
COMPARATIVE ANALYSIS OF THE INVERTASE ACTIVITY BY *Saccharomyces cerevisiae* ISOLATED FROM CANE JUICE WITH STANDARD INDUSTRIAL STRAIN

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Abstract

Microorganisms play a major role in solving various problems of the human needs in many ways. Invertase is one of the industrially valuable enzyme products, commonly obtaining from microorganisms. Invertases are intracellular as well as extracellular enzymes. The enzyme has wide range of commercial applications like, the production of confectionery with liquid. It also aids fermentation of cane molasses into ethanol. One of the best industrially applicable microorganism, is the beneficial yeast namely *Saccharomyces cerevisiae*. The organism has found its application in the field of brewing, baking, and also in medicine. The present study is focused on the isolation of the *Saccharomyces cerevisiae* from the sugar cane juice and comparison of its invertase activity along with that of the yeast strains got from the fermentation industries. The comparison was made with different parameters such as immobilized cells and the mutated cells. The study discloses the activity of invertase in relation to mutation and immobilization increased with respect to the time. Furthermore the invertase activity shows that the organisms subjected to mutation could be used for a large scale fermentation process on par with quick and efficient fermentation with relatively more productivity. These results showed for further analysis in the immobilization and or mutation mechanisms against *S. cerevisiae* for getting more metabolic product with low cost.

Keywords: Invertase, Cane Juice, Immobilization, Enzyme Activity, Sucrose.

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1. INTRODUCTION

It finds its application in scientific and engineering principles to the used in industries and improvement of the product produced by microbes. Industrial microorganisms have many commercial applications as they are used metabolic products like organic acids, acetone, enzyme, and many drugs. Though the microbes used in the industries provide a good outcome with beneficial results, the strain improvement and maintenance is yet to be devised with much better plans for getting a broad spectrum of useful products. On this basis the microorganisms are constantly being scrutinized for its ever changing characteristics and are being developed into better strains which would serve the process well for future industrial needs. These are unicellular, non chlorophyllous, spherical, saprophytic fungi

which reproduce by asexual reproduction (budding, and binary fission). They are used for the purpose of fermentation process in biopharmaceuticals.

Invertases are intracellular as well as extracellular (Nakano, et al., 2000). The enzyme has wide range of commercial applications like, the production of confectionery with liquid. It also aids fermentation of cane molasses into ethanol. Microbial invertase activity is used for the manufacture of cattles feed and food for honeybees (Weber, 20008). Invertase is a yeast derived enzyme and the official name for invertase is β -fructo furanosidase. Invertase is mainly used in the food (confectionery) industry where fructose is preferred over sucrose because it is sweeter and does not crystallize as easily (Sanchez and Gorbach, 2001). However, the use of invertase is rather

limited because another enzyme, glucose isomerase, can be used to convert glucose to fructose more inexpensively. For health and taste reasons, its use in food industry requires that invertase be highly purified.

Many microorganisms such as *Neurospora crassa*, *Candida utilis*, *Fusarium oxysporium*, *Phytophthora meganosperma*, *Aspergillus niger*, *S. cerevisiae*, *Schizosaccharomyces pombe*, and *Schwanniomyces occidentalis* produce invertase (Zarate and Belda, 1996). *Saccharomyces cerevisiae* is the organism of choice for invertase production because of its characteristic high sucrose-fermenting ability. A wide range of microorganisms produce invertase and can, thus, utilize sucrose as a nutrient. Commercially, invertase is biosynthesized chiefly by yeast strains of *S. cerevisiae*.

The present study deals isolation, characterization of *S. cerevisiae* from the sugar cane juice, and comparison of its metabolic product invertase with biopharmaceutical yeast strain invertases.

2. MATERIALS AND METHODS

Sterilization:

As per the Good Laboratory Practices (GMP) all glasswares were prepared and kept under aseptic condition.

Isolation and Identification of *S.cerevisiae* from Sugarcane Juice

The Sabouraud dextrose agar (SDA) plates were prepared and the culture *S. cerevisiae* was inoculated in to it using the standard spread plate technique. About 0.1 ml of the sample was placed at the centre of the solid agar surface and then spread uniformly with a sterile 'L rod' in a circular fashion. The plates were then incubated at 30°C for 1 day. Efficient invertase producer *S.cerevisiae* was isolated by using sucrose broth. First a loop full of culture was inoculated into the sucrose broth and after the incubation period of 3 days the broth was tested for invertase activity by boiling the sample with Benedict's reagent (green or brick red color indicates positive result). The strains

were identified using Grams' staining and Lactophenol cotton blue staining and the budding yeasts cells were observed under microscope.

Assay for Invertase Produced by Immobilized Yeast:

Column Method

The column of 1 m × 1.5 mm was taken. Sucrose broth of different concentration such as 1, 2, and 3% were prepared and added into the column slowly. 100 mL of the immobilized organism from the production medium was added into the column and aerated. The product was extracted for every 72 h of different concentration of sucrose solution as mentioned earlier. The product was assayed for various compounds using following methods.

Qualitative Analysis of Sugars by Benedicts Test:

About 5 mL of Benedict's reagent was taken in a five clean test tubes. Eight drops of product concentration such as 1, 2, and 3% were added into the three test tubes. Eight drops of substrate was added into one of the tube as a control. It was boiled for 100°C. The color change of the solution to the heat was observed and recorded.

Quantitative Estimation of Sugar by Folin Wu Method

A protein free filtrate of whole sample heated with tartarate solution. Glucose from the sample reduces the cupric ions in the soluble cupric tartrate to cuprous oxide produced was measured by the reduction of phosphomolybdate to molybdenum blue. The intensity of the blue color produced was proportional to the glucose in the sample and compared with the color given by a standard glucose (Zech and Goerish, 1995).

Identification of Sugars Using Thin Layer Chromatography (TLC)

Reagents

The sugars were identified using TLC method in which 100 mg of glucose is dissolved in 1 ml of distilled water. The samples used for

the identification are standard sugar, 1% product, 2% product, and 3% product. Butanol, acetic acid and water (2:1:1 ratio) was used as solvent system. The spraying reagent is 10% H₂SO₄ in methanol.

Purification and Characterization of Invertase

Crude extract was precipitated by 70% saturation with ammonium sulphate and then dialyzed against 100 mM Tris phosphate buffer (pH 7.5) for 24 h at 4°C.

Estimation of Protein by Lowry's Method:

The method combines the reactions of copper ions with the peptide bonds under alkaline conditions with the oxidation of aromatic protein residues. The Lowry's method is best used with protein concentrations of 0.01–1.0 mg/mL and is based on the reaction of Cu⁺, produced by the oxidation of peptide bonds, with Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid in the Folin-Ciocalteu reaction). The reaction mechanism is not well understood, but involves reduction of the Folin reagent and oxidation of aromatic residues (mainly tryptophan, also tyrosine). The concentration of the reduced Folin reagent is measured by absorbance at 750 nm. As a result, the total concentration of protein in the sample can be deduced from the concentration of Trp and Tyr residues that reduce the Folin reagent.

3. RESULTS AND DISCUSSION

The cane juice samples were serially diluted and inoculated on to the SDA plates and incubated. The industrial yeast strain, got from the industries was serially diluted and inoculated on to SDA plates and incubated. Optimum growth was observed at plates incubated at 37°C for one day. The morphological and cultural characteristic of the samples are similar to the standard *S. cerevisiae* (Table 1).

The organism was cultured in the SDA plates were analyzed for its invertase activity by inoculating it in the various concentrations of

sucrose broth. About 1, 2 and 3% of sucrose broth concentration was used for the inoculation and the estimation of the glucose produced in the broth as a result of fermentation due to invertase. Sucrose broth concentration was taken as 1, 2, and 3% respectively for the invertase enzyme activity and was analyzed using Benedict's method against industrial, wild and immobilized strains. In such case the industrial strains showed good sucrose utilization than the wild strain. Whereas, when industrial strain and immobilized strains were compared for the sucrose utilization the immobilized strains showed good sucrose utilization of about 1, 1.5, and 2% in a broth concentration of 1, 2.5 and 3% respectively. In the case of wild strain there is no change in sucrose concentration, and it remained the same in all broth concentration.

Table 1: Cultural characteristics of *S. cerevisiae* from cane juice.

Morphological and cultural characteristics	<i>S. cerevisiae</i> (std. strain)	<i>S. cerevisiae</i> (cane juice)
Colony morphology on SDA agar	Creamy white, umbonate, raised, circumscribed, dry colonies	Creamy white, umbonate, raised, circumscribed, dry colonies
Motility	Non motile	Non motile
Gram staining	Violet colored stout rods in clumps were found	Violet colored rods in clumps were found
LPCB staining	Slightly thin, oval shaped, budding organisms were found.	Stout, oval shaped, budding organisms were found.
Turbidity on SD Broth	Uniform Turbidity	Uniform Turbidity
Germ tube test	Negative	Negative

Ikram-ul-Haq et al. (2005) isolated yeast strains from dates available in a local market. Five hyper producing yeast strains (>100-fold higher invertase activity) were kinetically analyzed for invertase production. *Saccharomyces cerevisiae* strain GCA-II was found to be a better invertase-yielding strain

than all the other isolates. The values of Q_p and Y_p/s for GCA-II were economical as compared to other *Saccharomyces* cultures. The effect of sucrose concentration, rate of invertase synthesis, initial pH of fermentation medium and different organic nitrogen sources on the production of invertase under submerged culture conditions was investigated. Optimum concentrations of sucrose, urea and pH were 3%, 0.2 (w/v), and 6 respectively. The increase in the enzyme yield obtained after optimization of the cultural conditions was 47.7%.

Ballou, (1995) studied the invertase activity of the *S. cerevisiae* by mutating it for various time intervals and then assessing the invertase activity by the determination of free glucose level using the Anthrone method (Jagannathan et al., 2010). It was found that yeast cells after mutation failed to produce invertase due to aberration caused by the mutation. There was only about 36 mg/dL of glucose present in the sucrose broth after mutation which was greatly lesser than the non mutated strains that produced 280 mg/dL of glucose in the sucrose broth. The industrial strains convert sucrose into glucose and fructose efficiently than the wild strains. The concentration of glucose is high in broths inoculated with industrial strains than in broths inoculated with wild strains (Table 2).

Table 2: Thin Layer Chromatography analysis of invertase activity

Samples	Distance travelled by solute	Distance travelled by solvent	R_f Value
Standard invertase	1.6	5	0.32
Wild strain	1.5	5	0.30
Industrial strain	1.5	5	0.30
Immobilized wild strain	1.5	5	0.30
Immobilized industrial strain	1.5	5	0.30

Immobilization was performed using the sodium alginate and calcium chloride solutions, in such a way that the isolated yeast cells were entrapped in the gel matrix of calcium alginate.

The glucose level of the immobilized cells isolated from the cane juice was about 268 mg/dL in a 3% sucrose broth concentration. This shows that the amount of the invertase activity has slightly increased after immobilization. The glucose concentration of the immobilized industrial strains in the 3% sucrose broth medium was about 306.3 mg/dL which is evident from the Folin Wu method. This shows that the industrial strains were more capability of the invertase activity after immobilization, than the isolated strains. The glucose concentration in the 1% and 2% sucrose broth was of less significance for both the industrial and the isolated yeast strains as the variation was of a mere value.

In the present study 3% sucrose broth inoculated with mutated yeast strains, of various degree of exposure (1 min, 3mins, and 5mins) showed a gradual increase in the invertase activity which was evident from the increase in glucose concentration in various sucrose broth concentration from 1% to 3%. The amount of the glucose present in the sucrose broth of 5mins mutated strains after fermentation was 404 mg /dL and was greater than the wild strain which showed a glucose concentration of 240 mg/dL. This showed that the invertase activity had increased with the increase in the mutation period.

When the industrial strains were mutated for the same time interval and the invertase activity was assayed, it was noted that the 5 mins mutated industrial strains showed a glucose concentration of 417.8 mg/dL in the 3% sucrose broth concentration while the non mutated industrial strains showed about 266.6 mg/dL. This shows that the mutated industrial strains showed a twofold increase in the invertase activity which was evident from the glucose level. Silverira (Silverira et al. 1996) performed the mutation studies on the *S. cerevisiae* and did the estimation of glucose for the determination of the invertase activity as the enzyme hydrolyzed the sucrose in to glucose and fructose in the medium. When whole yeast cells are used in these assays, the monosaccharides formed by the action of the

periplasmic enzyme can be taken up and metabolized, leading to errors on the enzyme activity determination activity, under initial rate conditions was performed using cell concentrations up to 64 mg cell/ml. The results obtained showed that this method is particularly useful for cells with low invertase activity. In the present study the enzyme level for the wild, industrial and mutated *S. cerevisiae* were found to be 0.21 µg/ml, 0.32 µg/ml, 0.40 µg/ml respectively. This shows that the mutated strains have produced higher amount of enzyme which is obvious from the level of the enzyme estimated using Lowry's method. The amounts of the invertase present in the samples were determined as follow. The amounts of the protein present in the wild strain samples were found to be 0.26 µg/ml. The amount of the protein present in the industrial strain sample is found to be 0.32 µg/ml.

Sanchez et al. (2001) determined the amount of the invertase present in the sucrose broth using the Lowry's method. They found that the amount of the invertase produced by the yeast isolated from the sugar cane molasses were found to be in range from 0.24 to 0.46µg/ml.

After the determination of subsequently the proteins are retardation force value by thin layer chromatography (Ranjith et al., 2010) for the validation of extracted invertase with standard invertase. The Rf value of standard invertase is 0.32. The wild, industrial, immobilized wild and immobilized strains Rf values where near to the standard Rf values which is 0.30.

4. CONCLUSION

Invertase is one of the industrially valuable enzyme products, commonly obtained from microorganisms. The present investigation followed methodologies developed for enhancing the invertase production from *S. cerevisiae* using immobilization and mutation. It can therefore be concluded that the present

study on the invertase activity in relation to mutation and immobilization increased with respect to the time. Furthermore the invertase activity shows that the organisms subjected to mutation could be used for a large scale fermentation process on par with quick and efficient fermentation with relatively more productivity. These results showed for further analysis in the immobilization and or mutation mechanisms against *S. cerevisiae* for getting more metabolic product with low cost.

5. REFERENCES

1. Ballou C. (1995). *Schiosaccharomyces Pombe* mutants that is defective in glycoprotein galacto sylation. *Proceedings of the National Academy of Science of the United States of America*. 92 (7), 2790-2794.
2. Ikram-ul-Haq; Mirza Ahsen Baig; Sikander Ali. (2005). Effect of cultivation conditions on invertase production by hyperproducing *Saccharomyces cerevisiae* isolates. *App. Environ. Microbiol.* 21, 487-497.
3. Jagannathan, S.; Vijayakumar, R.; Rahul Gandhi, P.; Anandhi, M.; Chandra, C.; Premkumar, A.; Venkataramana, K.N. (2010). Analysis of carbohydrates in newly developed liquid state rabies vaccine. *J. Micro. Biochem. Technol.* 2 (6), 147-151.
4. Nakano, et al. (2000). Effective cultivation condition on invertase production by hyper producing *S. cerevisiae*. *World J. micro.* 43, 69-80.
5. Ranjith Kumar, R.; Vijayakumar, R.; Sathish Kumar, R.; Veerappan, C.; Jagannathan, S.; Rahul Gandhi, P. (2010). Isolation, purification and analysis of free radical scavenging activity of flavanoids and alkaloids from *Indigofera longercacemosa* Linn. *J. Med. Sci. Res.* 1 (2), 63-68.
6. Sanchez Silva, M.; Gorbach, S.L. (2001). Industrial activity of invertase produced by the yeast cells isolated from different fermented food. *J. Food Sci.* 20, 698-721.
7. Silverira M.C.F. et al. (1996). Assay for in vivo yeast invertase activity using NaF. *Ana Biochem.* 238(1), 26-28.
8. Weber; Roitsch. (2000). Enzyme activity of invertase on the different substrate. *J. Mycology.* 51, 619-623.
9. Zarate, V.; Belda, F. (1996). Characterization of the heterologous invertase produced by *Schizosaccharomyces pombe* from the SUC2 gene of *Saccharomyces cerevisiae*. *App. Bacter.* 80 (1), 45-52.
10. Zech, M.; Goerish, H. (1995). Invertase from *Saccharomyces cerevisiae*; reversible inactivation by components of industrial molasses media. *Enz. Micro. Tech.* 17 (1), 41-46.