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Effect of Nitrogen and Phosphate Sources on the Biosynthesis of β-Fructofuranosidase

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Abstract: *Saccharomyces* species GCA-II was used to investigate the effect of mineral constituents on the production of β-fructofuranosidase in submerged fermentation. Different organic nitrogen and phosphate sources in varied concentrations were tested for optimal production of enzyme. The results indicated that enzyme production increased from 107.42 to 168.58 U ml^{-1}. Thus, *Saccharomyces* strain GCA-II gave maximal β-fructofuranosidase in submerged fermentation when urea (3.0 g l^{-1}) as a nitrogen source and K_2HPO_4 (0.20 g l^{-1}) as phosphate source was supplied in fermentation medium.

Key words: β-fructofuranosidase, mineral constituents, *Saccharomyces*, organic nitrogen, phosphate, submerged fermentation

Introduction

β-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. β-fructofuranosidases are intracellular as well as extracellular enzymes (Shafiq ***et al.***, 2002). Different organic nitrogen sources and their concentrations have a major effect on the ability of yeast to synthesize β-fructofuranosidase. 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. Haq ***et al.*** (2002) used peptone as sole nitrogen source along with yeast extract. Ashokkumar ***et al.*** (2001) used urea and yeast extract as nitrogen source and reported marked increase in β-fructofuranosidase production. Silveira ***et al.*** (2000) have worked out nitrogen regulation of yeast β-fructofuranosidase.

Application of suitable inorganic phosphate source in appropriate concentration is also a determining factor for β-fructofuranosidase production. Different phosphate sources such as Na_2HPO_4, $(NH_4)_2HPO_4$ and K_2HPO_4 have been reported to be in direct relation with synthetic abilities of yeast (Gomez ***et al.***, 2000). The objective of the study was to investigate β-fructofuranosidase synthesis by *Saccharomyces* species GCB-AII in shake flask. The effect of different organic nitrogen sources, concentration of urea, different phosphate sources and concentrations of K_2HPO_4 was studied.

Materials and Methods

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 and agar 20.0 g l^{-1}; peptone 5.0 g l^{-1} and yeast extract 3.0 g l^{-1} 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^{-1} wv^{-1}) sucrose 30.0; peptone 5.0 and yeast extract 3.0 at pH 6. The flasks were cotton plugged and autoclaved at 15 lbs $inch^{-2}$ 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 h. The agitation rate was kept at 200 rev min^{-1}.

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 $inch^{-2}$ pressure for 15 min 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 h. The agitation rate was kept at 200 rev min^{-1}. The flasks were run parallel in duplicates.

Analytical methods

Dry cell mass

Dry cell mass of yeast was determined by centrifugation of fermented broth at 5000 rev min^{-1} using weighed centrifuge tubes. The tubes were oven dried at 105°C for 1 h.

Sugar estimation

Sugar was estimated spectrophotometrically by DNS method (Tasun ***et al.***, 1970) using scanning spectrophotometer (CECIL CE-7200, UK) for measuring colour intensity. Transmittance was measured at 546 nm using Scanning Spectrophotometer.

β-fructofuranosidase activity

Enzyme activity was determined according to the method of Sumner and Howell (1935).

Results and Discussions

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). Maximum β-fructofuranosidase activity (132.35 U ml^{-1}) was obtained when urea was used as nitrogen source. Application of appropriate nitrogen source is very important for optimal production of β-fructofuranosidase. Silveira ***et al.*** (2000) used a mixed nitrogen source (1% yeast extract and polypeptone, 0.5% ammonium chloride) for best β-fructofuranosidase production in flask culture. More enzyme production was obtained in the following study when urea was used in the medium. The reason might be positive influence of urease and β-fructofuranosidase on each other's secretion into the culture medium because various extracellular enzymes produced by the yeast *Saccharomyces cerevisiae* enhance each other's secretion (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 (147.26 U ml^{-1}) was observed at urea concentration of 3 g l^{-1}. Sugar consumption and dry cell mass were 24.72 and 0.82 g l^{-1}, respectively. Lesser urea concentration is not enough to fulfill nutrient requirement of the yeast, thus yielding less enzyme. Concentration of urea higher than

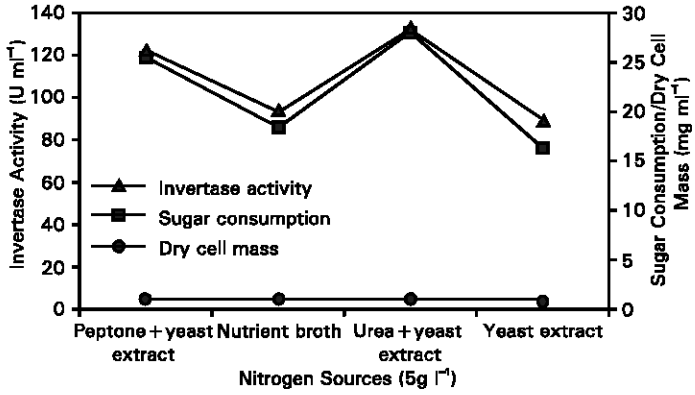

Fig. 1: Effect of organic nitrogen sources on the production of β-fructofuranosidase

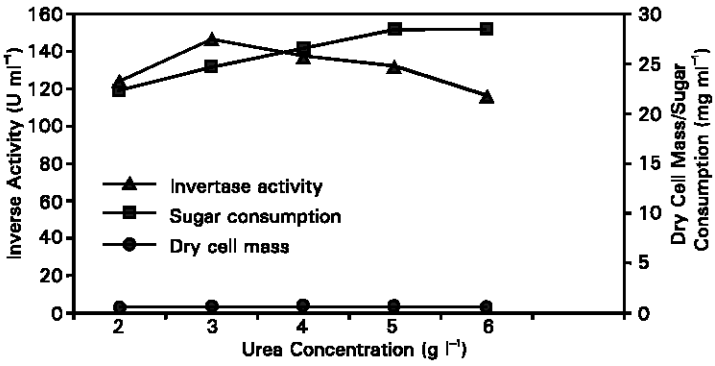

Fig. 2: Effect of urea concentration on the production of β-fructofruanosidase

optimum also produce less amount of β-fructofuranosidase. Higher concentrations of urea induce denaturation of β-fructofuranosidase (Pitombo ***et al.***, 1994). Ashokkumar ***et al.*** (2001) optimized concentration of urea and yeast extract as nitrogen source for β-fructofuranosidase production by submerged and solid state fermentation.

Effect of different phosphate sources

The effect of different phosphate sources (K_2HPO_4 and KH_2PO_4) on the production of β-fructofuranosidase by *Saccharomyces* species GCA-II was studied (Fig. 3). K_2HPO_4 was found to be the best phosphate source for maximal enzyme production. Dry cell mass and sugar consumption were 0.71 and 26.52 g l^{-1}, respectively. High enzyme activity might be obtained because phosphate was readily available to yeast cells when K_2HPO_4 was used as a phosphate source. Shafiq ***et al.*** (2002) optimized media for β-fructofuranosidase production and used K_2HPO_4 as phosphate source.

Effect of different concentrations of K_2HPO_4

The effect of different concentrations of K_2HPO_4 (0.10, 0.15, 0.20, 0.25 and 0.30 g l^{-1}) on the production of β-fructofuranosidase by *Saccharomyces* species GCA-II was studied (Fig. 4).

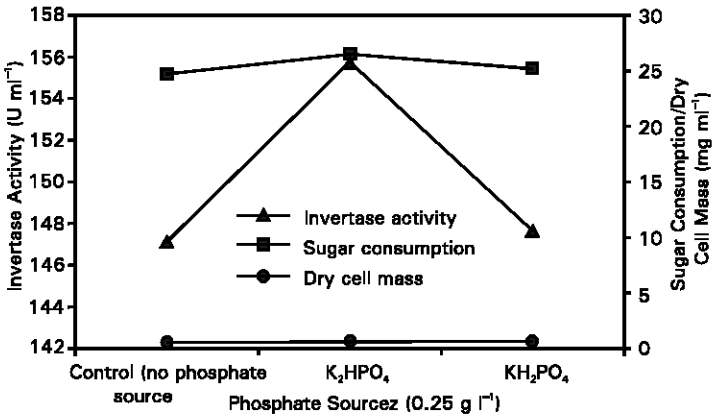

Fig. 3: Effect of phosphate sources on the production of β-fructofuranosidase

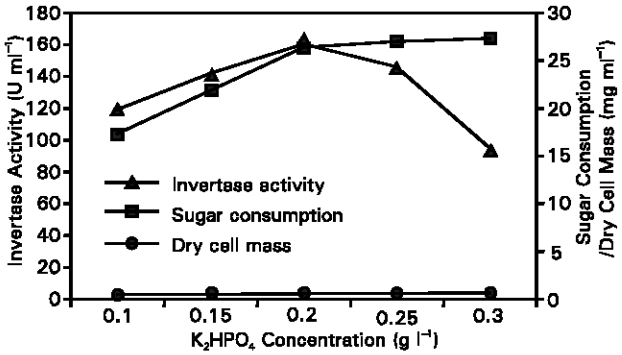

Fig. 4: Effect of K_2HPO_4 concentration on the production of β-fructofuranosidase production

Maximum enzyme yield was obtained at K_2HPO_4 concentration of 0.20 g l^{-1}. Dry cell mass and sugar consumption were 0.71 and 26.52 g l^{-1}, respectively. Final pH of the medium was 6.87. Amount of phosphate in the fermentation medium has great influence on β-fructofuranosidase production. Less amount of K_2HPO_4, cause nutrient limitation and improper growth of yeast, thereby reducing enzyme yield. Higher concentration of K_2HPO_4 result in overgrowth of ***Saccharomyces*** and an increase in alkalinity of the medium and resulting in less enzyme yield (Egorov ***et al.***, 2000).

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