

Hyper-solubilizing tricalcium phosphate mutants of klebsiella sp. gmd08

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Abstrak

Klebsiella sp. GMD08 is one of the bacteria that has the capability to dissolve insoluble inorganic phosphate into soluble phosphate ion through their organic acid production. Transposon is a genetic element agent usually used to generate mutant through mutagenesis. Thus it can be used to identify the genetic functions involved in those phosphate solubilizing mechanisms. This research was conducted to identify the genes of Klebsiella sp. GMD08 involved in phosphate solubilization through sequence detection obtained from a hyper-solubilizing phosphate mutant library. Mutation was conducted by inserting mini-Tn5 transposon hosted in Escherichia coli S17-1/Δpir [pBSL202] into Klebsiella sp. GMD08 chromosome by the filter mating conjugation method. Trans conjugant mutant candidates were then qualitatively and quantitatively analyzed for their solubilizing ability to dissolve tricalcium phosphate [Ca₃(PO₄)₂] using pikovskaya medium. The organic acid characteristics of transconjugant mutants were detected using High-performance liquid chromatography (HPLC). Meanwhile, suspected genes involved in phosphate solubilizing were detected using the sequencing method obtained from the transposon insertion result. Nucleotide Basic Local Alignment Search Tool (nucleotide BLAST) was used to identify the nucleotide base sequence similarity with the database. The results showed that PB116 and PB122 were the two main transconjugant mutants obtained from transposon mutagenesis which had higher tricalcium phosphate dissolving ability. Gluconic acid was the main organic acid produced by Klebsiella sp. GMD08 phosphate solubilizing mechanism. Moreover, arginine repressor (ArgR) and malate dehydrogenase gene (mdh) coding gene were involved in Klebsiella sp. GMD08 phosphate solubilizing mechanism.