INTISARI

Luciferase-like monooxygenase (LLM) merupakan enzim yang tergolong dalam kelompok Flavin-dependen monooksigenase. Kelompok enzim ini secara umum memiliki kemampuan dalam mengkatalisis berbagai macam reaksi oksigenasi dan memiliki peranan penting dalam biosintesis berbagai senyawa organik. Analisis *in silico* menunjukkan bahwa genom *Priestia megaterium* DSM319 diketahui memiliki lima (5) *open reading frame* (ORF) LLM. Terdapat empat (4) ORF yang berhasil diamplifikasi hingga saat ini, tiga (3) diantaranya telah berhasil dikloning dan diekspresikan. Penelitian ini dilakukan dengan tujuan untuk mendapatkan ORF LLM tipe 5 dari genom *Priestia megaterium* PSA 14 kemudian dikloningkan pada plasmid pET28a(+) serta diekspresikan dalam *Escherichia coli* BL21 (DE3). Isolasi ORF dilakukan menggunakan amplifikasi PCR, ORF lalu disisipkan ke dalam plasmid pET28a(+). Plasmid rekombinan kemudian ditransformasikan ke dalam *Escherichia coli* BL21(DE3) dan diamati hasil ekspresinya menggunakan SDS-PAGE. Hasil amplifikasi menunjukkan ORF LLM tipe 5 memiliki ukuran ±957 bp dan memiliki persentase similaritas sebesar 98.06% dengan LLM *class flavin dependent oxidoreductase* dari *Priestia megaterium.* Analisis ekspresi menunjukkan bahwa ORF LLM tipe 5 berhasil diekspresikan dan menghasilkan rekombinan LLM 5 dengan berat molekul ±35,8 kDa.

Kata kunci : Ekspresi, Kloning, *Luciferase-like monooxygenase* tipe 5, *Priestia megaterium* PSA14

ABSTRACT

Luciferase-like monooxygenase (LLM) is an enzyme that belongs to the class of Flavin-dependent monooxygenase. This group of enzymes in common has the ability to catalyze various oxygenation reactions and has an important role in the biosynthesis of various organic compounds. In silico analysis indicated that *Priestia megaterium* DSM319 genome had five types of LLM open reading frames. There are four ORFs that have been successfully amplified, and three of them have been successfully cloned and expressed. The objectives of this work were to obtain an open reading frame of type 5 of LLM from *Priestia megaterium* PSA 14 genome, cloned it into the pET28a(+) vector and expressed it in *Escherichia coli* BL21 (DE3). The open reading frame of type 5 of LLM was amplified by PCR technique and then cloned it into the pET28a(+) vector. The resultant plasmid was used to transform *Escherichia coli BL21*(DE3). SDS-PAGE was employed to analyse the recombinant proteins. The PCR product indicated that the ORF of type 5 of LLM had ±957 bp in size and showed ±98.06% similarity to common LLM class flavin dependent oxidoreductase from *Priestia megaterium*. Through this work, the open reading frame of type 5 of LLM was successfully cloned into the pET28a(+) and expressed in *Escherichia coli BL21*(DE3). The recombinant LLM5 exhibited molecular weight of 35.8 kDa in 12% SDS-PAGE analysis.

Keywords : Expression, cloning, *Luciferase-like monooxygenase* type 5, *Priestia megaterium* PSA14