PENINGKATAN KEMAMPUAN PELARUTAN FOSFAT *Klebsiella* sp. GMD08 MELALUI TRANSPOSON MUTAGENESIS

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INTISARI

Klebsiella sp. GMD08 merupakan bakteri pelarut fosfat yang diketahui memiliki kemampuan melarutkan fosfat anorganik tidak larut menjadi ion fosfat terlarut melalui produksi asam organik. Transposon merupakan elemen genetik yang dapat digunakan sebagai agen untuk meregenerasi mutan melalui mutagenesis sehingga dapat digunakan untuk mengidentifikasi fungsi genetik yang terllibat dalam mekanisme pelarutan fosfat. Penelitian ini bertujuan untuk mengetahui karakteristik fenotipik pelarutan fosfat mutan *Klebsiella* sp. GMD08, mengetahui karakteristik produk asam organik yang dihasilkan mutan, serta mengidentifikasi gen yang terlibat dalam pelarutan fosfat melalui deteksi sekuen hasil insersi transposon. Mutasi dilakukan dengan cara menginsersikan transposon *mini-Tn5* yang dipelihara dalam *Escherichia coli* S17-1/λ*pir*[pBSL202] ke dalam kromosom *Klebsiella* sp. GMD08 dengan metode konjugasi *filter mating*. Kandidat mutan transkonjugan diuji kemampuannya dalam melarutkan trikalsium fosfat [$Ca_3(PO_4)_2$] secara kualitatif dan kuantitatif menggunakan medium pikovskaya. Karakteristik produk asam organik mutan transkonjugan dideteksi dengan menggunakan metode *High-performance liquid chromatography* (HPLC). Gen yang diduga terlibat dalam pelarutan fosfat dianalisis dengan cara mendeteksi sekuen hasil insersi transposon menggunakan metode sekuensing. Urutan basa nukleotida hasil sekuensing dianalisis dengan menggunakan *nucleotide basic local alignment search tool* (*nucleotide BLAST*) untuk mengetahui similiritas sekuen dengan database. Hasil penelitian menunjukkan bahwa PB116 dan PB122 adalah dua mutan transkonjugan utama yang dihasilkan dari transposon mutagenesis yang memiliki kemampuan pelarutan trikalsium fosfat lebih tinggi dibanding *wild-type*. Asam glukonat merupakan produk asam organik utama yang terlibat dalam mekanisme pelarutan fosfat *Klebsiella* sp. GMD08. Gen pengkode represor arginin (*ArgR*) dan gen *malate dehydrogenase* (*mdh*) terlibat dalam mekanisme pelarutan fosfat *Klebsiella* sp. GMD08.

Kata kunci : pelarutan fosfat, *Klebsiella* sp. GMD08, transposon mutagenesis, asam glukonat, represor arginin (*ArgR*), *malate dehydrogenase* (*mdh*)

ENHANCEMENT OF PHOSPHATE-SOLUBILIZING CAPABILITY OF *Klebsiella sp.* GMD08 USING TRANSPOSON MUTAGENESIS

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ABSTRACT

Klebsiella sp. GMD08 is one of bacteria that have the capability to dissolve insoluble inorganic phosphate into soluble phosphate ion through their organic acid production. Transposon is a genetic element agent which usually used to generate mutant through mutagenesis, so it can be used to identify genetic functions involved in those phosphate solubilizing mechanism. This research aimed to determine the phosphate solubilizing phenotypic characteristics of *Klebsiella* sp. GMD08 mutant, its organic acid characteristics, as well as to identify gene involved in phosphate solubilization through sequence detection obtained from transposon insertion. Mutation was conducted by inserting *mini-Tn5* transposon hosted in *Escherichia coli* S17-1/λ*pir* [pBSL202] into *Klebsiella* sp. GMD08 chromosome by filter mating conjugation method. Transconjugant mutant candidates were then qualitatively and quantitatively analyzed for their solubilizing ability to dissolved tricalcium phosphate [$Ca_3(PO_4)_2$] using pikovskaya medium. Organic acid characteristics of transconjugant mutants were detected using *High-performance liquid chromatography (HPLC)*. Suspected genes involved in phosphate solubilizing were detected using sequencing method obtained from transposon insertion result. Nucleotide *Basic Local Alignment Search Tool* (nucleotide BLAST) was used to identify nucleotide base sequence similarity with the database. Results showed that PB116 and PB122 are two main transconjugant mutants which obtained from transposon mutagenesis which have higher tricalcium phosphate dissolving ability than wild-type. Gluconic acid was the main mutants' organic acid which involved in *Klebsiella* sp. GMD08 phosphate solubilizing mechanism. Arginine repressor (*ArgR*) and *malate dehydrogenase* gene (*mdh*) coding gene were involved in *Klebsiella* sp. GMD08 phosphate solubilizing mechanism.

Keywords : phosphate solubilization, *Klebsiella* sp. GMD08, transposon mutagenesis, gluconic acid, arginine repressor (*ArgR*), *malate dehydrogenase* (*mdh*)