

**ISOLASI, KLONING DAN EKSPRESI OPEN READING FRAME LUCIFERASE-LIKE MONOOXYGENASE ENZYME TIPE 2 DARI Priestia megaterium PSA14**

Abidah Tauchid

17/414717/PN/15298

Luciferase-like monooxygenase (LLM) memiliki kemampuan untuk mengkatalisis reaksi oksidasi Baeyer-Villiger khususnya dalam proses biosintesis senyawa organik. Analisis in silico, menunjukkan bahwa *Priestia megaterium* PSA 14 memiliki empat (4) open reading frame LLM. Penelitian ini bertujuan untuk mendapatkan open reading frame LLM tipe 2 *Priestia megaterium* PSA 14 dan klon tersebut pada vektor pET28a(+) serta mendapatkan protein rekombinan LLM tipe 2 *Priestia megaterium* PSA 14 pada bakteri *Escherichia coli* BL21 (DE3). Open reading frame LLM tipe 2 diamplifikasi dengan metode PCR kemudian dikloning dengan menyisipkan open reading frame kedalam vektor pET28a(+), kemudian vektor pembawa open reading frame ditransformasikan kedalam *Escherichia coli* BL21(DE3) serta diekspresikan menggunakan 12% SDS-PAGE. Hasil penelitian menunjukkan bahwa hasil amplifikasi open reading frame memiliki persentase similaritas sebesar 96.88% terhadap LLM class flavin dependent oxidoreductase *Priestia megaterium*. Open reading frame berhasil dikloning kedalam vektor pET28a(+) dan menunjukkan persentase similaritas sebesar 99.70% dengan MsnO8 family LLM class flavin-dependent oxidoreductase *Priestia megaterium*. Rekombinan LLM tipe 2 berhasil diekspresikan dalam system *Escherichia coli* BL21(DE3). Berat molekul protein rekombinan LLM tipe 2 sebesar  $\pm 43,95$  kDa bila dianalisis dengan 12% SDS-PAGE.

Kata kunci : LLM tipe 2, *Priestia megaterium*, kloning, ekspresi, protein rekombinan

**ISOLATION, CLONING AND EXPRESSION OPEN READING FRAME LUCIFERASE-LIKE MONOOXYGENASE ENZYME TYPE 2 FROM *Priestia megaterium* PSA14**

Abidah Tauchid

17/414717/PN/15298

Luciferase-like monooxygenase (LLM) is an enzyme that has capability to catalyze the Baeyer-Villiger oxidation reaction, in organic compounds biosynthesis. In silico studies showed that the *Priestia megaterium* genome contains four open reading frames of LLM. Therefore, the objectives of this work were to obtain an open reading frame type 2 LLM from *Priestia megaterium* PSA 14 genome and that clone into the pET28a(+) vector and to obtain protein recombinant type 2 LLM from *Priestia megaterium* PSA 14 genome in *Escherichia coli* BL21 (DE3). The open reading frame of type 2 LLM was amplified by using PCR and then cloned it into the pET28a(+) vector. The resultant plasmid was then used to transform *Escherichia coli* BL21(DE3). The expression of recombinant protein of type 2 LLM was examined by using 12% SDS-PAGE. The results showed that the open reading frame of type 2 LLM from *Priestia megaterium* PSA 14 was successfully amplified and showed percent similarity of 96.88% to LLM class flavin dependent oxidoreductase *Priestia megaterium*. Open reading frame was also successfully cloned into pET28a(+) vector and showed percent similarity of 99.70% to MsnO8 family LLM class flavin-dependent oxidoreductase *Priestia megaterium* and expressed in *Escherichia coli* BL21(DE3) system. The molecular weight of the recombinant type 2 LLM exhibited molecular weight of  $\pm 43.95$  kDa, when it was analyzed by 12% SDS-PAGE.

**Keywords :** LLM type 2, *Priestia megaterium*, isolation, cloning, expression, recombinant protein