INTISARI

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Kasus kejadian infeksi akibat bakteri yang tinggi di Indonesia perlu diimbangi dengan kemandirian dalam produksi bahan baku obat antibiotik, sehingga tidak ketergantungan impor dari luar negeri. Salah satu, antibiotik yang banyak digunakan yaitu golongan β-lactam seperti penisilin dan turunannya. Produksi antibiotik turunan penisilin membutuhkan katalis enzim Penisilin G Asilase (PGA). Enzim PGA dapat diproduksi dengan teknik rekayasa rekombinan. Pada penelitian terdahulu telah dilakukan ekspresi gen *pac* sintetik, pengkode enzim PGA pada *E. coli* BL21(DE3), dengan hasil aktivitas enzim PGA yang rendah (0,01754 U/mg). Sehingga, pada penelitian saat ini dilakukan ekspresi gen *pac* sintetik pada inang yang berbeda dengan menggunakan tiga jenis inducer berbeda. Tujuan yang ingin dicapai dalam penelitian ini, untuk mendapatkan produksi protein rekombinan PGA dengan aktivitas enzim yang tinggi.

Penelitian kali ini dilakukan transformasi dan sekuensing utuh gen *pac* sintetik dalam konstruk plasmid rekombinan pET22b-*pgaEc* pada inang *E. coli* HB101 dan *E. coli* BL21(DE3) untuk mengetahui urutan basa nitrogen setelah ditransformasi. Inang dengan plasmid rekombinan mengandung gen *pac* sintetik tanpa adanya mutasi kemudian diinduksi ekspresi protein rekombinan PGA dengan tiga inducer yang berbeda IPTG 0,05 mM, laktosa 0,2%, dan arabinosa 1,5% dengan suhu inkubasi 20°C, pada kecepatan agitasi 150 rpm selama 17 jam. Protein rekombinan PGA diisolasi dengan metode sonikasi serta dilakukan treatment Freeze-Thawing untuk mendapatkan kembali protein rekombinan PGA yang aktif secara biologis. Verifikasi protein rekombinan dilakukan dengan elektroforesis SDS-PAGE 12%. Protein rekombinan PGA dilakukan uji aktivitas enzim dengan menggunakan pDAB.

Hasil dari penelitian ini, sekuensing gen *pac* sintetik dari inang *E. coli* HB101 dan *E. coli* BL21(DE3) tidak ada perubahan urutan basa nitrogen. *E. coli* HB101 mampu menghasilkan ekspresi protein rekombinan PGA dengan aktivitas tertinggi (10,17 U/mg) dibanding dengan inang *E. coli* BL21(DE3) (6,67 U/mg). Jenis inducer yang paling optimum untuk induksi ekspresi protein rekombinan PGA dengan aktivitas enzim yang tinggi yaitu menggunakan arabinosa 1,5%.

Kata kunci: Gen *pac* Sintetik, PGA, *E. coli* BL21(DE3), *E. coli* HB101

ABSTRACT

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The high incidence of bacterial infections in Indonesia needs to be balanced with independence in the production of raw materials for antibiotic drugs so that it does not depend on imports from other countries. One of the most widely used antibiotics is the β-lactam group, such as penicillin and its derivatives. The production of penicillin-derived antibiotics requires the enzyme Penicillin G acylase (PGA) as a catalyst. PGA enzymes can be produced by recombinant engineering techniques. In a previous study, the expression of the synthetic pac gene, encoding the PGA enzyme in *E. coli* BL21(DE3), was carried out, with the result that the PGA enzyme activity was low (0.01754 U/mg). Thus, in the current study, the expression of the synthetic pac gene was carried out in different hosts using three different inducers. The aim of this research is to obtain the production of PGA recombinant protein with high enzyme activity.

In this study, the complete transformation and sequencing of the synthetic pac gene in the pET22b-pgaEc recombinant plasmid construct in the host *E. coli* HB101 and *E. coli* BL21(DE3) was carried out to determine the sequence of nitrogen bases after transformation. The host whose recombinant plasmid contained the synthetic pac gene without any mutations was then induced to express PGA recombinant protein with three different inducers IPTG 0.05 mM, lactose 0.2%, and arabinose 1.5% with an incubation temperature of 20°C, at agitation speed. 150 rpm for 17 hours. PGA recombinant protein was isolated by sonication method and Freeze-Thawing treatment was performed to recover biologically active PGA recombinant protein. Verification of recombinant protein was carried out by SDS-PAGE 12% electrophoresis. PGA recombinant protein was tested for enzyme activity using pDAB.

The results of this study, sequencing of the synthetic pac gene from the host *E. coli* HB101 and *E. coli* BL21(DE3) did not change the nitrogen base sequence. *E. coli* HB101 was able to produce PGA recombinant protein expression with the highest activity (10.17 U/mg) compared to the host *E. coli* BL21(DE3) (6.67 U/mg). The most optimum type of inducer for the induction of PGA recombinant protein expression with high enzyme activity was using 1,5% arabinose.

Keywords: Synthetic *pac* gene, PGA, *E.coli* BL21(DE3), *E.coli* HB101