INTISARI

Antibiotik turunan penisilin merupakan golongan antibiotik yang umum dibutuhkan untuk menangani infeksi bakteri. Salah satu contoh antibiotik turunan penisilin adalah amoksisilin yang dapat dihasilkan melalui ketersediaan bahan baku yaitu *6-aminopenicillanic acid* (6-APA) dari hasil katalisis oleh enzim penisilin G asilase (PGA). Pemberdayaan enzim PGA perlu digencarkan supaya Indonesia mampu memproduksi bahan baku obat seperti 6-APA secara mandiri. Penelitian terdahulu telah berhasil memproduksi enzim rekombinan PGA melalui pemanfaatan vektor ekspresi. Meskipun demikian, aktivitas enzim termasuk rendah sehingga produksi 6-APA kurang maksimal. Mutasi melalui pendekatan *site-directed mutagenesis* pada enzim PGA dapat diterapkan untuk mengoptimalkan ikatan antara enzim dengan substrat saat bereaksi.

Penelitian ini memprediksi nilai afinitas berbagai variasi PGA mutan dalam berikatan dengan substrat penisilin G melalui tahapan *homology modeling* dan *molecular docking*. Selanjutnya, salah satu PGA mutan yang terseleksi kemudian dilanjutkan ke percobaan laboratorium. Plasmid rekombinan pET-22b(+)-pgaEcβThr68Tyr telah ditransformasikan ke *E. coli* BL21(DE3) dan ekspresi gen diinduksi oleh perlakuan *inducer* IPTG dan arabinosa secara terpisah. Selain itu, isolasi dan solubilisasi protein dengan sarkosyl dilakukan sehingga diperoleh beberapa fraksi protein dimana masing-masing fraksi dilakukan uji aktivitas enzim dan performa reaksi enzimatis dibandingkan.

Perbandingan hasil *molecular docking* menunjukkan empat PGA mutan memenuhi interaksi katalitik dan mempunyai nilai *binding affinity* yang lebih baik berbanding model wild type. Salah satu dari empat PGA mutan tersebut yaitu βThr68Tyr yang diseleksi untuk diekspresikan, menghasilkan tingkat aktivitas spesifik PGA yang lebih rendah berbanding PGA *wild type* namun perbedaan tidak signifikan. Diduga bahwa aktivitas spesifik PGA mempunyai korelasi dengan jenis *inducer* yang digunakan. Berdasarkan data aktivitas enzim, arabinosa merupakan *inducer* yang lebih berpotensial berbanding IPTG dalam menghasilkan protein dengan aktivitas enzim yang lebih tinggi. Dugaan sementara terkait aktivitas enzim PGA mutan yang lebih rendah berbanding PGA *wild type* disebabkan maturasi protein yang kurang optimal karena residu pengganti yang memicu pembentukan inclusion bodies.

Kata kunci: *6-aminopenicillanic acid, aktivitas enzim, molecular docking, penisilin G, penisilin G asilase, site-directed mutagenesis*

ABSTRACT

Penicillin-derivative antibiotics are the class of antibiotics commonly needed to overcome bacterial infections. One of the penicillin derivative antibiotics is amoxicillin, which can be produced through the availability of raw materials, namely 6-aminopenicillanic acid (6-APA) after the catalysis by penicillin G acylase (PGA). Empowerment of the PGA enzyme needs to be intensified so that Indonesia can produce medicinal raw materials such as 6-APA independently. Previous research has succeeded in producing recombinant PGA enzymes with expression vectors. However, enzyme activity is low, which is why 6-APA production is less than optimal. A mutation approach via site-directed mutagenesis of the PGA enzyme can be applied to optimize the bond between the enzyme and the substrate in an enzymatic reaction.

This research predicts the affinity values of various PGA mutants in binding to penicillin G substrates through homology modeling and molecular docking. One of the selected mutant PGAs, βThr68Tyr, was then subjected to laboratory experiments. The recombinant plasmid pET-22b(+)-pgaEcβThr68Tyr was transformed into *E. coli* BL21(DE3) and gene expression were treated by IPTG and arabinose inducer separately. In addition, protein isolation and solubilization with sarkosyl were carried out to obtain several protein fractions where each fraction was tested for enzyme activity and the performance of the enzymatic reaction was compared.

The comparison of molecular docking result shows four mutant PGAs that fulfill the catalytic interaction and has better binding affinity than the wild-type model. One of the four potential mutants, βThr68Tyr, which was selected to be expressed generates lower specific activity than that of wild-type PGA but the difference was not significant. It is suspected that the specific activity of PGA is correlated with the type of inducer used. Based on enzyme activity, arabinose possesses better potential as an inducer compared to IPTG in producing proteins with higher enzyme activity. The current assumption now concerning the lower enzyme activity of the mutant βThr68Tyr compared to the wild type is due to non-optimal protein maturation because the substitute residue triggers the formation of inclusion bodies.

Key words*: 6-aminopenicillanic acid, enzyme activity, molecular docking, penicillin G, penicillin G acylase, site-directed mutagenesis*