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Mutagenesis and protoplast fusion of *Arthrobacter* sp. for improved glucose isomerase production

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In attempts to construct superior glucose isomerase-producing strains, four bacterial strains (i.e., *Arthrobacter* sp. B-3728, *Actinoplanes missouriensis* B-3342, *Streptomyces phaeochromogenes* B-1131 and B-1517) were screened for their glucose isomerase (GI) synthesis. Both *Arthrobacter* sp. and *A. missouriensis* were proved as the highest producers (16.8 and 15.6 U.ml-1, respectively). Ultra Violet (UV) and Ethyl Methane Sulfonate (EMS) were used for mutagenesis. Induced mutants having antimicrobial resistance markers generated from *Arthrobacter* sp. and *A. missouriensis* (wild types) were screened for their GI production and compared with wild types. About 8 mutants from each treatment and each strain were examined. The mutant EMS 60-28 D generated from *Actinoplanes missouriensis* exhibited the highest activity (33.6 U.ml-1) amongst the isolated mutants from this strain with 1.99-folds. While, the mutant EMS 60-25 D generated from *Arthrobacter* sp. exhibited the highest GI production in this study (49.7 U.ml-1) with 3.2 folds improvement than its wild type. Protoplast fusion technology was successfully applied using hyper-producing GI mutants generated from *Arthrobacter* sp. according to their antimicrobial responses, 4 mutants were selected to perform 6 crosses. Eight fusants were obtained from each cross and their GI activities were determined. The fusant (C 3-2) exhibited the highest GI synthesis (2.75 folds the wild type). For optimal GI synthesis by the mutant EMS 60-25 D and fusant C 3-2, batch fermentation system was optimized. Optimization of production fermentation resulted in an additional 10% improvement in enzyme synthesis by mutant EMS 60-25 D. On the other side, GI of fusant C 3-2 was increased after optimization from 42.4 to 60.1 U.ml-1 with 3.85 times the activity of the wild type. Enhanced glucose conversion ratio (48 and 48.8%), respectively was also noted by the studied strains compared to 35.3% for the wild type. Glucose isomerase of fusant C 3-2 was extracted, then purified by ammonium sulfate fractionation followed by gel filtration on Sepharose 4-B. The total yield was 17.8%. SDS-PAGE of the purified GI showed one band with a molecular weight of 47 kDa. Optimum temperature; pH; substrate and Mg+2 concentration of the purified enzyme were 75 °C, 8500 mM and 0.05 M, respectively. Km value as calculated from Lineweaver-Burk plot was 285 mM. The enzyme was stable for 1 hour at 80-90 °C and pH 5. The highest GI producing fusant C 3-2 was successfully immobilized within K-carrageenan gel, hardened with glutaraldehyde, for the continuous production of HFCS. The immobilized preparation exhibited a maximal glucose conversion of 36% after 12 hours of isomerization at 60 °C and the half life of such beads was 408 hours of continuous operation.

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