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KEY WORDS : DENGUE VIRUS SEROTYPE 2 / NS2B-NS3 PROTEIN / POLYHISTIDINE AFFINITY TAG

RABUESAK KHUMTHONG : EXPRESSION AND PURIFICATION OF THE PROTEASE COMPLEX NS2B-NS3 FROM THE DENGUE VIRUS TYPE 2; GERD KATZENMEIER, Ph.D., CHANAN ANGSUTHANASOMBAT, Ph.D., CHARTCHAI KRITTANAI, Ph.D., 108 p. ISBN 974-662-711-2

Dengue virus is a major causative agent of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The positive polarity RNA genome encoded a polyprotein precursor of 3391 amino acids which requires proteolytic processing to the individual proteins by host and viral proteases. A two-component protease, the NS2B-NS3 protein, is responsible for cleavage at several dibasic sites in the polyprotein.

In this study, the gene fragment for the full length NS2B-NS3 protein from dengue virus serotype 2 strain 16681 was cloned in pTrcHis expression vector. The recombinant plasmid pTHNS2B3 encoding the two-component protease fused N-terminally with the PolyHis tag was transformed into different strains of *Escherichia coli*. Being expressed under IPTG-inducible control of the pTrc promoter, the 88 kDa fusion protein was produced in the form of inclusion bodies and cross-reacted with Ni-NTA conjugated to alkaline phosphatase in western blot analysis. Expression of the fusion product in *ompT* protease deficient strains *i.e.* BL21 and C41 gave relatively higher expression levels when compared to the JM109 strain. The NS2B-NS3 fusion protein could be partially purified by immobilized metal affinity chromatography and was successfully renatured on-column after purification step to 75 % homogeneity. Yield obtained of the renatured NS2B-NS3 fusion protein was 1 mg/liter of culture as determined by Bradford's assay. However, the PolyHis affinity tag could not be removed by enterokinase enzyme under the conditions used. Autocleavage of the NS2B-NS3 fusion protein at the NS2B-NS3 junction or within the helicase domain could not be detected.