Expression of prM/ E Protein of Dengue Virus Serotype 3 in *E. Coli* as a Sub Unit Recombinant Vaccine Candidate

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Dengue virus (DV) infection is an endemic disease in tropical and subtropical countries especially Indonesia. Secondary infections with different serotype of dengue virus may cause severe clinical manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Vaccine development is the promising approach for DV infection prevention. Challenge associated with development of tetravalent subunit vaccines led many researches to improve recombinant vaccines for overcoming this disease. Pre membrane and envelope genes (prM/ E) have been reported as an immunogenic antigen. Several studies revealed that prM/E could give higher immune responses than either structural or non structural proteins. Development of subunit vaccines base on Indonesian local circulating DV is challenging. In this study, prM/ E genes of DENV serotype 3 will be cloned into pET-32b and transforms into E. Coli DH5α then expressed in E. Coli BL21. Western Blot method will be used to detect prM/ E protein and predict epitomes to B cell and T cell using B Cell Epitome Prediction Tools and Genetyx.

Keywords: dengue virus, serotype 3, prM/ E, recombinant

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Introduction

Dengue virus (DENV), transmitted by *Aedes aegepty*, is a virus that cause broad spectrum clinical manifestations of dengue virus infection in Indonesia. According to Suwandono et. al. (2006), Indonesia is a one of dengue virus infection endemic country in the world. Ministry of Health of Indonesia reported that, there are 158.912 dengue infection cases in 2009.

DENV, a member of family Flaviviridae, is a single strand RNA which has approximately 11 kb genome. DENV genome contains two untranslated regions (UTRs) and encode three structural genes: capsid (C), precussor membrane (prM) and envelope (E) and seven non-strucutral

genes: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Mosiman et. al., 2009). DENV is classified immunologically into four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Lanciotti et. al. (1994) reported that DENV-3 is the most common causative agent of dengue virus infection in Indonesia .

Dengue virus can cause dengue fever (DF) while secondary infection with different serotype may cause severe manifestations such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). DF produces non-specific symptoms while DHF is a more serious illness, with symptoms including severe fever, liver enlargement, plasma leakage, haemorrhage and thrombocytopenia. DHF can progress to potentially lethal DSS if plasma leakage is profound (Herrero *et. al*., 2013). The main virulence factors of DENV are viral protein, virion structure, virus genotype and serotype virus . To date, there are no vaccine or antiviral registered yet to prevent and treat this disease. Further characterization of dengue virus is very important for development of anti-viral compound or vaccine.

An E protein, viral surface protein, contains epitopes and prM protein is essential protein for expression process of E. Both genes are used to be a target antigen because they produce protective imune responses. Guirakhoo et. al. (2001) reported that YF 17D virus express successfully DENV-2 prM/E protein which has imunogenicity and protective ability. In addition, Lua (2013) also reported that DNA vaccine expressing prM/E induce anti-DENV-2 antibody titer.

We report expression of Indonesian local DENV-3 derived prM/ E protein and prediction of epitopes for B cell and T cell using *B Cell Epitope Prediction Tools* and *Genetyx* sofware.

2. Materials and methods

2.1. Dengue virus serotype 3 (DENV-3)

Local isolate of dengue virus serotype 3 generously provided by Institute of Tropical Disease Airlangga University Surabaya was used in this study. Virus RNA was extracted from cell culture supernatant using the high pure viral nucleic acid kit (Roche, Germany) according to the manufacturer's instructions. RT-PCR was performed to confirm DENV serotype with DENV-3 primer. A spesific primer set for prM/E was used to amplify prM/E gene. Agarose gel electrophoresis and DNA sequencing were employed to confirm prM/E gene of the DENV-3.

2.2. Preparation of competent cells

The competent cells, E. coli DH5α and BL21, were developed by using TSS

(Transformation and Storage Solution) method (Sambrook and Russel, 2001). Composition of TSS solution are 85% LB medium, 10% PEG (wt/vol, BM 8000), 5% DMSO (vol/vol), dan 50 mM $MgCl_2$ (pH 6,5). Cell cultures were incubated overnight with LB medium (Luria Berthani) at 37° C, 200 rpm. After incubation, each cell cultures were incubated again at 37° C, 200 rpm for 3 hours until OD_{600} 0,5-0,6. Then cell cultures were taken about 1-1,5 ml and centrifuged at 8,000 rpm for 30 seconds. Supernatants were removed then pellet were resuspended with 100µl and stored at 4° C.

2.3. Cloning and expression of DENV-3 prM/E protein

The PCR product, prM/E gene, was purified using Ron's gel extraction kit. It was cloned into pET 32b vector (Novagen) using flanking EcoRV and BamHI restriction sites, sequenced, and then transformed in DH5α. Ligation was performed using method described by Sambrook and Russel (2001), in brief, mix vector and target gene (ratio 1:3). Transformation was performed using heat shock method. In brief, ligation product was incubated in ice for 5 minutes then put into waterbath at 42° C for 90 seconds. After that, it was incubated again in ice for 5 minutes. Transformation product was added with 1 ml SOC liquid media then incubated in shaker incubator at 37° C for 60 minutes.. Sample was centrifuged at 8000 rpm for 30seconds. Supernatant was removed and then platted on LB agar medium that contains amphicylin (50 µg/ml) then incubated overnight at 37° C. Plasmids were extracted from positive cell clones using Plasmid DNA Purification kit (Geneaid Biotech Ltd) then comfirmed using PCR method and sequencing . Sequencing result was used to predict the persence of candidate epitope for T cell and B cell. Plasmids were transformed into E. coli BL21 for expressing prM/E protein.

2.4. Induction of protein and purification

Positive clone cells were incubated in LB liquid medium overnight that contains antibiotic at 37° C. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added for inducing prM/E protein.

2.5. SDS-PAGE and Western Blot

Sodium dedocyl sulfate-polyacrylamide gel electroforesis was used to confirm prM/E based on moleculer weight and continued with Western blot to confirm the prM/E protein using antibody anti-his.

2.6. Data analysis

Sequencing result was analyzed by BLAST

(http://blast.ncbi.nlm.nih.gov/Blast.cgi). Epitope predictions of T cell was performed using software *Genetyx* based on IAd and IEd pattern (Sette et. al., 1989) and Rothbard/Taylor pattern (Rothbard and Taylor, 1988). Predicted epitope of B cell were analyzed using *B cell epitope prediction tools* from IEDB (http://tools.iedb.org/main/html/bcell_tools.html).

3. Results

Primer was designed to amplify the full gen of prM/E encoded genome of DENV-3. However the primer did not suceed to amplify the gene, since the band showed sorter (480 bp) comparing to the expected size (1987 bp). (Figure 1).

Fig 1. Band showed in 450 bp

4. Discussion

Optimizing PCR to get prM/E segment was not succeed with first primer. It is likely because primer was not appropriate with Indonesian local isolate.

We have designed new primers. Now, we are optimimizing primers for getting a prM/ E segment.

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