NOTES

High-Potency Amyloglucosidase-Producing Mold of the Aspergillus niger Group

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Amyloglucosidase is known to be produced by a variety of species belonging to the Aspergillus genus. This enzyme is being used commercially to make glucose from corn starch. In addition to amyloglucosidase, crude enzyme preparations usually contain varying amounts of transglucosidase, which is undesirable in the manufacture of crystalline glucose because the oligosaccharides formed are not readily hydrolyzed to glucose and interfere in the crystallization process. It would be desirable to find an organism that would form relatively large amounts of amyloglucosidase without the formation of objectionable quantities of transglucosidase.

Numerous cultures of Aspergillus have been tested from the ARS Culture Collection of the Northern Regional Research Laboratory. Culture number NRRL 3112, a member of the A. niger series but not A. niger in the strict sense, was chosen for further study. When this culture was propagated in 20-liter stainless-steel fermentors, it produced relatively large quantities of amyloglucosidase when compared with A. niger NRRL 337. Comparison was made with this strain of A. niger because it has been widely distributed and gives fairly typical amyloglucosidase yields.

The two organisms were grown in unbaffled 20-liter stainless-steel fermentors with an air rate of 1 vol per vol per min and an agitator speed of 600 rev/min. The medium consisted of 2 kg of corn meal, 40 g of gibberel- lin barley malt, and 8 liters of water. The pH was adjusted to 5.5. During the sterilization process, the temperature was held at 60 C for 20 min to allow the barley malt to thin the corn starch. The temperature was then raised to 120 C for 20 min to sterilize the mash and in- activate the barley malt enzymes. The fermentors were inoculated with 800 ml of culture and run for 4 days at 35 C. The inoculum was grown in Fernbach flasks on a rotary shaker in a medium consisting of 5% ground corn and 0.25% yeast extract.

Under these conditions, A. niger NRRL 337 produced from 3.0 to 3.5 amyloglucosidase units per ml, whereas NRRL 3112 produced from 10 to 12 units per ml. One amyloglucosidase unit is the amount of enzyme necessary to form 1 g of glucose from 4 g of starch substrate in 1 hr at 60 C (Miles Chemical Co., Tech. Bull. No. 2-122, p. 21, 1962).

Not only was the potency of the enzyme produced by NRRL 3112 three to four times greater than that of the enzyme from A. niger NRRL 337, but also the 3112 enzyme contained considerably less transglucosidase activity. Transglucosidase was determined by incubating 0.5 M maltose substrate with appropriate amounts of culture filtrates from the two cultures at 60 C for 24 hr. The hydrolyzed materials were lyophilized to remove water, and the dry solids were dissolved in dry pyridine. Trimethylsilyl derivatives of the carbohydrates present were prepared (Sweeley et al., J. Am. Chem. Soc. 85: 2497, 1963). The derivatized products were then separated by gas chromatography. The comparative amyloglucosidase and transglucosidase activities are illustrated in Table 1. In 24 hr, NRRL 337 has appreciable isomaltose formation, and the hydrolysis of maltose is not yet completed. At 24 hr, NRRL 3112 shows nearly complete conversion of maltose, with only slight production of isomaltose.

A high amyloglucosidase-potency enzyme with a low transglucosylase potency can be prepared in a simple medium (Table 1). Consequently, such a culture should be of potential use to the enzyme and corn sugar industry.