

INTISARI

EKSPRESI DAN PURIFIKASI PROTEIN *RECOMBINANT ENVELOPE* (rE) VIRUS DENGUE PADA *Escherichia coli* BL21(DE3)

Demam Berdarah Dengue (DBD) yang disebabkan oleh Virus Dengue telah menjadi kasus endemi di negara-negara dengan iklim tropis dan subtropis, sehingga dikategorikan menjadi sebuah tantangan global. Salah satu pendekatan biologi molekuler untuk pencegahan DBD adalah dengan vaksinasi. Pendekatan vaksin berbasis protein rekombinan dapat dilakukan dengan menggunakan protein *Envelope* (E) dari Virus Dengue, karena protein tersebut berperan penting dalam pengikatan dengan reseptor inang, sehingga menjadi target yang ideal sebagai kandidat vaksin. Dalam penelitian ini, sekuens gen *Recombinant Envelope* (rE) yang mengkode protein *Recombinant Envelope* (rE) yang telah dilakukan optimasi, selanjutnya dilakukan uji karakteristik dan struktur pemodelan protein secara *in silico*. Hasil yang didapatkan menunjukkan bahwa protein rE memiliki nilai indeks ketidakstabilan, indeks alifatik, dan titik isoelektrik yang masing-masing bernilai 32,14; 75,08; dan 7,17. Hasil analisis plot Ramachandran protein rE menunjukkan sebaran residu asam amino bernilai 95,4% dan 4,7% masing-masing di *allowed region* dan *disallowed region*. Dengan demikian, hasil uji secara *in silico* membuktikan bahwa prediksi pemodelan protein rE dinilai akurat. Gen pengkode protein rE kemudian diinsersikan ke dalam plasmid vektor pET-15b untuk selanjutnya diekspresikan melalui sistem inang ekspresi *Escherichia coli* BL21(DE3). Koloni *E. coli* yang positif membawa gen *rE* diinduksi dengan 1 mM IPTG. Hasil ekspresi dianalisis menggunakan SDS-PAGE, dan dipurifikasi menggunakan kolom Ni-NTA, dilanjutkan dengan analisis SDS-PAGE dan *western blot*. Hasil penelitian menunjukkan keberhasilan insersi plasmid rekombinan pET-15b-rE pada *E. coli* BL21(DE3). Protein rE yang berukuran 50,68 kDa dapat diekspresikan di *E. coli* BL21(DE3), dibuktikan dengan hasil analisis SDS-PAGE yang menunjukkan adanya pita di rentang 50–60 kDa. Protein rE berhasil dipurifikasi menggunakan kolom Ni-NTA. Konsentrasi hasil purifikasi bernilai 129 mg/L. Berdasarkan hasil penelitian, disimpulkan bahwa gen *rE* Virus Dengue keempat serotipe dapat dikloning dan diekspresikan proteinnya pada *E. coli* BL21(DE3).

Kata Kunci: Virus Dengue, Envelope Dengue, Vaksin DBD, *E. coli* BL21(DE3)

ABSTRACT

EXPRESSION AND PURIFICATION OF RECOMBINANT ENVELOPE PROTEIN (rE) OF DENGUE VIRUS IN *Escherichia coli* BL21(DE3)

Dengue Hemorrhagic Fever (DHF) caused by the Dengue Virus has become an endemic problem in countries with tropical and subtropical climates, thus categorizing it as a global challenge. One molecular biology approach for DHF prevention is through vaccination. A protein-based recombinant vaccine approach can be employed by utilizing the Envelope protein (E) of the Dengue Virus, as this protein plays a crucial role in binding to host receptors, making it an ideal target as a vaccine candidate. This study optimized the gene sequence of Recombinant Envelope (*rE*) coding for the Recombinant Envelope protein (rE), followed by *in silico* testing of protein characteristics and structure modeling. The obtained results revealed that the rE protein exhibited instability index, aliphatic index, and isoelectric point values of 32.14, 75.08, and 7.17, respectively. The Ramachandran plot analysis indicated that 95.4% of amino acid residues were within the allowed region, while 4.7% were within the disallowed region, demonstrating the accuracy of the *in silico* protein modeling for rE. Consequently, the *in silico* testing results demonstrated that the rE protein possessed a stable and high-quality structure. The *rE* gene was then inserted into the pET-15b vector plasmid for subsequent expression using the *Escherichia coli* BL21(DE3) expression host system. Positive *E. coli* colonies carrying the *rE* gene were induced with 1 mM IPTG. The expression results were analyzed using SDS-PAGE, followed by purification using a Ni-NTA column, and further analyzed by SDS-PAGE and western blot. The research findings demonstrated the successful insertion of the recombinant pET-15b-rE plasmid into *E. coli* BL21(DE3). The rE protein, with a size of 50.68 kDa, was successfully expressed in *E. coli* BL21(DE3), as evidenced by the SDS-PAGE analysis showing a band within the 50-60 kDa range. The rE protein was purified using the Ni-NTA column, with a 129 mg/L purification yield. In conclusion, this study successfully achieved the expression and purification of the recombinant Envelope protein (rE) of Dengue Virus in *Escherichia coli* BL21(DE3).

Keywords: Dengue Virus, Dengue Envelope, Dengue Vaccine, *E. coli* BL21(DE3)