INTISARI

Potensi pengembangan antibiotik golongan sefalosporin masih sangat luas, diantaranya melalui modifikasi rantai samping dari prekursor bernama 7-ACA *(7-aminocephalosporanic acid)*. Sintesis enzimatik 7-ACA yang mulanya dilakukan dua tahap dapat digantikan dengan sintesis satu tahap langsung dari substrat sefalosporin C dengan bantuan enzim bernama sefalosporin C asilase (CCA). Aktivitas CCA secara alami sangat rendah sehingga diperlukan upaya-upaya untuk meningkatkan aktivitasnya agar sesuai dengan kebutuhan industri.

Beberapa peneitian telah dilakukan untuk meningkatkan aktivitas CCA, diantaranya CCA dari *Pseudomonas* SE83. Telah dilakukan mutasi 6 titik menghasilkan CCA varian S12 dengan aktivitas sebesar 1207 U/L serta mutasi satu titik A675G dengan aktivitas sebesar 5349 U/L. Terinspirasi dari dua penelitian sebelumnya tersebut, pada penelitian ini didesain sekuens penyandi enzim CCA mutan dari *Pseudomonas* SE83 yang mengandung gabungan kedua mutasi di atas. Desain sekuens tersebut dikirim ke Genscript®, sebuah perusahaan sintetik gen, untuk dilakukan optimasi kodon menyesuaikan dengan *codon usage E. coli*, disintesis basa DNA-nya dan diinsersikan ke dalam plasmid pET28b hingga diperoleh rekombinan gen sintetik pET28b-*CCAs12-A675G*.

Dalam penelitian ini, terhadap rekombinan gen sintetik tersebut dilakukan konfirmasi guna memastikan kesesuaiannya sebelum diekspresikan dengan cara diperbanyak dalam *Escherichia coli* DH5α dilanjutkan analisis menggunakan PCR dan sekuensing. Rekombinan klon yang telah terkonfirmasi sesuai kemudian diekspresikan dalam *Escherichia coli* BL21 (DE3). Fermentasi dilakukan dengan teknik *batch* menggunakan *semi-defined medium* dengan variasi induser (laktosa 3 g/L dan arabinosa 15 g/L) dan suplementasi osmolit glisin-betain untuk mengoptimalkan ekspresi CCA mutan. Hasil fermentasi menggunakan induser laktosa 3 g/L menghasilkan aktivitas total sebesar 8602 U/L, mengalami kenaikan sebesar 8,2% dibandingkan kontrol tanpa induser (7949 U/L). Sementara menggunakan induser arabinosa 15 g/L menghasilkan aktivitas total sebesar 6624 U/L, mengalami penurunan sebesar 16,7% dibandingkan kontrol tanpa induser. Suplementasi osmolit glisin-betain dengan konsentrasi 0; 0,5; 1; 1,5; 2 g/L dilakukan pada kultur yang menggunakan induser laktosa. Hasilnya, aktivitas total tertinggi diperoleh pada konsentrasi glisin-betain 0,5 g/L, yaitu sebesar 33623 U/L. Nilai aktivitas ini menunjukkan kenaikan sebesar 239,9% dibandingkan kultur tanpa pemberian glisin-betain. Dengan demikian, dari hasil penelitian ini, dapat diperoleh kesimpulan bahwa sekuens penyandi CCA mutan yang didesain dapat diekspresikan dengan baik dalam *Escherichia coli* BL21 (DE3), dimana ekspresi optimal tercapai dengan pemberian induser laktosa 3 g/L dan osmolit glisin-betain 0,5 g/L.

Kata kunci : sefalosporin C asilase, 7-ACA, laktosa, arabinosa, glisin-betain

ABSTRACT

The potential for the development of cephalosporin antibiotics is still very broad, such as through modification of the side chain of the precursor named 7-ACA (7-aminocephalosporanic acid). The enzymatic synthesis of 7-ACA, which was initially carried out in two stages, can be replaced by a one-stage synthesis directly from the cephalosporin C substrate with the help of an enzyme called cephalosporin C acylase (CCA). The activity of CCA is naturally very low so efforts are needed to increase its activity to suit industrial needs.

Several studies have been conducted to increase the activity of CCA, in particular CCA from *Pseudomonas* SE83 (*AcyII*). A 6-point mutation resulted in CCA variant S12 with an activity of 1207 U/L and a one-point mutation A675G with an activity of 5349 U/L. Inspired by those two previous studies, this study designed a sequence encoding a mutant CCA enzyme from *Pseudomonas* SE83 (*AcyII*) containing both mutations above. The designed sequence was sent to Genscript®, a gene synthesis company, for codon optimization in accordance to *E. coli* codon usage, then the DNA bases were synthesized and inserted into the pET28b plasmid to obtain the recombinant synthetic gene pET28b-*CCAs12-A675G.*

In this study, the recombinant synthetic gene was confirmed to ensure its appropriateness before being expressed by propagating it in *Escherichia coli* DH5α followed by analysis using PCR and sequencing. Recombinant clones that have been confirmed to be appropriate were then expressed in *Escherichia coli* BL21 (DE3). Fermentation was carried out by batch technique using semi-defined medium with various inducers (lactose 3 g/L and arabinose 15 g/L) and glycine-betaine osmolyte supplementation to optimize mutant CCA expression. Fermentation results using lactose 3 g/L inducer produced a total activity of 8602 U/L, which increased by 8.2% compared to the control without inducer (7949 U/L). Meanwhile, using arabinose inducer 15 g/L produced a total activity of 6624 U/L, which decreased by 16.7% compared to the control without inducer. Glycine-betaine osmolyte supplementation with concentrations of 0; 0.5; 1; 1.5; 2 g/L was performed on cultures using lactose inducers. As a result, the highest total activity was obtained at a glycine-betaine concentration of 0.5 g/L, which reached 33623 U/L. This activity value showed an increase of 239.9% compared to the culture without glycine-betaine. Thus, from the results of this study, it can be concluded that the designed synthetic sequences encoding mutant CCA can be well-expressed in *Escherichia coli* BL21 (DE3), where optimal expression is achieved by lactose 3 g/L and glycine-betaine 0.5 g/L supplementation.

Keywords: cephalosporin C acylase, 7-ACA, lactose, arabinose, glycine-betaine