that detached to form the free living population were completely down-expressed for chiA/chiB gene activity, while the highly up-expressed subpopulation remained associated with the chitin surface.

Evaluation of chiA expression and ELF®-97 crystal location at the single cell level revealed two physiologically distinct subpopulations of S91 on the chitin surface: one that was chitinase-active and remained associated with the surface and another that was chitinase-inactive and released daughter cells into the bulk aqueous phase. Following detachment from the chitin surface, cells appeared to enter a starvation survival mode of existence. The ability of a population to coordinate chitinase activity, cell reproduction and surface detachment among different cells on a surface may enhance their ability to locate new sources of nutrients in the pelagic marine environment.

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MONTANA STATE UNIVERSITY-BOZEMAN
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APPROVAL

of a thesis submitted by

Ace Melton Baty III

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Gill Geesey — (Signature) — Dec 20, 1999 — Date

Approved for the Department of Microbiology

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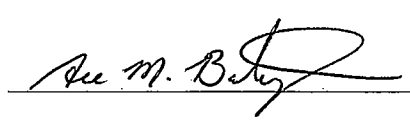
Signature 

Date Dec. 20, 1999

This work is dedicated to Kristi, Ellie, and Madigan

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