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SUBSTRATE-INDUCED REPRESSION OF INVERTASE SYNTHESIS BY *SACCHAROMYCES CEREVISIAE* IN SUBMERGED CULTURE

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Abstract

Invertase enzyme is used traditionally in the production of inverted sugars for industry, especially in the manufacture of candies and preserves, production of lactic acid and ethanol production from fermentation of cane sugar molasses. The present study deals with the substrate-induced changes in invertase formation by *Saccharomyces cerevisiae* KR_{18}. The maximal production of invertase during the course of study i.e. 5.63±0.9 U ml^{-1}, was achieved using initial sucrose concentration of 15.0 g l^{-1} after 48 h of fermentation. The sugar consumption and mycelial dry weight were 9.65±1.3 and 2.62±0.3 mg ml^{-1}, respectively. Higher concentrations of sucrose in fermentation medium induce catabolite repression of yeast invertase. All the kinetic parameters *i.e.*, product and growth yield coefficients ($Y_{p/s}$, $Y_{p/x}$ and $Y_{x/s}$), and specific rate constants, μ (h^{-1}) were highly significant.

Introduction

Saccharomyces cerevisiae produces an extracellular beta-D-fructofuranoside fructohydrolase (invertase) when grown on a medium containing the beta-fructofuranosides sucrose or raffinose, indicating that synthesis is subject to induction by the substrate. Expression of invertase in the yeast *Saccharomyces cerevisiae* is greatly delayed when derepression occurs in a medium that lacks a usable carbon source (Martinez & Estruch, 1996). *Saccharomyces* inverts sugar but inversion is often endocellular, without enzyme released into the medium. Kirillova (1997) observed the invertase activity of sucrose tolerant and osmophilic micromycetes. Strains with high invertase activity and capable of growing and developing on media containing 20, 30, 40 or more % of sucrose were found. Various sugars have been investigated for induction of invertase, but only the two beta-fructofuranosides were found to induce high production levels; with the other sugars, the enzyme was produced only at a low constitutive level. Sucrose is considered as the best source because yeast hydrolyses sucrose into glucose during growth to use glucose as substrate. It was found that invertase is more synthesized in medium supplemented with sucrose rather than glucose (Ashokkumar & Gunasekaran, 2002). In *Saccharomyces cerevisiae*, the expression of invertase, which is the hydrolyzing enzyme of sucrose, is controlled by the presence of monosaccharides, such as glucose and fructose, and referred to as carbon catabolite repression (Herwig *et al.*, 2001). Catabolite repression of invertase synthesis produced by glucose operates at the levels of transcription and translation and produces an increase in the rate of mRNA degradation (Elorza *et al.*, 1977). Present study deals with the optimization of inducible level of sucrose for enhanced production of yeast invertase.

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Organism and culture media: *Saccharomyces cer*evisiae KR_{18}, isolated locally at Government College University, Lahore, Pakistan, was used for invertase production by submerged fermentation. Yeast culture was maintained on sucrose-yeast extract-peptone-agar medium (Sucrose 20.0 g l^{-1}, Peptone 5.0 g l^{-1}, Yeast extract 3.0 g l^{-1} and Agar 20.0 g l^{-1}) at initial pH 6.0.

Vegetative inoculum and fermentation: Cell suspension was prepared from 2-3 days old slant culture of yeast strain. Twenty-five ml of the medium containing (g l^{-1}, wv^{-1}) sucrose 30.0; peptone 5.0 and yeast extract 3.0 at pH 6, was transferred to each 250 ml Erlenmeyer flask. 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 cell suspension (1.2×10^3 cells) from the slant culture was aseptically transferred into the growth medium. The flask was incubated at 30°C in an incubator (Gallenkamp, UK) for 12 h. The agitation rate was kept at 200 rev min^{-1}. The vegetative inoculum was transferred (1.0 ml per 250 ml) to the production medium, same as used for growth medium. 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^{-1}. The flasks were run parallel in duplicates.

Assay protocol: 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 one hour. Supernatant was used for further analysis. Sugar was estimated spectrophotometrically by DNS method (Tasun *et al.*, 1970). A UV/VIS spectrophotometer (Cecil-700, UK) was used for measuring % color intensity at 546 nm. Invertase activity (saccharolytic) in supernatant was assayed as described by Sumner & Howell (1935) based on dinitrosalicylic acid method a test for reducing sugar determination: 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.

Kinetics and statistical studies: The kinetics of the research work was studied after Pirt (1975). Statistical analysis of the data were determined following the procedures of Snedecor & Cochran (1980). Standard deviation among the replicates was presented in the form of probability (< p >) values.

Results and Discussion

The initial sugar concentration plays an important role in determining the maximum amount of enzyme produced and also residual sugars produced after hydrolysis by *Saccharomyces cerevisiae* (Haq *et al.*, 2002). Initial sucrose concentration was varied from 5.0 g l^{-1} to 40.0 g l^{-1}. *Saccharomyces cerevisiae* strain KR_{18} secreted optimal amount of invertase (5.63±0.9 U ml^{-1}) in the medium containing 15.0 g l^{-1} sucrose (Fig. 1). The sugar consumption and mycelial dry weight were 9.65±1.3 and 2.62±0.3 mg ml^{-1}, respectively. Further increase in concentration of sugar resulted in the gradual decrease in product formation. It might be due to repeated budding of yeast cells, which resulted in more viscous medium and less efficient mineral availability. A concentration higher than 15.0 g l^{-1}, however, lead to greater amount of residual sugars, making the process uneconomical, while on the other hand a lower concentration of sugar lead to lower enzyme secretion due to accumulation of hydrolysed monosaccharides. Accumulation of monosaccharides in medium causes glucose repression of invertase enzyme.

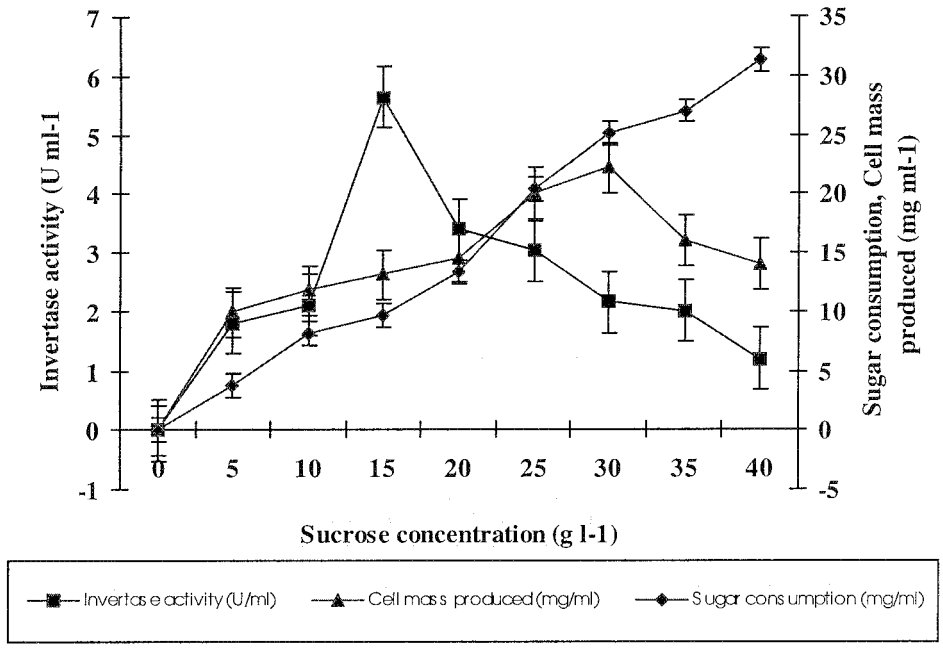

Fig. 1. Inductive effect of sucrose concentration on invertase production by *Saccharomyces cerevisiae*.

Y error bars indicate the standard error of means among the three parallel replicates. The values differ significantly at $p \leq 0.05$.

The values for kinetic parameters i.e., $Y_{p/s}$, $Y_{p/x}$, and $Y_{x/s}$ were more significant at 15.0 g l^{-1} initial sucrose level than all other concentrations (Fig. 2). It is evident from the results that increase in sucrose concentration has a repressive effect both on product formation and cell mass production in relation with sugar consumption. Glucose, hydrolysis product of sucrose by yeast for its growth, plays an important regulatory role in the yeast *Saccharomyces cerevisiae*, which is mostly reflected at the transcriptional level by glucose repression. The signal that initiates glucose repression is unknown, but data indicate that it is located at or above the level of glucose 6-phosphate, suggesting the involvement of either the intracellular or extracellular glucose concentration or the glucose flux in triggering glucose repression (Meijer *et al.*, 1998). Figure 3 shows the comparison of specific product and growth rates, μ (h^{-1}) at different concentrations of sucrose. The more significant value of the μ (h^{-1}) was calculated at 15.0 g l^{-1} sucrose level. Pejin & Razmovski (1993) investigated the influence of sugar concentration in nutrient media on the specific growth rate and biomass yield in the course of continuous fermentation of *Saccharomyces cerevisiae*. It was found that an increase of sugar content in media decreased the specific growth rate and the biomass yield. Sugar concentration has significant effects on protein and phosphate contents of cells. Shafiq *et al.*, (2002) reported 25 g l^{-1} sucrose concentration for optimal production of invertase by *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* KR_{18} is more industrially feasible strain based on the fact that use of lower amount of sugar in fermentation medium makes process more economical.

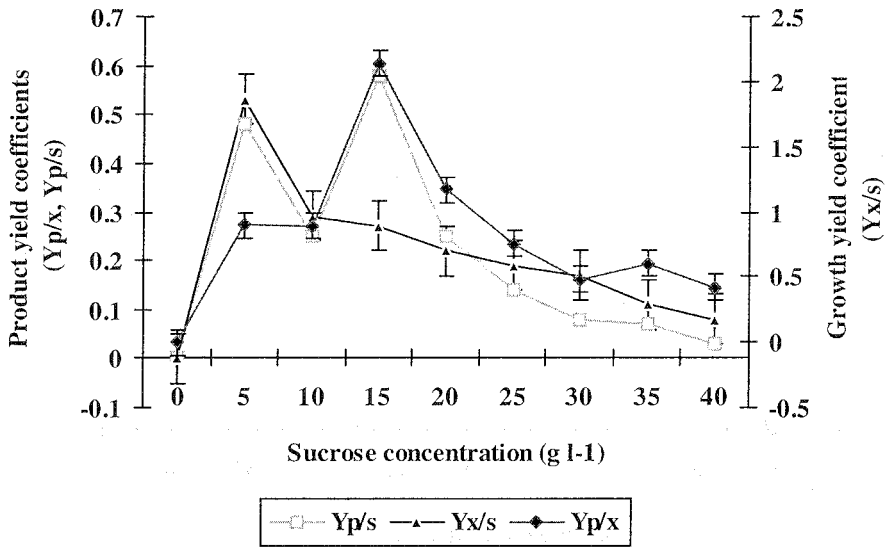

Fig. 2. Comparison of product and growth yield coefficients for invertase production by *Saccharomyces cerevisiae*.

Kinetic parameters

$Y_{p/s}$ = Amount of enzyme produced mg^{-1} substrate consumed; $Y_{p/x}$ = amount of enzyme produced mg^{-1} cell mass; $Y_{x/s}$ = mg cell mass formed mg^{-1} substrate consumed. Y error bars indicate the standard error of means among the three parallel replicates. The values differ significantly at $p \leq 0.05$.

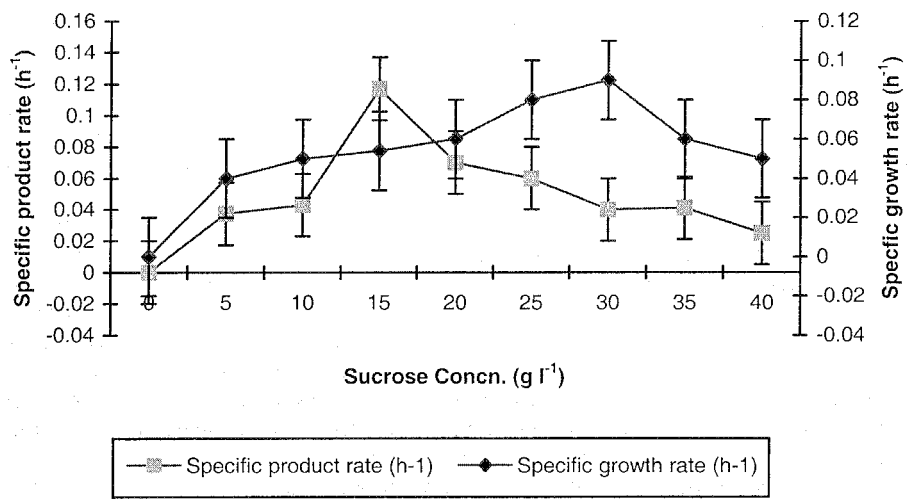

Fig. 3. Comparison of specific rate constants for invertase production.

Kinetic parameters
Specific growth rate, μ (h^{-1}) = g cell mass produced ml^{-1} min^{-1}
Specific product rate, μ (h^{-1}) = amount of enzyme produced ml^{-1} min^{-1}
Y error bars indicate the standard error of means among the three parallel replicates. The values differs significantly at $p \leq 0.05$.

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