

INTISARI

Polyhydroxyalkanoate (PHA) merupakan salah satu bahan sangat menjanjikan untuk plastik *biodegradable*. Salah satu family PHA yaitu Poli- β -hidroksibutirat (PHB) disintesis melibatkan tiga enzim utama yaitu *acetyl-CoA acetyltransferase*, *acetyl CoA reductase* dan *phaC* sintase. *Acetyl-CoA acetyltransferase* mengkatalisis dua molekul asetil-CoA menjadi asetoasetil-CoA. Asetoasetil CoA merupakan salah satu precursor penting untuk proses biosintesis polihidroksialkanoat. Tujuan penelitian ini yaitu kloning dan ekspresi *open reading frame* gen *phaA* dari *Priestia megaterium* PSA 14 serta mendapatkan klon dan ekspresi *open reading frame* gen *phaA* yang mengkode *acetyl-CoA acetyltransferase* pada bakteri *E.coli* BL21 (DE3). Amplifikasi ORF *phaA* dilakukan dengan PCR kemudian dilakukan kloning ke dalam plasmid pET28a(+) dengan menggunakan enzim restriksi *NcoI* dan *EcoRI*. Proses penempelan antara ORF gen *phaA* dan plasmid pET28a(+) yang terpotong menggunakan T4 ligase. Rekombinan protein dianalisis menggunakan 12% SDS PAGE. Dari penelitian ini, *orf phaA* berhasil diamplifikasi dan dikloning kedalam sistem pET. Rekombinan protein *Orf phaA* berhasil diekspresikan dan diproduksi dengan berat molekul ± 43 kDa.

Kata kunci: *E.coli* BL21 (DE3), ORF *phaA*, *Priestia megaterium*, dan *acetyl CoA acetyltransferase*.

ABSTRACT

Polyhydroxyalkanoate (PHA) is one of the most promising materials for biodegradable plastics. One of the PHAs families is Poly- β -hydroxybutyrate (PHB) which is synthesized by involving three main enzymes, i.e. acetyl-CoA acetyltransferase, acetyl CoA reductase and phaC synthase. β –ketothiolase catalyzes the formation of acetoacetyl-CoA from two molecules of acetyl-CoA. Acetoacetyl CoA is one of important precursor for polyhydroxyalkanoate biosynthesis process. This study was aimed to clone and overexpress an *open reading frame* of the *phaA* gene (β -ketothiolase encoding gene) from *Priestia megaterium* PSA14. The ORF of *phaA* gene was amplified by PCR then the PCR product was cloned into pET28a(+) following *NcoI* and *EcoRI* double digestion. The ligation process of the ORF of *phaA* and the plasmid was carried out by T4 DNA ligase. The ligation product was then used to transform *E. coli* BL21(DE3). The recombinant protein was examined by 12% SDS-PAGE. From this work, the ORF of *phaA* was successfully amplified and cloned into pET system. The ORF of *phaA* was successfully overexpressed and produce the recombinant protein with molecular weight of ± 43 kDa.

Keywords: *E.coli* BL21 (DE3), ORF *phaA*, *Priestia megaterium*, and *acetyl CoA acetyltransferase*.