Isolation Screening and Optimization of Invertase Production under Submerged Fermentation

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Abstract: **- Invertase producing 17 isolates of yeast were isolated and characterized from different sample of fruits in which isolate R5 give maximum invertase production. The enzyme activity reached to maximum when incubation time was 48 hrs, and pH 5. Different Carbon and Nitrogen source were investigated for the enzyme production and sucrose and yeast extract was found to be best Carbon and Nitrogen source for invertase production. Incubation temperature 30ºC, inoculum size 5%, were found to be optimum temperature and inoculam size for invertase production.**

Key word**: Invertase, Yeast, Sucrose, Submerged fermentation.**

I. INTRODUCTION

Invertase is one of the beneficial enzyme that provides many products for industrial purpose such as pharmaceutical, food etc. (Guimaraes et al., 2007). Invertase (β.D. fructofuranosidase, E.C. 3.2.1.26) cleaves α-1,4 glycosidic linkage between α-D-glucose and β-D-fructose molecules of sucrose by hydrolysis and releases monosaccharides (Li *et al.*, 1998; Mobini-Dehkordi et al., 2008). The enzyme attacks beta-D-fructofuranoside (sucrose, raffinose, stachyose and inulin) from the fructose end (Rubio *et al*., 2002; Rubio and ; Gore *et al.*, 2009).

Invertase catalyzes the hydrolysis of sucrose into two equimolar mixtures of glucose and fructose. The resulting mixture of fructose and glucose is called inverted sugar syrup which is sweeter than sucrose (Talekar *et al.,* 2010). Alternative name for invertase include saccharase, glucosucrase, beta-h-fructosidase, invertin, sacrase, maxinvert L1000, fructosylinvertase, alkaline invertase, acid invertase and the systematic name: beta-fructofuranosidase (Hubert., 2007).

Invertases are enzymes that are used in food industries for the production of fructose syrup from sucrose solutions (Husain *et al.*, 1996). Invertase being major part (95 %) of the total enzyme is located chiefly outside the cytoplasmic membrane (Lampen *et al.,* 1967).Various microorganism like fungi, bacteria, yeast produce invertase in huge amount. The most common species produce invertase are *Arthrobacterglobiformis* (Win *et al.,* 2004), *Lactobacillus reuteri* (de Gines *et al.,* 2000), *Neurosporacrassa*, *Phytophthorame ganosperma* (Nishizawa *et al.,* 1980), *Pichiaanomal* (Perez *et al.,* 2001), *Kluyveromyces fragilis* (Workman and Day., 1983), *Saccharomyces cerevisiae* (Andjelkovic *et al*., 2010).

II. MATERIAL AND METHOD

Reagents and Media

Agar (Hi Media, India), Sucrose (Rankem Mumbai ,India) Yeast extract(High Purity Laborites Chemical Pvt. Ltd), Peptone (Hi Media Laborites Pvt. Ltd.), Sodium acetate (Rankem , Mumbai ,India), DNSA (HPLC Pvt. Ltd., Mumbai) used were of analytical grade. All this reagent and media were prepared in distilled water.

Isolation and Screening of yeast strains

Different environmental samples i.e. fruits like apple, plum, peach, date, banana, mango and grapes collected from Vapi Gujarat – 396195, India. The environmental samples were suspended in sterile water and appropriate dilutions were plated by spread plate technique in the glucose yeast extract plate containing (gm/liter) yeast extract 5g, Peptone 10g, agar 32g, and pH 5. The inoculated plates were incubated at 30°C for 24-48 hours. The isolated culture were further purified by sub culturing on GYE plate .The purified culture was further confirmed by gram staining and lacto phenol cotton blue staining.

Submerged Fermentation

The medium used for enzyme production under submerged fermentation was Sucrose yeast extract peptone medium. The Erlenmeyer flask (250 ml) containing 100 ml of the SYE medium was autoclaved at 121°C and 15 lbs pressure for 15 min and cooled at room temperature. The sterilized fermentation flask inoculated with 5% inoculum of the isolated yeast strain and incubated at 30ºC, 150 rpm for 48 hrs. The samples were collected at regular intervals and centrifuged at 5000 rpm for 20 min. The cell-free supernatant was used as the source of crude invertase enzyme

Preparation of inoculums

The inoculum was prepared in 25 ml of sucrose yeast extract peptone medium containing (gm/liter) sucrose 30.0, peptone 5.0 and yeast extract 3.0 at pH 6. A single colony of yeast from the slant was aseptically transferred into the 25 ml medium. The flask was incubated at 30°C at 150 rpm for 24 h.

A 5% inoculum with 1 O.D. was used to inoculate in fermentation flasks.

Enzyme Assay

Invertase activity was determined using the method of Sumner and Howells(Sumner and Howell, 1935) with slight modification by incubating 1 ml of enzyme solution with 2 ml of sucrose in 0.1M acetate buffer (pH 5.0) and incubated at 55°C for 10 minute. To stop the reaction, 1ml of the dinitrosalicylic acid reagent was added and heated for 10 minutes in boiling water bath. Finally, the absorbance was read at 540 nm in spectrophotometer (Miller, 1959). One unit of enzyme activity (IU) is defined as the amount of enzyme which liberates 1 micro mole of glucose/minute/ml under standard assay condition.

Optimization of invertase production

The present work involves optimization of different parameters governing invertase production. The effects of various nitrogen sources, carbon source, incubation temperature, etc on invertase production were examined by one factor at a time method.

Effect of time period on invertase production

The production medium Sucrose yeast extract peptone medium at pH 5 was inoculated with 5% of the inoculums of selected yeast strain. The broth was incubated for different time period (0h, 6h, 12h, 18h ,24h ,30h,36h ,42h, 48h) at 30°C with shaking at 100rpm. The samples were withdraw at regular time interval, centrifuge at 5000 RPM for 20 min and supernatant was used as crude enzyme source.

Effect of pH on invertase production

Effect of initial pH of the medium on invertase production was studied by adjusting the pH of the production medium in the range of 3-9 using 1 N sterile NaOH and 1N sterile HCL after sterilization. Then each flask was inoculated with 5% of the inoculum and incubated at 30°C for 48 hrs under shaking conditions at 100 rpm. The samples were collected after 48 hrs of incubation and centrifuged at 5000 rpm for 20 min. The cell-free supernatant was used as the source of crude invertase enzyme.

Effect of temperature on invertase production

Effect of temperature on protease production was studied by incubating the production medium with 5% of the inoculums at different temperatures including 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, for 48 hours under shaking conditions at 100 rpm. The samples were collected after 48 hrs of incubation and centrifuged at 5000 rpm for 20 min. The cell-free supernatant was used as the source of crude invertase enzyme.

Effect of carbon source on invertase production

Various carbon sources including fructose, maltose, lactose, glucose, sucrose, mannitol and galactose were used in production media to asses the effect of carbon source on invertase production media. The flasks were inoculated with 5% of the inoculum and incubated at 30°C for 48 h with shaking at 100 rpm. The samples were collected after 48 hrs and centrifuged at 5000 rpm for 20 min. The cell-free supernatant was used as the source of crude invertase enzyme. The samples were collected after 48 hrs of incubation and centrifuged at 5000 rpm for 20 min.

Effect of nitrogen source on invertase production

Effect of different nitrogen sources including yeast extract, peptone, beef extract, ammonium sulphate, urea, ammonium chloride, and sodium nitrate was determined by replacing peptone in the production medium. A control is represented with peptone and yeast extract use as nitrogen source was also performed. Each flask was inoculated with 5% of the inoculum and incubated at 30°C for 48 hrs under shaking conditions at 100 rpm. The samples were collected after 48 hrs of incubation and centrifuged at 5000 rpm for 20 min. The cell-free supernatant was used as the source of crude invertase enzyme.

III. RESULT AND DISCUSSION

Isolation and screening of invertase producing organisms

The environmental samples were collected from Vapi Gujarat – 396195, India used for isolation of invertase producing yeast. Appropriate dilution of the sample were plated on GYE plate and incubated for 48 hrs. A total of 17 yeasts culture were isolated in pure form and characterized for their morphological characteristics.

Table 1: Morphological and cultural characteristic of isolates from environmental sample.

Sample	Isolate	Colony characteristics	Motility	Gram staining	Enzyme activity (U/ml/min)
Apple	R1	Creamy white, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	1.8
Plum	R2	Creamy white umbonate, raised, circumscribed, dry colonies	Non –motile	Gram positive stout rods	1.65
Peach	R3	Creamy white, umbonate, raised,	Motile	Gram positive cocci	1.2

		circumscribed, dry colonies			
Date	R4	Creamy white umbonate, raised, circumscribed, dry colonies	Non –motile	Gram positive stout rods	1.78
Grapes	R5	Creamy white, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	2.03
Banana	R6	Creamy white, irregular, smooth, moist colonies	Non- motile	Gram positive stout rods	1.25
Mango	R7	Creamy white, umbonte, raised, circumscribed, dry colonies	Motile	Gram negative rods	1.1
Orange	R8	Creamy white, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	1.602
Tomato	R9	Creamy white, umbonate, raised, circumscribed, dry colonies	Motile	Gram positive stout rods	1.32
Sugar dump(soil)	R10	Creamy white ,regular ,raised, dry colonies	Motile	Gram positive cocci	1.0
Sugar industries(soil)	R11	Creamy white, umbonate, raised, circumscribed, dry colonies	Non –motile	Gram positive stout rods	1.63
Keera	R12	Creamy white, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	0.5
Curd1	R13	Creamywhite, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	0.9
Pickle	R14	Creamy white, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	0.932
Idly	R15	Creamy white, umbonate, raised, circumscribed, dry colonies	Non- motile	Gram positive stout rods	0.863
Spoilage fruits	R16	Creamy white, umbonate, raised, circumscribed, dry colonies	Motile	Gram negative short rods	1.5
Curd2	R17	Creamy white, umbonate, raised, circumscribed, dry colonies	Motile	Gram positive stout rods	1.1

Isolates were identified on the basis of characteristic features such as Creamy white, umbonate, raised, circumscribed, dry colonies. From LPCB stating slightly thin, oval shaped budding yeast are observed in the microscope (Ikram-ul-Haq et al., 2005).

From 17 isolates, screened 1 isolate R5 gives maximum invertase activity 2.03U/ml/min after 48 hrs .Therefore isolate No R5 was selected for further studies.

Effect of time course on invertase production

Time course study is one of the most critical factors, which governs the value of the process along with product formation. The invertase production was increased as incubation time increase and maximum amount of invertase was observed at 48 hrs of incubation 2.0623U/ml/min. However, further incubation of fermentation flask result in decreased invertase production. The maximum amount of invertase production was observed in 48 hours incubation time (Fig. 1).

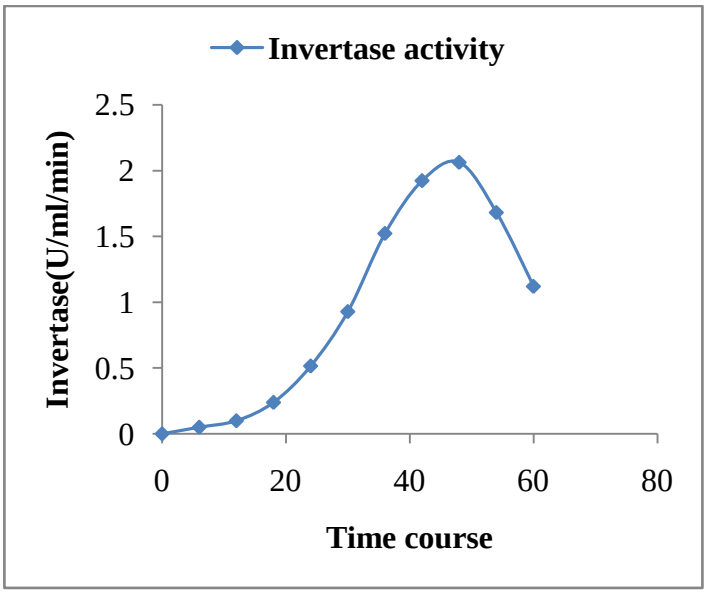

Fig. 1 Effect of time course on invertase production

Effect of pH on invertase production

In order to determine optimum pH for invertase production. The invertase production was assesed at pH range 3-10. The invertase production after 48 hours of incubation period at 30°C maximum amount of invertase production was achived at pH 5.0, 3.56U/ml/min. And minimum invertase production was achived at pH 10. Similarly, Kaur and Sharma, (2005) reported that the initial pH 5.0 gave the best invertase activity by an *actinomycetes strain*. Ul-Haq and Ali, (2003) also specified that the maximum production of invertase was obtained while early pH of the fermentation medium was retained at 6.0.

A less enzyme production at higher developed pH was due to blocked enzyme secretion from the yeast cells (Costaglioli *et al.*, 1997). In contrast, Uma *et al.* (2012) reported maximum invertase activity recorded at pH 6.0 by *Cladosporium cladosporioides*.

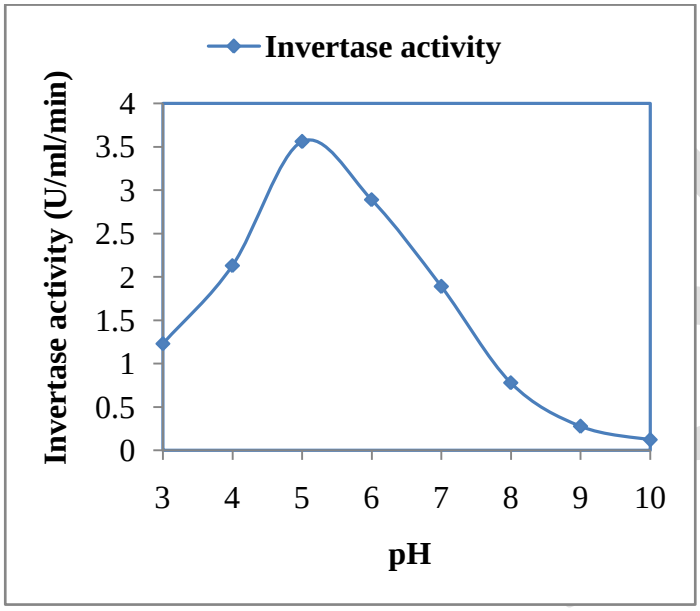

Fig.2 Effect of pH on invertase production

Effect of temperature on invertase production

The effect on incubation temperature on invertase production shows that as incubation temperature increased, invertase production was increased and maximum invertase production was observed at 30°C 3.21U/ml/min. However, further increase in tempreture results in decreased invertase production. Similarly, temperature 30°C for invertase production was also reported as optimum temperature for invertase activity by *Saccharomyces cerevisiae* NRRLY 12623 (Nooman *et al.*, 2009). Higher temperature caused decrease in rate of invertase production due to thermal denaturation of enzyme as reported by Resa *et al.* (2009).

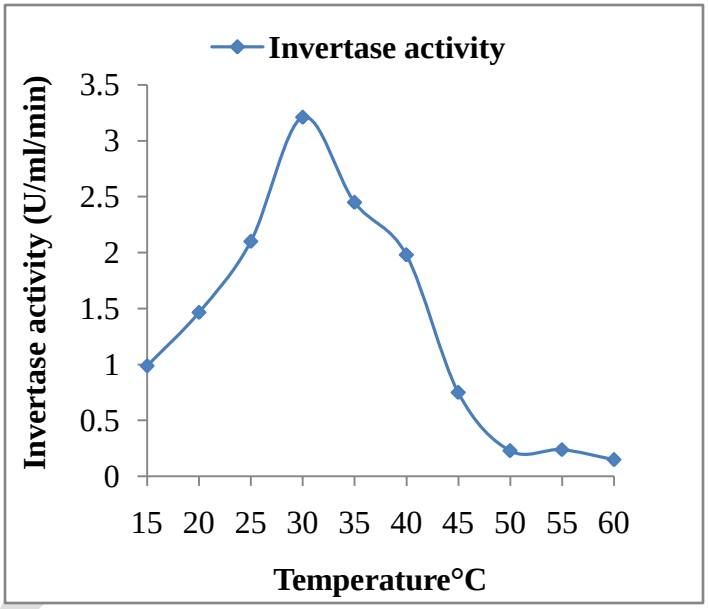

Fig. 3 Effect of temperature on invertase production

Effect of carbon sources on invertase production

The effect of carbon sources on invertase production by yeast after 48 hours of incubation period at 30°C is given. In figure the maximum invertase production was recorded in sucrose 3.75U/ml/min supplemented medium. The minimum invertase production was recorded in lactose added medium. The similar result was also reported by (Cairns *et al.*, 1996) that invertase production was induced when sucrose used as a 'C' source.

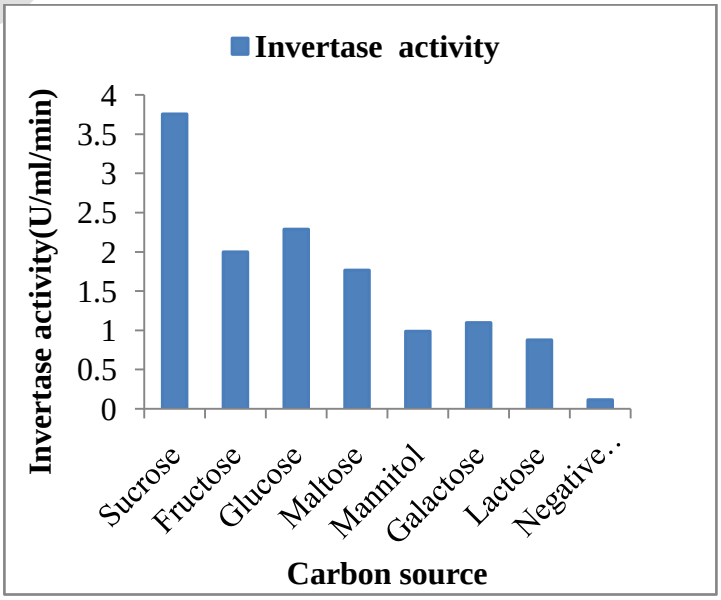

Fig.4 Effect of carbon sources on invertase production

Effect of nitrogen sources on invertase production

The effect of different nitrogen sources on invertase production after 48 hours of incubation period at 30°C showed maximum amount of enzyme production in yeast extract supplemented medium and minimum amount of invertase production in urea supplemented medium. In contrast Shafiq *et al*. reported that among all the nitrogen source tested peptone gave maximum production of invertase activity using *saccharomyces cerevisae* under the temperature of 30°C and pH 6.0 and agitation rate 200 rpm.

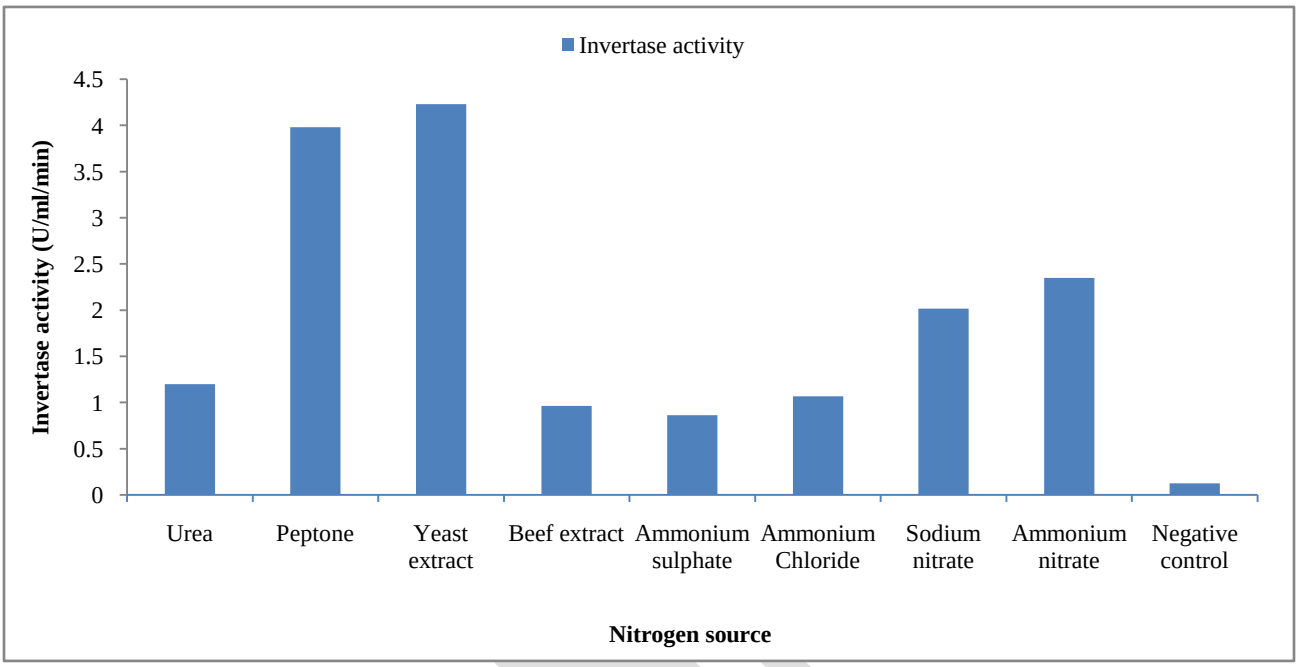

Fig. 5 Effect of nitrogen sources on invertase production

IV. CONCLUSION AND SUMMARY

This study indicates the potential of food wastes such as spoilage grapes as efficient source of invertase producing yeast. Optimum temperature and pH for invertase production was achived at pH 5 and temperature 30°C. The best 'C' and 'N' source for invertase production was found to be sucrose and yeast extract respectively.

ACKNOWLEDGEMENT

I would like to thank to my principle and all the faculty members of **K.B.S. commerce & Nataraj Professional Science College** for providing all the laboratory facilities and for their support provided at all the steps of this study.

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