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NOPPAKUN MOOLSIN: CLONING AND SEQUENCE ANALYSIS OF A PUTATIVE GENE RELATED TO PROTEASE SECRETION IN MARINE BACTERIUM *PSEUDOALTEROMONAS* SP.STRAIN S91 THESIS ADVISORS: SAKOL PANYIM, Ph.D., SOMCHAI PONGPATTANAKITSHOTE, Ph.D. 126 p. ISBN 974-663-649-9

Pseudoalteromonas sp. strain S91 is a Gram-negative marine bacterium which is able to survive in an oligotrophic environment. Extracellular enzyme secretion is an important mechanism that marine bacteria such as S91 use to survive in a limited nutrient habitat. Research into the area of extracellular enzyme secretion is scanty especially in non-pathogenic bacteria. In this study S91 mutant strains, P2.6 and P4.15, which were defective in protease activity as a result of mini-Tn*10:lac:kan* insertion were cloned and sequenced. One recombinant plasmid derived from P4.15 was designated pMO1 and the other one derived from P2.6 was designated pMO2. These two clones were verified by restriction endonuclease analysis. pMO1 and pMO2 contained a 15.7 and 8.8 kb of insert of genomic DNA. Since both recombinant clones from plasmid vector did not contain the full–length of putative gene, to obtain a complete gene, plaque lift hybridization of S91 wild type library was conducted. The DNA probe of approximately 400 bp from in a recombinant clone was generated using DIG-dUTP incorporated during PCR reaction. A positive plaque was excised from Lambda phage by coinfecting with Exassist helper phage. The entire nucleotide sequences flanking the transposon were combined and analyzed. The nucleotide sequence of the putative gene interrupted by mini-Tn*10:lac:kan* contained an open reading frame of 546 bp and was deduced to 183 amino acid residues. Comparison of DNA and amino acid sequences revealed that the putative gene was homologous to FimH subunit of type I pili. Complementation of the protease reduced strains demonstrated that no restoration of a protease activity was observed in both P4.15 and P2.6. Likewise, no distinct difference in pattern of protein band was detected when supernatant and cell lysates of both mutants and wild type were separated on a SDS-PAGE gel.

In conclusion, the putative genes involved in protease secretion from S91mutants were successfully cloned and sequenced. These findings suggest ORF2 may function as a molecular chaperone playing an important role in the secretion of exoprotease.