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Production of invertase enzymes from *Saccharomyces cerevisiae* strain isolated from sugarcane and grape juices

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ABSTRACT

Aim of the present studySaccharomyces cerevisiaewas isolated from sugarcane and grape samples by using the standardize immobilization and non-immobilized technique. To test the sucrose hydrolysis activity ofimmobilized and non-immobilized form of Saccharomyces cerevisiae in sucrose broth, and quantitative and qualitative analysis of glucose by using Benedict's method, Folin Wu method and Thin layer Chromatography. The isolated strain is better than the MTCC strain or similar then it could be possible to get a fresh stain for the industrial application at least after following strain improvement techniques. In conclusion the grape and sugarcane juice can be more potent used as invertase for the production of enzymes by using standard technique viz,immobilized and non-immobilized.

Keywords: Sucrose, Invertase, *Saccharomyces cerevisiae*, Grape, Industry.

INTRODUCTION

Immobilized cells currently whole cells are gaining importance as a source of immobilized enzymes. Whole cells can be immobilized either in a viable or non- viable form. An important limitation in the utilization of whole cells as an intracellular source of enzyme is the diffusion of substrate and products through the cell membrane. One of the ways to obviate this problem is to use Permeabilization cells. Alternatively in the case of periplasmic enzymes such as invertase and Catalyst in yeast whole cells can be used as a source of enzymes without Permeabilization. The immobilization of biocatalysts can be immobilized either through adsorption, entrapment, covalent binding, cross-linking or a combination of all three techniques. Invertase can be immobilized via its carbohydrate moiety. Invertase or β-fructofuranosidase (EC 3.2.1.26) can be immobilized by using solution of 10g sodium alginate [1]. Yeast cells could be immobilized on to wool by treating either the yeast cells or wool or broth with gluteraldehyde [2]. Recombination *Saccharomyces cerevisiae* cells with invertase activity were immobilized in liquid-core alginate capsules [3]. The enzyme invertase catalyses the cleavage of the disaccharide sucrose to the monosaccharaides glucose and fructose. Glucose and fructose are reducing sugars which yield a deep red colour when reacted with Benedict's reagent. Sucrose is a non-reducing sugar and therefore develops no red coloration with Benedict's reagent. Sucrose commonly known as alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1, 4-glucosidic bond. When this bond is cleaved in a hydrolysis reaction, an equimolar mixture of glucose and fructose is generated. This mixture of monosaccharide is called as invertase (or) invert sugar and enzyme that

hydrolysis is called sucrose. Commercially fructose and glucose syrups are produced from sucrose by the action of invertase. Invertase which occur in higher plant tissues are mostly extracellular or in soluble form. In plant tissues invertase are usually classified as acid, neutral or alkaline depending on the basis of the range required for their maximum activity. The acid invertase is widely distributed in plants such as beet root, carrot, potato and red beet root whereas acid neutral type of invertase have been detected in sugarcane. However both acid and alkaline invertase has been isolated from soybean nodules. The highest enzyme activities can be attained at pH-4, DO-4mg 0-2/L and Temperature 35-45^{0}C [4]. In the present study production of invertase enzymes from *Saccharomyces cerevisiae* strainisolated from sugarcane and grape juices by using standard techniques like immobilized and non – immobilized.

MATERIALS AND METHODS

The sugarcane and grape juice were collected from in and around Vellore district. To isolation of *Saccharomyces cerevisiae* from sugarcane juice and grape juice for enzyme invertase production. The samples were inoculated in to SDA media for isolation. The SDA plates were prepared and culture *Saccharomyces cerevisiae* was inoculated into it. The MTCC (Microbial Culture Collection Centre, Chandigarh) Strain of *Saccharomyces cerevisiae* (3252) was also analysed by following methods.

Isolation of efficient invertase producer
Efficient invertase producer (*Saccharomyces cervisiae*) was isolated by inoculation of culture into sucrose broth. After the incubation period of three days the broth was tested for invertase activity by boiling the sample with Benedict's reagent (green to brick red colour indicates positive result).

Identification of *Saccharomyces cerevisiae* Gram's staining
A loopful broth cultures was subjected to Gram's staining produced and observed microscopically under oil immersion objective. Grams positive budding yeast cells were observed.

Lacto phenol cotton blue staining
A loopful of broth culture was subjected to Lacto phenol Cotton Blue Staining producer and observed under high power objectives. Budding yeast cells were observed.

Quantitative estimation of sugar [Benedict's method]
Take a 5ml of Benedict's reagent in clean test tube then add eight drops of concentration such as1% 2% 3% into the three test tubes. And eight drops of substrate was added into one of the tube as a control. Finally it was boiled at 100^{0}C.

Quantitative estimation of sugar [Folin Wu method]
A protein free filtrate of whole sample heated with in alkaline tartrate 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 colour produced was proportional to the glucose in the sample and compared with the colour given by a standard glucose.

Identification of sugars using thin layer chromatography (TLC)
Silica gel G plates were prepared by using distilled water. Standard sugars 100mg glucose dissolved in 1ml – distilled water (loaded 5 X 1 micro litre). Sample 3% substrate 1% product 2% product 3% product.

Solvent system (v/v)
N – Butanol/ Acetic acid / Water (2:1:1)

Spray reagent
Sprayed with 10% H_2SO_4 in methanol [Sugar gave black colour].

RESULTS

In the present study immobilized and non – immobilized forms of *Saccharomyces cerevisiae* were assessed to identify its invertase productivity. The immobilization was carried out using sodium alginate entrapped method. For

testing the product of invertase (i.e., glucose and fructose) initially sucrose hydrolysis test was used conducted. Various concentrations (1% 2% 3%) of sucrose broth medium were used for both techniques like immobilized and non - immobilized cells. When increasing the sucrose concentration, the amount glucose productivity also increased in both of us immobilized and non – immobilized forms after 3 days of incubation period. However the amount of glucose level varied among the two forms. Higher amount of glucose production was observed in immobilized forms.

Further thin layer chromatography , Qualitative sugar analysis [Benedict's method] and Quantitative sugar analysis [Foli Wu method] were also conducted to identify the invertase activity in both immobilised forms and non-immobilized forms of *Saccharomyces cerevisiae* produced more invertase than the non-immobilized forms. The reason for the enhanced productivity of invertase in immobilized forms of *Saccharomyces cerevisiae* may be due to the entrapment of the organisms. The increases in the substrate without break were produce continuously with equilibrium. Similar findings were also observed. **Table 1, 2** shows that the immobilized cells converts sucrose into glucose and fructose efficiently than the non- immobilized cells. The concentration of glucose is high in broths inoculated with immobilized cells than in broths inoculated with non-immobilized cells. The production of invertase isolated strain are present in the **Table 3, 4.**

Table: 1-Glucose Qualitative Analysis (Benedicts Method) For Invertase Activity of Isolated Strain of *Saccharomyces Cerevisiae*

S.No.	Sucrose concentration	Immobilized form		Non-immobilized Form	
		Colour	Glucose concentration	Colour	Glucose Concentration
1.	1%	green	1%	no change	-
2.	2%	brick red	1.5%	green	1%
3.	3%	orange	2%	green-red	1.5%

Table: 2-Glucose Quantitative Analysis (Folin Wu Method) For Invertase Activity of isolated Strain of *Saccharomyces cerevisiae*

S.NO	Sucrose broth concentration	OD Value				Amount of glucose(mg/dl)	
		Before fermentation		After fermentation			
		Standard	Test sample	Standard	Test sample	Before fermentation	After fermentation
1.	1%	0.15	0.07	0.15	0.11	93.3	146.6
2.	2%	0.15	0.09	0.15 0.15	0.16	120.0	220.0
3.	3%	0.15	0.10		0.22	146.6	293.3

Table: 3-Glucose Qualitative Analysis (Benedict's Method) For Invertase Activity ofMTCC Strain of*Saccharomyces cerevisiae*

S.No.	Sucrose Concentration	Immobilized Form		Non Immobilized Form	
		Colour	Glucose Concentration	Colour	Glucose Concentration
1.	1%	Green	1%	No Change	1.5%
2.	2%	Brick Red	1.5%	Green	2%
3.	3%	Orange	2%	Green-Red	25%

Table: 4-Glucose Quantitative Analysis (Folin Wu Method) For Invertase Activity of MTCC Strain Of*Saccharomyces cerevisiae*

S.NO	Sucrose broth concentration	OD Value				Amount of glucose(mg/dl)	
		Before fermentation		After fermentation			
		Standard	Test sample	Standard	Test sample	Before fermentation	After fermentation
1.	1%	0.15 0.15	0.07	0.15	0.18	123.3	176.6
2.	2%	0.15	0.09	0.15 0.15	0.21	160.0	260.0
3.	3%		0.10		0.32	196.6	343.3

DISCUSSION

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents provide good and services [6]. The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological application of capabilities of microorganisms, cultured tissues and biochemical cultured cells. Industrial biotechnology is a part of biotechnology deals with study of microorganism used in industries and improvement of the product produced by microbes [7]. Industrial microorganisms have many

commercial applications they are used in the synthesis of products like organic acids , acetone, enzyme, and many drugs. Immobilization technique for yeast cells was standardized comparative studies were conducted between immobilized form and non-immobilized forms of *Saccharomyces cerevisiae* in related with their invertase production. Invertase is one of the industrially valuable enzyme produce commonly obtaining from microorganisms [8].

In the current investigation following methodologies was developed for enhancing the invertase production from *Saccharomyces cerevisiae*. The concentration of the sucrose broth medium was directly influenced the invertase formation (i.e glucose formation) from yeast cells. Among the three sucrose concentrations (1% 2% and 3%) maximum amount of invertase product formation was observed at sucrose level. Among the two forms of *Saccharomyces cervisiae* (Immobilized and non-immobilized forms), higher amount invertase product formation was observed in immobilized form. Qualitative analysis (Benedict method) and quantitative (Folin Wu method) were revealed. That immobilized forms of *Saccharomyces cerevisiae* was best for higher amount of invertase production than non-immobilized forms. However, the MTCC strain was identified as superior one compared to the isolated strain both in immobilized and non-immobilized form. But, after subjecting the isolated strain to various kinds of strain improvement form. But, after subjecting the isolated strain to various kinds of strain improvement technique such as, mutation etc., it could be possible that the isolates could be converted into a standard industrial strain.

CONCLUSION

In the present study reveals thatgrape and sugarcane juice can be more potent used as invertase for the production of enzymes by using standard technique *viz*,immobilized and non-immobilized

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