

ABSTRACT

ISOLATION, CLONING AND EXPRESSION OF OPEN READING FRAME POLYHYDROXYALKANOATE SYNTHASE ENCODING GENE FROM *Bacillus* sp. PSA10 AND CHARACTERIZATION OF THE PRODUCT BY *IN* *SILICO* ANALYSIS

Listia Pradani

17/419978/PMU/09189

Polyhydroxyalkanoate (PHA) is biopolymer produces by microorganism under nutrient limited condition such as nitrogen and excess of carbon source. PHA is biodegradable so it has the potential to be a material for making bioplastics. PHA biosynthesis involves the multiple enzymes such as PhaC synthase. In order to improve PHA production and quality, it is noteworthy to further study the PhaC synthase enzymes. The objectives of this study were to get the *open reading frame* of *phaC* from *Bacillus* sp. PSA10, get a clone of *open reading frame* *phaC*, express it to *E.coli* BL21 (DE3) and characterize its products by *in silico* analysis. To convince that the *Bacillus* sp. PSA10 produces PHA polymer, the microscopic experiment was performed. Following the PHA polymer production confirmation, the genomic isolation, amplification, cloning and expression of *open reading frame* of *phaC* were carried out. The properties and structure of PhaC synthase were examined by *in silico* analysis. BlastX analysis to the *open reading frame* of *phaC* exhibited that the isolated *open reading frame* encoded the class III of PhaC synthase with 99.44% identity to the class III of PhaC synthase from *Bacillus megaterium*. Overexpression of the *open reading frame* in *Escherichia coli* BL21(DE3) showed that the *open reading frame* could produce the recombinant protein with molecular weight of ± 43.6 kDa, when it was run on 12% SDS-PAGE gel. *In silico* analysis showed that the class III of PhaC synthase from *Bacillus* sp. PSA10 had the physicochemical properties, which was similar to the common class III PhaC synthase. The structure of the class III PhaC synthase from *Bacillus* sp. PSA10 showed that the enzyme had similar structure with the class I PhaC synthase from *Cupriavidus necator*.

Keywords : *Bacillus* sp. PSA10, *in silico* analysis, PHA, PhaC synthase.

INTISARI

ISOLASI, KLONING DAN EKSPRESI *OPEN READING FRAME* POLIHIDROKSIALKANOAT SINTASE DARI *Bacillus* sp. PSA10 DAN KARAKTERISASI PRODUKNYA SECARA *IN SILICO*

Listia Pradani

17/419978/PMU/09189

Polihidroksialkanoat (PHA) merupakan biopolimer yang diproduksi oleh mikroorganisme di bawah kondisi keterbatasan nutrisi seperti nitrogen dan berlebihnya sumber karbon. Sifat PHA yang *biodegradable* membuat PHA berpotensi dijadikan sebagai bahan untuk membuat plastik ramah lingkungan. Biosintesis PHA melibatkan peran beberapa enzim salah satunya polihidroksialkanoat (PhaC) sintase. Produksi dan kualitas PhaC sintase dapat ditingkatkan melalui pengkajian lebih mendalam tentang enzim tersebut. Tujuan penelitian ini adalah untuk mendapatkan *open reading frame* gen penyandi PhaC sintase dari *Bacillus* sp. PSA10, mendapatkan klon *open reading frame phaC*, mengetahui ekspresi *open reading frame phaC* pada bakteri *E.coli* BL21 (DE3) serta melakukan karakterisasi produk PhaC sintase melalui analisis *in silico*. Konfirmasi produksi PHA oleh *Bacillus* sp. PSA10 dilakukan melalui pengamatan mikroskopik. Selanjutnya dilakukan isolasi genom, amplifikasi, kloning dan ekspresi *open reading frame phaC*. Karakterisasi dan analisis struktur PhaC sintase dilakukan dengan analisis *in silico*. Analisis BlastX terhadap *open reading frame phaC* menunjukkan bahwa *open reading frame* isolat tersebut memiliki identitas 99.44% dengan PhaC sintase kelas III bakteri *Bacillus megaterium*. Overekspresi *open reading frame* pada *E.coli* BL21 (DE3) menunjukkan bahwa *open reading frame* tersebut dapat menghasilkan protein rekombinan dengan berat molekul $\pm 43,6$ kDa pada gel SDS PAGE 12%. Hasil analisis *in silico* menunjukkan bahwa PhaC sintase kelas III *Bacillus* sp. PSA10 memiliki karakteristik fisikokimia yang sama dengan PhaC sintase kelas III pada umumnya. Hasil analisis struktur PhaC sintase kelas III dari *Bacillus* sp. PSA10 menunjukkan bahwa enzim tersebut memiliki kesamaan struktur dengan PhaC sintase kelas I dari *Cupriavidus necator*.

Kata kunci : *Bacillus* sp. PSA10, analisis *in silico*, PHA, PhaC sintase.