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Effect of Different Mineral Nutrients on Invertase Production by *Saccharomyces cerevisiae* GCB-K5

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Abstract: The effect of different mineral nutrients was studied on the production of extracellular invertases by *Saccharomyces cerevisiae* GCB-K5. Nutritional studies were carried out in 250ml Erlenmeyer flasks by submerged fermentation. The culture medium (pH 6.0) was incubated for 48 hours at 30°C. The optimum levels of peptone and dipotassium hydrogen phosphate were found to be 4.0 and 0.2 g/l, respectively. Maximum invertase activity was found to be 12.68 U/ml. The amount of sugar consumed and dry cell mass were 21.08 and 5.88g/l, respectively.

Key words: Invertase, *Saccharomyces cerevisiae*, mineral nutrients, production, nitrogen and phosphorous limitation

Introduction

Invertases are special kind of enzymes that catalyze the hydrolysis of sucrose into glucose and fructose. Invertase finds uses 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. (Sanchez *et al.*, 2001). The organism showing greatest ability to secrete invertase is yeast (Moreno *et al.*, 1979; Silveira *et al.*, 2000). Different substrates can be used in submerged fermentation for the production of invertase (Gomez *et al.*, 2000). Different organic nitrogen sources and their concentrations have a major effect on the ability of yeast to synthesize the invertase. There exists a specific physiological response of sucrose metabolism to the presence of nitrogen source (Silveira *et al.*, 1996). The present study describes the relation between different nutrient sources and invertase secretion in submerged culture.

Materials and Methods

Organism and inoculum preparation: The project was performed in Biotechnology Laboratories, Govt. College, Lahore. in six months duration. Yeast strain *Saccharomyces cerevisiae* GCB-K5 was used for the production of invertase. The culture was maintained on sucrose (2%), yeast extract (0.3%), peptone (0.5%) and agar (2.0%) medium and stored at 4°C in the refrigerator. Inoculum was

prepared from 2-3 days old slant culture of *Saccharomyces cerevisiae* GCB-K5. Ten ml of sterilized distilled water was added to the slant having optimal growth. Cells were then scratched with sterilized inoculating needle and the tube was shaken gently. The number of cells (1.0×10^7 cells/ml) was counted with the help of Hemocytometer slide.

Fermentation conditions: Production of yeast invertase was studied by shake flask technique using 250 ml Erlenmeyer flasks. Twenty-five ml of fermentation medium (sucrose 30.0 g/l, peptone 5.0 g/l and yeast extract 3.0 g/l) was transferred to each cotton wool plugged Erlenmeyer flask. The flasks were sterilized in an autoclave at 15 lbs/inch^2 pressure (121°C) for 15 minutes and cooled at room temperature. The fermentation media was varied in composition according to parameters under study. Different nitrogen and phosphate sources were tested for their effect on the production of invertase. Nitrogen sources and phosphate sources were added at the rate of 0.5 and 0.015g/100 ml fermentation medium, respectively. Enhanced production of invertase in response to applied supplements was recorded. One ml of inoculum was aseptically transferred to each flask. Flasks were then rotated in a rotary incubator shaker (Model: GLSC 051.HR.196-11) at 30°C for 48 hours. The agitation rate was kept at 200 rev/min. After 48 hours, fermented broth was subjected for the estimation of invertase produced.

Analytical methods: "One invertase unit is defined as the amount of enzyme, which releases one milligram of inverted sugar in 5 minutes at 20°C, at pH 4.5". Invertase activity was determined according to the method of Sumner and Howell (1935). Dry cell mass of yeast was determined 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 was estimated colorimetrically by DNS method (Tasun *et al.*, 1970). Transmittance was measured at 546nm on photoelectric colorimeter (Model: AE-11M Erma, Japan).

Results and Discussion

Effect of different nitrogen sources: Nitrogen constituent has a profound effect on the production of invertase in culture medium because there exists a strong correlation between nitrogen equilibrium and productivity of yeast cells (Rouwenhorst *et al.*, 1991; Neto *et al.*, 1996). The data in Table 1 shows the effect of different nitrogen sources (Nutrient broth, peptone, yeast extract) on invertase production by *Saccharomyces cerevisiae* GCB-K5. Among all the nitrogen sources tested, peptone gave maximum production of invertase (8.75 U/ml). It enhanced the growth of yeast and in turn increased the invertase production. The dry cell mass and sugar consumption were 5.32 and 20.54g/l, respectively.

Effect of different concentrations of peptone: The effect of different concentrations of peptone

(0.30-0.60 g/100 ml) on the production of invertase was studied (Table 2). Maximum production of invertase (9.13 U/ml) was achieved at 0.40 g/100 ml level of peptone. Dry cell mass and sugar consumption were 5.61 and 20.60 g/l, respectively. Maximum production of invertase at optimized concentration of peptone might be due to favourable nutrient supply for yeast growth (Silveira *et al.*, 2000). At low concentration, less invertase production might be due to lower supply of nitrogen, which was insufficient for yeast growth. Dworschack and Wickerham (1960) used 0.5 g/100 ml peptone in culture medium for maximum invertase production.

Effect of different phosphate sources: The data in Table 3 shows the effect of different phosphate sources like NaH_2PO_4, $(NH)_4\ H_2PO_4$, KH_2PO_4, KH_2PO_4 and Na_2HPO_4 on invertase production by *Saccharomyces cerevisiae* GCB-K5. Among all the phosphate sources tested, K_2HPO_4 gave maximum production of invertase i.e., 12.04 U/ml. Dry cell mass and sugar consumption were 5.84 and 20.42 g/l, respectively. Final pH of medium was 5.8. Maximum production of invertase by using K_2HPO_4 as phosphate source might be due to fact that phosphate was readily available to yeast. Similar type of work was reported by Gomez *et al.* (2000).

Effect of different concentrations of K_2HPO_4: The data in Table 4 shows the effect of different concentrations (0.010-0.030 g/100 ml) of dipotassium hydrogen phosphate (K_2HPO_4) on the production of invertase by *Saccharomyces cerevisiae* GCB-K5. Maximum production of invertase (12.68 U/ml) was achieved when 0.020 g/100 ml K_2HPO_4 was used. The dry cell mass and sugar consumption were 5.88 and 21.08 g/l, respectively. Any increase or decrease in the phosphate concentration beyond optimum, greatly reduced the invertase units due to overgrowth or improper growth of yeast. At high concentrations, phosphate reduces cell mass and increases the alkalinity of medium, which is highly unfavourable for yeast growth and enzyme stability (Underkofler and Hickey, 1954).

It was estimated that the strain of *Saccharomyces cerevisiae* GCB-K5 gave 12.68 units of invertaseper ml of fermentation medium, when supplied with 4.0g/l peptone as nitrogen supplement

Table 1: Effect of organic nitrogen sources on the production of invertase by *Saccharomyces cerevisiae* GCB-K5.

Organic nitrogen sources (0.5 g/100 ml)	Dry cell mass (g/l)	Final pH	Sugar consumption (g/l)	Invertase activity (U/ml)
Nutrient broth	4.84	5.7	19.21	5.85
Peptone	5.32	5.8	20.54	8.75
Yeast extract	3.01	5.6	17.16	4.28

Sucrose concentration, 25 g/l; incubation period, 48 hours; temperature, 30°C; initial pH, 6.0; agitation rate, 200 rev/min.

Table 2: Effect of different concentrations of peptone on the production of invertase by *Saccharomyces cerevisiae* GCB-K5.

Concentration peptone (g/100 ml)	Dry cell mass (g/l)	Final pH	Sugar consumption (g/l)	Invertase activity (U/ml)
0.30	4.26	5.3	19.28	7.01
0.40	5.61	5.8	20.60	9.13
0.50	5.20	5.7	20.47	8.74
0.60	5.01	6.0	21.63	7.21

Sucrose concentration, 25 g/l; incubation period, 48 hours; temperature, 30°C; initial pH, 6.0; agitation rate, 200 rev/min.

Table 3: Effect of different phosphate sources on the production of invertase by *Saccharomyces cerevisiae* GCB-K5.

Phosphate sources (0.5 g/100 ml)	Dry cell mass (g/l)	Final pH	Sugar consumption (g/l)	Invertase activity (U/ml)
NaH_2PO_4	4.86	6.0	18.96	9.37
$(NH_4)_2HPO_4$	5.21	5.5	19.45	10.25
K_2HPO_4	5.84	5.8	20.42	12.04
KH_2PO_4	4.92	5.8	19.53	10.20
Na_2HPO_4	4.20	5.2	18.01	8.82

Sucrose concentration, 25 g/l; incubation period, 48 hours; temperature, 30°C; initial pH, 6.0; agitation rate, 200 rev/min.

Table 4: Effect of different concentrations of K_2HPO_4 on the production of invertase by *Saccharomyces cerevisiae* GCB-K5.

Concentration of K_2HPO_4 (g/100 ml)	Dry cell mass (g/l)	Final pH	Sugar consumption (g/l)	Invertase activity (U/ml)
0.010	4.03	5.6	19.16	9.66
0.015	5.78	5.8	20.50	12.08
0.020	5.88	5.9	21.08	12.68
0.025	4.63	6.0	19.34	9.82
0.030	3.82	6.2	18.82	8.64

Sucrose concentration, 25 g/l; incubation period, 48 hours; temperature, 30°C; initial pH, 6.0; agitation rate, 200 rev/min.

and 0.2 g/l dipotassium hydrogen phosphate as phosphate source n the fermentation m edium. The incubation temperature and initial pH were 30°C and 6.0, respectively while incubated for 48 hours. All these conditions were concluded as optimal for invertase biosynthesis by the yeast strain.

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