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Effect of Urea as an Inducer of β -Fructofuranosidase in *Saccharomyces* Fermentation

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Abstract: The primary application of invertase from *Saccharomyces cerevisiae* is in the confectionary industry. In Pakistan, much of this enzyme is imported. The influence of the carbon source during invertase production is well known, but little is understood about the nitrogen source influence. We examined three different nitrogen sources using *Saccharomyces cerevisiae* strain for invertase production. *Saccharomyces* species GCA-II was used to investigate the effect of urea and other nitrogen sources on the production of β -fructofuranosidase in submerged fermentation. It was found that when a very little amount of urea was added to the fermentation medium it showed marked increase in β -fructofuranosidase production i.e., from 121.35 to 158.26 U/ml.

Key words: β -fructofuranosidase, urea, *Saccharomyces*, fermentation, nitrogen source

Introduction

Beta-fructofuranosidases (invertases) are enzymes that cleave α -1, 4-glucosidic linkage between α -D-glucose and β -D-fructose molecules of sucrose by hydrolysis producing glucose and fructose (Shafiq *et al.*, 2003). Beta-fructofuranosidases are intracellular as well as extracellular enzymes. It is used in the production of confectionery with liquid or soft centers, fermentation of cane molasses into ethanol, in calf feed preparation and also in manufacture of inverted sugars as food for honeybees (Haq *et al.*, 2002). Different organic nitrogen sources and their concentrations have a major effect on the ability of yeast to synthesize β -fructofuranosidase (Nakano *et al.*, 2000). Nitrogen equilibrium in yeast cell results in an increased β -fructofuranosidase synthesis. So there exists a specific physiological response of sucrose metabolism to the presence of nitrogen source. Shafiq *et al.* (2002) have worked out nitrogen regulation of yeast β -fructofuranosidase, peptone at the level of 4.0 g/l was found as best nitrogen source for enzyme secretion by yeast strain.

Present work describes the nutritional studies for β -fructofuranosidase synthesis by *Saccharomyces* species GCA-II in shake flask. The effect of different organic nitrogen sources and concentration of urea on the growth of yeast and enzyme production was studied. Specific product rate and specific growth rate were also studied in relation to nitrogen source induction.

Materials and Methods

Organism: *Saccharomyces* species was used for production of β -fructofuranosidase in the present study. The organism was isolated from dates (fruit of date palm, *Phoenix dactylifera*), cultured and maintained on the medium containing sucrose 20.0 g/l; agar 20.0 g/l;

peptone 5.0 g/l and yeast extract 3.0 g/l at pH 6.0 (Dworschack and Wickerham, 1960).

Preparation of vegetative inoculum: Cell suspension was prepared from 2-3 days old slant culture of *Saccharomyces* species. Twenty-five ml of seed medium was transferred to each 250 ml Erlenmeyer flask. The medium was consisted of (g/l, w/v) sucrose 30.0; peptone 5.0 and yeast extract 3.0 at pH 6. The flasks were cotton plugged and autoclaved at 15 lbs/inch² pressure (121 °C) for 15 minutes and cooled at room temperature. One ml of inoculum was aseptically transferred to each flask. Flasks were then incubated in a rotary incubator shaker (SANYO Gallenkamp PLC, UK.) at 30 °C for 24 hours. The agitation rate was kept at 200 rev/min.

Fermentation technique: Production of β -fructofuranosidase was carried out by shake flask technique using 250 ml Erlenmeyer flasks. Same medium composition was used for vegetative inoculum preparation and for fermentation. Twenty-five ml of fermentation medium was transferred to each Erlenmeyer flask. The cotton-plugged flasks were autoclaved at 15 lbs/inch² pressure for 15 minutes and cooled at room temperature. One ml of vegetative inoculum was aseptically transferred to each flask. Flasks were then incubated in a rotary incubator shaker (SANYO Gallenkamp PLC, UK) at 30°C for 48 hours. The agitation rate was kept at 200 rev/min. The flasks were run parallel in duplicates.

Analytical methods

Dry cell mass: Dry cell mass of yeast was determined

Baig *et al.*: Nitrogen Source Effect on Yeast β -fructofuranosidase Production

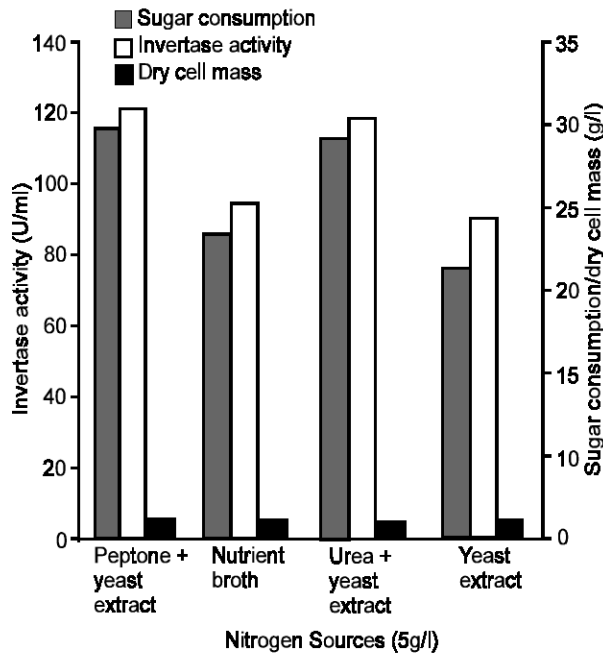


Fig. 1: Effect of organic nitrogen sources on the production of β -fructofuranosidase

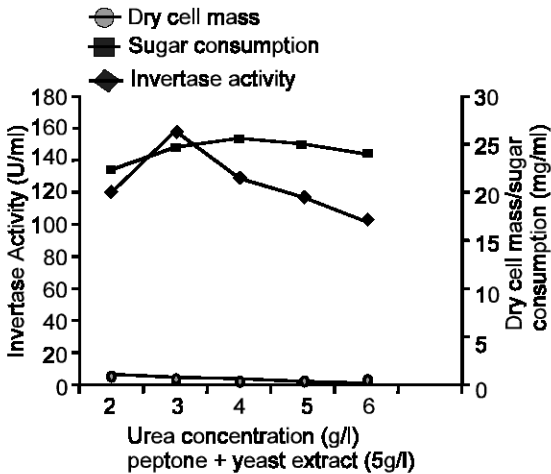


Fig. 2: Effect of urea concentration on the production of β -fructofuranosidase

by centrifugation of fermented broth at 5000 rev/min using weighed centrifuge tubes. The tubes were oven dried at 105 °C for one hour.

Sugar estimation: Sugar was estimated spectrophotometrically by DNS method (Tasun *et al.*, 1970). Transmittance was measured at 546 nm using Scanning Spectrophotometer (CECIL CE-7200, UK).

Beta-fructofuranosidase activity: Enzyme activity was determined according to the method of Sumner and Howell (1935). "One invertase unit is defined as the

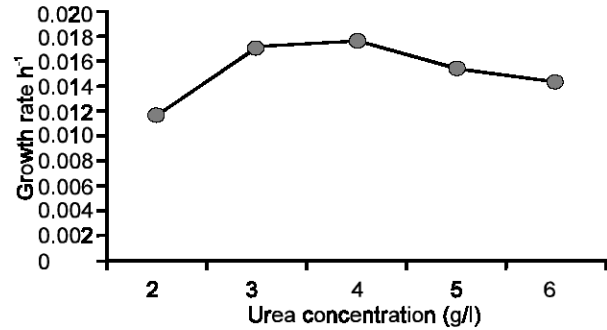


Fig. 3: Effect of urea concentration on specific growth rate

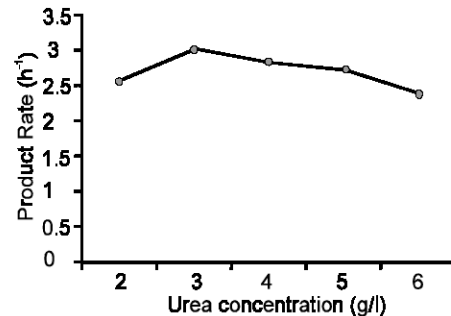


Fig. 4: Effect of urea concentration on specific product rate

amount of enzyme, which releases one milligram of inverted sugar in 5 minutes at 20 °C, at pH 4.5". Specific product and growth rates for fermentation were also determined.

Results and Discussion

Effect of different organic nitrogen sources: Effect of different organic nitrogen sources (nutrient broth, peptone + yeast extract (control), urea + yeast extract and yeast extract only) on the production of β -fructofuranosidase by *Saccharomyces* species was studied (Fig. 1). Application of appropriate nitrogen source is very important for optimal production of β -fructofuranosidase. In the following study, maximum β -fructofuranosidase activity and dry cell mass (121.35 U/ml and 1.2 g/l respectively) was obtained when "peptone + yeast extract" was used as nitrogen source. Least dry cell mass was obtained when urea was used in the medium (0.77 g/l) whereas enzyme production was closer to maximum i.e. 117.3 U/ml. Reduced cell mass production might be due to denaturing effect of urea on yeast cells (Pitombo *et al.*, 1994). The reason for high enzyme yield might be positive influence of urease and β -fructofuranosidase on each other's production because various extracellular enzymes produced by the yeast *Saccharomyces* enhance each other's secretion into the culture medium (Egorov *et al.*, 2000).

Effect of urea concentration: The effect of urea concentration in the fermentation medium on the production of β -fructofuranosidase by *Saccharomyces* species GCA-II was studied (Fig. 2). Maximum enzyme activity (158.26 U/ml) was observed at urea concentration of 2 g/l. Sugar consumption and dry cell mass were 24.72 and 1.02 g/l, respectively. Lesser urea concentration is not enough to induce urease in amount sufficient to promote β -fructofuranosidase production, thus yielding lesser enzyme. Concentration of urea higher than optimum also produce less amount of β -fructofuranosidase. Higher concentrations of urea induce denaturation of *Saccharomyces* cells thereby reducing enzyme production (Pitombo *et al.*, 1994). Specific product and growth rate also support the fact that urea at the level of 2.0 g/l acts as an inducer of yeast β -fructofuranosidase in shake flask (Fig. 3 and 4).

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