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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (4): 562-563, 2000

Degradative Activity of Enzyme from *Synnematous* sp. Endophytic Fungus on Raw Starches

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Abstract: The degradative activity on various raw starches by enzyme from *Synnematous* sp., an endophytic fungus was studied. The enzyme hydrolyzed raw starches to produce maltose and glucose. Maximum maltose and glucose were produced from raw rice and tapioca starch were 18.8 and 3.6%, respectively. The yields were dependent on accessibility of granules surface to be attacked by the enzyme. The results of this study suggest that enzyme from *Synnematous* sp. has potential for the production of glucose and maltose using raw starches as substrates.

Key words: Endophytic fungus, glucose, maltose, *Synnematous* sp.

Introduction

Endophytic fungi occur within plant tissues without producing any apparent symptoms and their presence may confer certain advantages to the host plant. Endophytic fungi have also been recognized as a repository of novel secondary metabolites, some of which have beneficial biological activities (Monaghan *et al.,* 1995; Carroll, 1995; Schulz *et al.,* 1995). In conventional method for the production of glucose, maltose, cyclodextrins and other products derived from starch the process had been carried out by two stages; liquefaction of starch and then saccharification or transglycosylation. However, the traditional method has shortcoming such as high energy consumption, low product yield and complexity in separation and purification of product. Direct conversion of raw starch to sugars would produce high purity of products without accumulation of undesirable oligosaccharides. This paper describes degradative activity of enzyme from *Synnematous* sp. endophytic fungus on raw starches as substrates.

Materials and Methods

Organisms growth and enzyme preparation: *Synnematous* sp. was grown in broth medium containing 2 g $NaNO_3$, 0.4 g yeast extract, 0.5 g KH_2PO_4, 0.5 g KCI, 0.5 g $MgSO_4$, $7N_20$, 0.01, g $FeSO_4$, $7H_20$ and 20 g raw sago starch in 1000 ml of deionized water autoclaved at 121°C for 15 min. The raw sago starch was sterilized separately at 140°C for 1 h. A 500 ml Erlenmeyer flask containing 200 ml of the broth medium, inoculated with a loopful of the fungus taken from a stock slant was incubated at 30°C on a rotary shaker at 120 rpm for 5 days. Mycelia and residual starch granules were then removed by centrifugation at 15 000 g for 20 min at 4°C. The culture was filtered through a Whatman filter paper No. 4 and the enzyme filtrate was used as the crude enzyme.

Determination of optimum enzyme and substrate concentration: Optimum concentrations of substrate and enzyme were determined at pH 5.5 using various concentrations of substrate (2 to 20% (w/v) ad different amounts of enzyme (4 units to 40 units) in a total reaction volume of 2 ml. The reaction mixture was incubated at 55°C for 30 min. The reaction was then stopped by heating in boiling water for 5 min and after centrifugation the reducing sugar in the supernatant was measured by the dinitrosalicylic acid method of Miller (1959).

Enzymatic degradation of starch: Enzymatic degradation of starch was carried out under optimum conditions (pH 5.5, temperature 65°C) in the presence of various raw starches. An appropriate amount of enzyme prepared from *Synnematous* sp. was mixed with an appropriate concentration of raw starch in a total reaction volume of 10 ml. Two ml samples were taken at regular intervals (12, 24, 48 and 96 h) and the reactions was stopped by heating in a boiling water for 5 min.

Identification of sugars: Sugars produced were analyzed by HPLC using the NH2-18C column (25 cm mm, Merck-Germany). The column was maintained a 38°C with 80% (v/v) acetonitrile in delonized water (HPLC grade) as the mobile phase at 1.2 ml/min. Sugar standards used were glucose, maltose and maltotriose.

Results and Discussion

The enzyme produced from synnematous sp. was able to hydrolyze raw starch tested. The effects of amount of substrate and enzyme on the production of sugars after 30 min. reactions are presented in Fig. 1 and 2. The amount of sugars produced was detected as reducing sugar. The highest amount of reducing sugars produced was detected as reducing sugar. The highest amount of reducing sugar produced (0.92 mg/ml) was obtained at a raw sago starch concentration of 16% (w/v). As shown in Fig. 2, optimum amount of enzyme was 24 units. At concentrations above this value, the reducing sugar produced was reduced, possibly due to the lack of the active site of the enzyme to interact with raw starch granule surface, or due to the lack of the suspension water caused by the penetration of added water molecule into raw starch granules, which prohibited maintaining the suspension state for enzyme reaction (Lee and Park, 1996). Hydrolysis of various raw starches produced a mixture of glucose and maltose. The relationship between the enzyme action to raw starches of different granules sizes is shown in Fig. 3. Quantitatively, somewhat more of small granules such as rice, wheat and corn starch were converted to maltose by the hydrolysis stages, though on percentage basis there was no difference between the two groups of granules. A possible explanation is that the initial reaction rate of the raw starch is not only dependent on the types of the starch and amount of the enzyme that adsorbed to the granules but also unique action of the enzyme involved.

Glucose was also produced at a lower amount as minor product during the course of the reaction (Fig. 4). It has been demonstrated previously by Kimura and Robyt (1996) that lower glucose production was obtained from native starches using commercial enzyme from *Aspergillus rhizopus.* Among the raw starches tested, those with large granules sizes (raw sago, potato and tapioca starch) were degraded more efficiently compared to those with small granules sizes (raw corn, wheat and rice starch) (Fig. 4). This observation indicates that the enzyme action is not exclusively a surface phenomenon; but enzyme molecules penetrating freely into the granule molecules are limited to certain accessible surfaces. Based on these observations, we considered

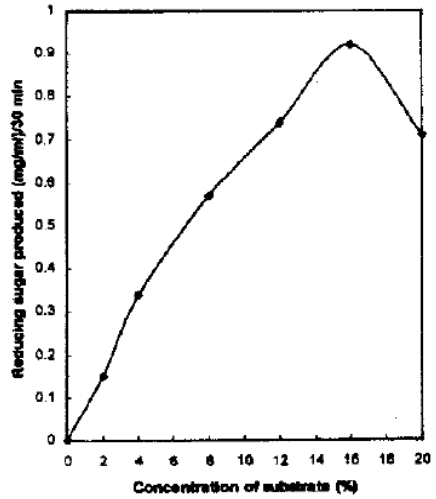

Fig. 1: Effect of substrate concentration on the reducing sugar produced by the enzyme of *Synnematous* sp.

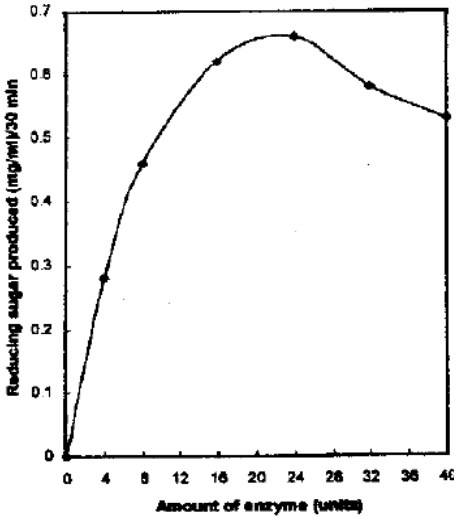

Fig. 2: Effect of amount of enzyme on the reducing sugar produced by the enzyme of *Synnematous* sp.

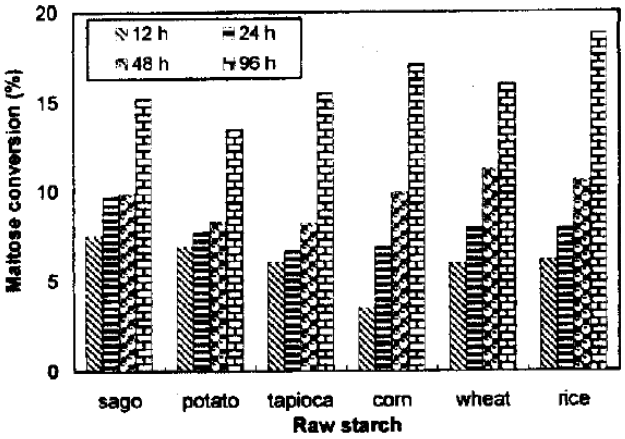

Fig. 3: Maltose conversion (%) from various raw starches using enzyme produced by *Synnematous* sp. The reaction was carried out 12, 24, 48 and 96 hrs under the optimum conditions (pH 5.5, incubation temperatur 55°C, substrate concentration: 16%)

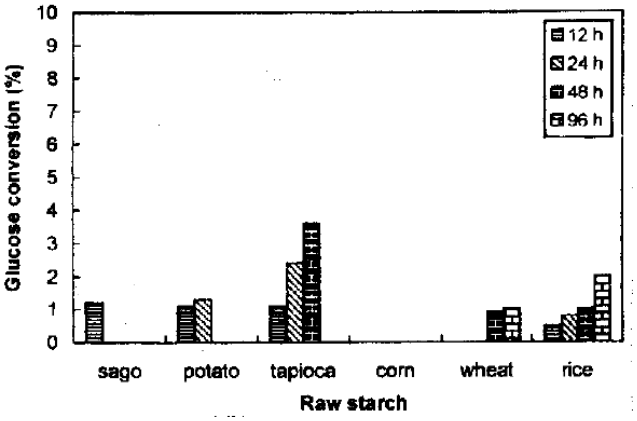

Fig. 4: Glucose conversion (%) from various raw straches using enzyme produced by *Synnematous* sp. The reaction was carried out for 12, 24, 48 and 96 hrs under the optimum conditions (pH 5.5, incubation temperature 55°C, substrate concentration: 16%)

the enzyme to be a kind of a-amylase. The a-amylase hydrolyzed alpha-1,4-glycosidic bonds randomly and by pass alpha-1, 6 glycosidic bonds (James and Lee, 1997). In conclusion, the *Synnematous* sp. examined showed a spectrum of degradation of a variety of compounds of raw starches. To the authors' knowledge, this is the first report of a raw starch degrading enzyme produced by *Synnematous* sp., an endophytic fungus isolated from forest trees in Malaysia.

Acknowledgments

This work was supported by award from the Ohgushi Yoshika Foundation in memory of Mrs. Yoshika Ohgushi. We thanked Dr. Katsuhiko Ando from Tokyo Research Laboratories, Kyowa Hakko, Japan for his assistance in the identification of the endophytic fungus.

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