

Jiruthakorn Thiantanunkul 2011: Cloning and Expression of Envelope Protein E2 of Chikungunya Virus for Application of Diagnosis. Master of Science (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Captain Chaivat Kittigul, M.Sc. 93 pages.

Chikungunya is a viral disease which is a major impact on public health in many countries. Envelope protein E2 of Chikungunya virus (CHIKV) is shown to be a predominant epitope for stimulating immune responses. In this study, the amplified E2 gene of virus was cloned into an expression vector pET21a to construct the recombinant plasmid, pET21a-CHK-E2. Nucleotide and its deduced amino acid sequences of the recombinant plasmid were found to be 99% identity to those of Chikungunya virus Ross strain retrieved from GenBank AF490259.2. To produce a recombinant E2 protein, the recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) pLysS, and the transformed *E. coli* strain was induced with 0.5 mM IPTG at 20°C. After 3 hours of induction, a 46-kDa recombinant protein was detected as an inclusion body. After protein solubilization using 0.5% laurylsarcosine, the solubilized recombinant protein was characterized by western blot analysis using CHIKV infection patients' serum. The western blot result showed a specific binding of recombinant E2 protein to antibody against E2. The dot blot analysis showed recombinant protein E2 did not bind to antibody from normal serum. Both two results suggesting that the recombinant E2 protein is possibly used in serological diagnostic methods such as ELISA, immunochromatography (IC). In this study we used recombinant E2 as a raw material to pre-study for developing diagnostic test kit of Chikungunya disease as IC. The IC showed recombinant protein E2 reacted to CHIKV infection patients' serum, and recombinant protein E2 did not react to normal serum. All of results showed the potential of recombinant protein E2 for using as a raw material for developing diagnostic test kit.

Student's signature

Thesis Advisor's signature