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Chitobiase and Chitinase from *Vibrio harveyi*

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biology

by

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ABSTRACT OF THE DISERTATION

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The *Vibrio harveyi N,N'*-diacetylchitobiase (chitobiase) and chitinase genes were cloned in *E. coli*. Chitobiase activity was found to be strongly induced by chitobiose in *V. harveyi*, whereas, when cloned in *E. coli*, chitobiase gene (*chb*) expression was constitutive. Chitobiase was localized to the outer membrane in *E. coli* clones harboring the *chb* gene, and it was exported with concomitant removal of a signal peptide. Maturation of chitobiase may follow a pathway similar to that of the major outer membrane lipoprotein (Lpp) of *E. coli*. A region six amino acids in length surrounding the Lpp processing site was found to be identical to a corresponding region of chitobiase, and processing of chitobiase is inhibited by the signal peptidase II specific inhibitor, globomycin. A protein purification scheme was developed where chitobiase was obtained in up to 30% yield by detergent removal from the surface of *E. coli* cells containing the *chb* gene, followed by a HPLC purification step using ion-exchange chromatography. The purified protein had a specific activity of 104 U/mg and an

apparent M_r of 92,000 deduced by SDS-PAGE. Transcriptional analysis of the *chb* gene promoter revealed three mRNA start sites in *E. coli* harboring the *chb* gene, two of these are also used by *V. harveyi*. An amino acid sequence comparison is made between chitobiase and the human hexosaminidase A. Two chitinase genes from *V. harveyi* were cloned, and the nucleotide sequence of one of these genes (*chiA*) is described. Chitinase expressed from a truncated (missing ~580 nucleotides at the 3' end) version of the *chiA* gene was excreted from *E. coli* cells. A comparison is made between the deduced amino acid sequences of two *S. marcescens* chitinases and *V. harveyi* chitinase.