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ABSTRACTS

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Bioconversion of Cassava Starch to Nutrient Sources for Slow-Growing Rhizobium Cultivation

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Rhizobia are effective nitrogen-fixing bacteria in symbiosis with legumes. Rhizobial inoculants are currently produced for growing several economic legumes. The utilization of carbon compounds by rhizobia varied with their species and strains. Most slow-growing rhizobia cannot use the simple sugar, glucose, as well as sucrose. Glycerol and mannitol, particularly mannitol, are mainly used for the cultivation of these slow-growing rhizobia. Prices of the carbohydrate compounds are about 12-20 times more expensive than glucose. Consequently, the production of these legume inoculants is costly. This study aims to produce nutrient sources containing mannitol and/or glycerol by the conversion of cassava starch, a cheap raw material, using yeast for slow-growing rhizobium cultivation. Bacterial growth factors can be obtained from yeast cells. Thus, the crude product from yeast could be directly utilized for the rhizobium cultivation. The bacterial inoculant production cost might be reduced. A total of 147 yeast isolates from natural habitats and 19 yeast strains from culture collections were screened for their glycerol and mannitol production capabilities using both glucose and cassava starch as carbon sources. The accumulation of glycerol and mannitol was detected from both cultured filtrate and cell extracts. The yeast isolate KAY1 isolated from rozelle fruit and identified as belonging to the genus *Rhodotorula* was selected. It could efficiently utilize cassava starch, and accumulate mannitol in its cell. The maximum yield of mannitol was 1.23-1.48 grams per litre of cultured medium in the laboratory scale (500-mililitre working volume) when the yeast was cultivated in its suitable medium containing 2, 0.3, 0.5, 0.05, and 0.1% of cassava starch, yeast extract, ammonium sulphate, magnesium sulphate, and mono-potassium phosphate respectively, at 30°C on the rotary shaker (200 rpm) for 4 days. The heat-shock treatment of KAY1 cells at 45°C for 20 minutes prior to inoculating into the cassava starch medium could increase the mannitol accumulation of 10.5% higher than untreated cells. The maximum mannitol yield was obtained on the third day of cultivation. When cultured two slow-growing rhizobium strains, *Bradyrhizobium japonicum* USDA 110 and *Bradyrhizobium* sp. THA 5, in the medium containing only 0.6, 0.05, and 0.01% of mannitol in crude KAY1 cell lysate, di-potassium phosphate, and sodium chloride respectively; both *Bradyrhizobium* strains gave their good growth of about 10^8 cells per mililitre. Similar results were achieved when cultivated the two bacterial strains in Yeast extract-mannitol broth (YMB) composed of 0.5, 0.05, 0.02, 0.05, and 0.01% of mannitol (commercial grade), yeast extract, magnesium sulphate, di-potassium phosphate, and sodium chloride respectively. The cost of chemicals used for the rhizobium growth medium preparation was basically calculated. The total cost of chemicals used for preparing the rhizobium growth medium containing crude KAY1 cell lysate and the starch medium (KAY1 growth medium) which gave sufficient concentration of mannitol for cultivating *Bradyrhizobium* strains was around 34% lower than the cost of chemicals used for preparing YMB.