Thesis Title	Molecular Cloning and Expression of the NS4 and NS5 Genes From the Thai Isolates of Hepatitis C Virus
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ABSTRACT

Hepatitis C virus (HCV) is the major causative agent of posttransfusion non-A, non-B hepatitis. HCV genome consists of 9,500 nucleotides encoding a single open reading frame. The viral genome is coded for structural proteins (core and envelopes) and non-structural protein which are involved in viral replication. Substantial nucleotide diversity of HCV was observed throughout the genome. This led to the classification of HCV into genotype based on nucleotide sequence homology. Reports from previous studies showed that HCV isolated from Thailand can be categorised into genotypes 3a, 1b, 1a, and 6 group variants. By using the recombinant DNA approach, the first generation serological assay for detecting anti-HCV antibody was developed using NS4 protein as antigen. The second and third generation assays were developed by including structural protein (nucleocapsid) and other non-structural proteins (NS3 and NS5) in order to increase the sensitivity and specificity of test. Furthermore, recent data demonstrated that genotype-specific immunodominant epitopes specific for each genotype were located in NS4 region

The objectives of this study were to sequence and clone the non-structural regions 4 and 5 (NS4 and NS5) of the HCV genotypes commonly found in Thailand and to express the recombinant proteins containing NS4 and NS5 proteins.

In this study, the NS4 and NS5 genes of the Thai isolates of HCV were isolated from anti-HCV-positive-blood donors. Both the NS4 and NS5 genes were sequenced, analysed and compared with other sequences deposited in GenBank database. Nucleotide sequences analysis showed 74.9% and 60.4% homology in the NS4 and NS5 region, respectively, between the Thai isolates and other isolates.

The fragment of NS4 gene of about 600 bp of HCV genotype 3a, 1a, and 6 group variants were cloned into pGEX-3X expression vector. Transformed bacteria did not express the expected proteins even optimising the expression conditions. Cloning into pGEX-4T-1 and pPRO-EX-1, as well as truncation at 3'-end, also failed to express NS4 protein. The correct in-frame cloning was validated by nucleotide sequencing. The mechanism of the inability to express NS4 protein is still unknown

The fragment of NS5 gene of HCV genotype 3a was cloned into pGEX-3X expression vector. The recombinant protein can be expressed as a fusion protein with glutathione S transferase (GST). The GST-NS5 fusion protein was purified using glutathione affinity giving a yield of approximately 26.3 μg/ml of bacterial culture. The GST-NS5 fusion protein was immunogenic as detected by immunoblot assay and can specifically reacted with HCV-infected human plasma.

The information obtained from this study and the recombinant plasmid containing HCV NS5 gene of the Thai isolate will be valuable for the development of screening assays for detecting antibodies to HCV in Thailand.