KLONING DAN EKSPRESI GEN HOMOLOG ORF 007L *Infectious Spleen and Kidney Necrosis Virus* SERTA PURIFIKASI PROTEINNYA SEBAGAI KANDIDAT VAKSIN

INTISARI

Protein envelope yang disandi oleh ORF 007L *Infectious Spleen and Kidney Necrosis Virus* telah diketahui bersifat imunogenik. Tujuan dari penelitian ini adalah mengkloning gen, mengetahui potensi protein sebagai kandidat vaksin berdasarkan prediksi epitop, mengekspresikan gen serta mempurifikasi protein homolog ORF 007L. Penelitian ini menggunakan sampel metagenom Ikan Kerapu terinfeksi ISKNV yang berasal dari Keramba Jaring Apung di Wilayah Bali, Indonesia. Primer didesain untuk mengamplifikasi gen homolog ORF007L ISKNV. Kloning dan ekspresi gen menggunakan vektor pET-32a. Prediksi epitop sel B dan T dilakukan secara *in silico*. Selanjutnya protein yang telah diproduksi dipurifikasi menggunakan kromatografi afinitas Ni-NTA. Hasil menunjukkan bahwa kloning berhasil dilakukan. Sekuen gen berukuran 1.441 bp menunjukkan similaritas 98% dengan ORF 007L ISKNV (Acc. No: AF371960.1). Analis *in silico* menunjukkan sekuen asam amino memiliki daerah epitop yang dikenali oleh sel B dan sel T dan memiliki konservasi tinggi pada genus *Megalocytirus*. Protein homolog ORF 007L ISKNV berhasil diekspresikan dengan induksi *Isopropyl β-D-1-thiogalactopyranoside* (IPTG). Protein rekombinan berhasil dipurifikasi dengan Ni-NTA agarose. Berdasarkan hasil penelitian ini dapat disimpulkan bahwa protein homolog ORF 007L ISKNV berpotensi untuk dikembangkan sebagai kandidat vaksin.

Kata kunci: *Megalocytivirus*, homolog ORF 007L ISKNV, kloning, ekspresi protein, purifikasi

CLONING AND EXPRESSION OF HOMOLOGOUS GENE OF ORF 007L *Infectious Spleen and Kidney Necrosis Virus* AND ITS PROTEIN PURIFICATION AS A VACCINE CANDIDATE

ABSTRACT

Envelope protein which was encoded by *Infectious Spleen and Kidney Necrosis Virus* ORF 007L has immunogenic reaction. The aims of this study were to clone a gene of ORF 007L homologous, to determined potential of protein of ORF 007L homolous as a vaccine candidate which is based on epitope prediction, to express it's gene and to purify it's protein. This study used Grouper infected ISKNV metagenom sample from cage culture at Bali, Indonesia. Primer was designed to amplify the homologous ORF007L ISKNV gene. Cloning and gene expression used pET-32a vector. Prediction of B and T cell epitopes performed by in silico method. Furthermore, the produced protein was purified by Ni-NTA affinity chromatography. The results showed that ORF 007 homologous gene was succesfully cloned. It has 1,441 bp and showed 98% similarity with the ORF 007L ISKNV (Acc. No: AF371960.1). In silico analysis showed that the amino acid sequence had an epitopes region which was recognized by B cells and T cells and highly conserved in Megalocytivirus genus. Homologous proteins of ORF 007L ISKNV was successfully expressed with induction of isopropyl β-D-1-thiogalactopyranoside (IPTG). Recombinant proteins was successfully purified by Ni-NTA agarose. Based on these results, it were concluded that the homologous protein of ISKNV ORF 007L was potential to be developed as a vaccine candidate.

Keyword : Megalocytivirus, homologous of ORF 007L ISKNV, cloning, protein expression, purification