INTISARI

KLONING DAN EKSPRESI *OPEN READING FRAME* (ORF)
ACETOACETYL-COA REDUCTASE DARI *Priestia megaterium* PSA14

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Polihidroksialkanoat (PHA) merupakan biopoliester yang berpotensi sebagai bahan pembuatan plastik ramah lingkungan. PHA terdiri dari berbagai *hidroksialkanoat* (HA) yang diproduksi oleh mikroorganisme dibawah kondisi substrat (P,Fe,Mg,N) yang terbatas dan sumber karbon yang berlebih. Proses biosintesis PHA melibatkan tiga komponen enzim utama, yaitu PhaA, PhaB, dan PhaC. Diantara ketiga enzim tersebut, *acetoacetyl-CoA reductase* (PhaB) merupakan enzim yang dapat mereduksi gugus 3-ketone asetoasetil-CoA untuk mensintesis (R)-3-*hydroxybutyryl-CoA* dengan oksidasi NADPH sebagai kofaktor. Tujuan dari penelitian ini adalah untuk mendapatkan klon *open reading frame pha*B dari bakteri *Priestia megaterium* PSA14 dan mengekspresikan rekombinan PhaB pada bakteri *Escherichia coli* BL21 (DE3). *Open reading frame pha*B diamplifikasi dari genom *Priestia megaterium* PSA 14 kemudian dikloning dengan menyisipkan ORF kedalam vektor pColdIV dan ditransformasikan pada *E.coli* BL21 (DE3) serta diekspresikan pada SDS-PAGE 12%. Hasil penelitian menunjukkan bahwa ORF hasil amplifikasi memiliki identitas sebesar 99.60% terhadap Acetocaetyl-CoA reducatase dari *Priestia megaterium*. Rekombinan PhaB berhasil diekspresikan dalam system *E. coli* BL21(DE3). Rekombinan protein hasil ekspresi memiliki berat molekul ± 26 kDa bila dianalisis pada gel SDS-PAGE 12%.

Kata kunci : *polyhydroxyalkanoate* (PHA), *pha*B, *Priestia megaterium* PSA14, kloning, ekspresi.

ABSTRACT

CLONING AND EXPRESSION OF OPEN READING FRAME (ORF) ACETOACETYL-COA REDUCTASE FROM *Priestia megaterium* PSA14

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Polyhydroxyalkanoates (PHA) are biopolyester that can be used as environmentally friendly plastic materials. PHA consists of various hydroxyalkanoates (HA) which are produced by microorganisms under conditions of substrate (P, Fe, Mg, N) limitation but in the excess of carbon sources. PHA biosynthesis involves three main enzymes, which consist of PhaA, PhaB, and PhaC. Among the three enzymes, PhaB or acetoacetyl CoA reductase has an important role for synthesizing of (R) -3-hydroxybutyryl-CoA in parallel with NADPH oxidation. The objectives of this study were to clone open reading frame of PhaB encoding gene (*pha*B) from *Priestia megaterium* PSA14 and express the recombinant PhaB in *Escherichia coli* BL21 (DE3) system. Open reading frame of PhaB (*pha*B) was amplified from *Priestia megaterium* PSA14 genome by using PCR technique and then cloned it into pColdIV vector. The resultant vector was then used to transform *E.coli* BL21 (DE3). The expression of recombinant acetoacetyl-CoA reductase was carried out by IPTG induction of *E. coli* BL21(DE3) harbouring the vector carrying the orf of *pha*B on 12% SDS-PAGE gel. The results showed that the orf of PhaB from *P. megaterium* PSA14 was successfully amplified and showed percent identity of 99.60% to acetoacetyl-CoA reductase from *Priestia megaterium.* The orf of PhaB was also successfully cloned in pColdIV vector and expressed in *E. coli* BL21(DE3) system. The expression analysis indicated that the recombinant acetoacetyl-CoA reductase had molecular weight of ± 26 kDa when it was analysed on 12% SDS-PAGE gel.

Keywords : polyhydroxyalkanoate (PHA), phaB, *Priestia megaterium* PSA14, cloning, expression.