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Cassava as a cheap source of carbon for rhizobial inoculant production using an amylase-producing fungus and a glycerol-producing yeast

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Summary

The aim of this research was to develop methods to use low-cost carbon compounds for rhizobial inoculant production. Five raw starch materials; steamed cassava, sticky rice, fresh corn, dry corn and sorghum were tested for sugar production by an amylase-producing fungus. Streamed cassava produced the highest amount of reducing sugar after fermentation. *Bradyrhizobium japonicum* USDA110, *Azorhizobium caulinodans* IRBG23, *Rhizobium phaseoli* TAL1383, *Sinorhizobium fredii* HH103, and *Mesorhizobium ciceri* USDA2429 were tested on minimal medium supplemented with reducing sugar obtained from cassava fermentation. All strains, except *B. japonicum* USDA110, could grow in medium containing cassava sugar derived from 100 g steamed cassava per litre, and the growth rates for these strains were similar to those in medium containing 0.5% (w/v) mannitol. The sugar derived from steamed cassava was further used for production of glycerol using yeast. After 1 day of yeast fermentation, the culture containing glycerol and heat-killed yeast cells, was used to formulate media for culturing bradyrhizobia. A formulation medium, FM4, with a glycerol concentration of 0.6 g/l and yeast cells ($OD_{600} = 0.1$) supported growth of *B. japonicum* USDA110 up to 3.61×10^9 c.f.u./ml in 7 days. These results demonstrate that steamed cassava could be used to provide cheap and effective carbon sources for rhizobial inoculant production.

Introduction

Legume productivity can be increased by inoculation of the seeds with rhizobia, which form effective nitrogen-fixing nodules on leguminous hosts. For effective nodulation of legumes, seeds are generally inoculated with an appropriate rhizobial strain before sowing. For industrial production of rhizobial inoculants, it is important to identify inexpensive and easily available sources of nutrients for culture medium. In such media preparations, a single source of carbon cannot be used for all strains, because rhizobial strains of different genera often differ in carbon utilization. Generally, fast-growing rhizobia can utilize a variety of sugars such as glucose, sucrose, maltose, whereas bradyrhizobia appear to be nutritionally fastidious (Stowers 1985). Bradyrhizobia can use arabinose, gluconate and some sugar alcohol, such as mannitol and glycerol as a preferred carbon source (Stowers 1985; Lie *et al.* 1992). Generally, mannitol is universally used for cultivation both fast- and slow-growing rhizobia (Stowers 1985).

For large-scale production of rhizobial inoculant, it is desirable to identify alternative sources of carbon that are less expensive than mannitol. It is known that raw starch material can be changed to sugar by using a biotechnological process (Chumkhunthod *et al.* 2001). Starch is composed of two α-glucan polymers, amylose and amylopectin, as linear and branched structures, respectively. Some groups of bacteria and fungi produce α-amylase, β-amylase or amyloglucosidase (Zeikus & Johnson 1991) that convert starch to maltose and glucose (saccharification). It should be possible to convert some inexpensive starch material, such as cassava tubers, sticky rice, corn or sorghum, into sugars by microbial fermentation for large-scale inoculant production of fast-growing rhizobia. It is also known from the literature that glycerol can be produced from glucose through yeast fermentation (Radler & Schutz 1982; Parekh & Pandey 1985; Vijaikishore & Karanth 1987; Romano *et al.* 1997). Glycerol production using yeast fermentation can be enhanced by adding sodium sulphite or alkaline reacting salts into medium (James 1928). Based on this information, we hypothesized that the unpurified sugars derived from microbial fermentation of low cost starch material could be used directly for producing glycerol, which could be further used as a carbon source for the large-scale production of a bradyrhizobial inoculant. The objectives of this research