Research Paper

ISSN: 2321-1520

Isolation, Production, and Optimization, of Invertase from *Pichiakudriavzevii*.

Varsha S Makwana, Rajput K N and Dr. R R Panchal*.

Department of microbiology and biotechnology, University school of sciences,
Gujarat University, Ahmedabad,Gujarat.
E-mail:panchalrrce@yahoo.com

*Corresponding Author

Received Date: 10-1-2019

Published Date: 15-3-2019

Abstract

In present study invertase producing yeast were isolated from various fruits samples.Total 16 invertase producing organisms were isolated of which *Pichiakudriavzevii*is the choice of organism for invertase production because of its characteristic high fermentability.*Pichiakudriavzevii*produced 2.02 U/ml invertase. Optimization of production conditions were studied, where 37^{o}C temperature, 6.0 pH, Glucose as carbon source, peptone as nitrogen source, incubation period of 24 hoursand 2.0 % inoculum size could produce 2.79 U/ml invertase.

Keywords: Invertase, Submerge fermentation, *pichiakudriavzeii*,

Introduction

Enzymes are complex globular proteins found in living cells, acting as a bio-catalyst facilitating metabolic reactions in an organism's body. Enzymes catalytic nature is responsible for the functioning. In 1878 Kuhne coined the term 'enzyme' from the Greek word, "enzumas", which

refers to the leavening of bread by yeast. Catalytic properties of a bifunctional enzymes is responsible for the molecular arrangement of chemical compound and get involves in a reaction without being consumed in the reaction, produce excessive rate of product conversion by slow down the Gibb's free energy required for the conversions to occur, Due to their precise nature enzymes can discriminates between chemical compound with identical structures and can induce reactions over a broad range of temperatures[0°C - 110°C] and in the pH range [2 - 14]. The primary source of energy in all living organisms is carbohydrates. Even non-reducing disaccharides like trehalose or sucrose also have other roles like acting as signaling molecule as well as stress protectants [SamarthKulshresth*a et al,* 2013]. Monosaccharides produced by microbial enzyme hydrolysis are more preferred because of high functionalities like similar taste as of sucrose and good bulking properties. The ofûcial name for Invertase is beta-fructofuranosidase (EC.3.2.1.26), which implies that the reaction catalyzed by the enzyme, is the hydrolysis of the terminal non-reducing beta-fructofuranoside residues in beta-fructofuranosides. Invertase is widely distributed among the biosphere. Invertase is characterized in plants and microorganisms. *Saccharomyces cerevisiae* commonly called Baker's yeast is the important strain used for the production of Invertase.*Saccharomyces cerevisiae* is found in wild growing, on the skin of grapes and other fruits [SamarthKulshresth*a et al,* 2013 and Romero-Gomez S *et al,* 2000]. Generally, microorganisms like *S. cerevisiae, Candidautilis, A. niger* are considered ideal for their invertase production study. The other common species produce invertase are *Aspergillusochraceus, Aspergillusniger, Aspergillus japonicas, and Thermomyceslanuginosu* [A.C.P. Alegre*et al* 2009], *Saccharomyces cerevisiae*and *Saccharomyces carlsbergensis, Penicillium*[F. Veana*et al,* 2011], *Candida utilis, Fusariumoxysporium, Phytophthorameganosperma*, Schwanniomycesoccidentalis [M. C. Silveira *et al*, 2000]. An equimolar mixture of fructose and glucose (invert syrup) obtained by sucrose hydrolysis by invertase is sweeter than sucrose due to high degree of sweetness of fructose, as a result the sugar content can be increased without crystallization of the material [Uma C *et al* 2010]. Growth conditions have a great influence on invertase production capacity of *Saccharomyces cerevisiae* [K. Shafiq*et al,* 2004].Cultivation conditions are essential in successful production of an enzyme and optimization of parameters such pH, temperature and media composition is important in developing the optimum fermentation

conditions [T.Sivakumar1 *et al*, 2013]. There is a broad range of attributes i.e. temperature resistance and optimum pH, accessible for selection and there are chances of greatly optimizing enzyme production by strain preference and growth parameters.

Material and Methods

Isolation of Invertase producing Microorganisms:

For Isolation of Invertase producing microorganisms 'Glucose Yeast Extract Agar' medium was used for yeasts and 'Nutrient agar' medium was used for bacteria. Isolation was done using serial dilution method. Different ripped fruits such as pineapple, orange, banana, papaya, apple, grapes, kiwi, sapodilla, sugar apple, and guava are collected from the Gandhinagar and Ahmedabad fruit markets.

Primary screening of Invertase producing microorganisms in broth medium:

Invertase production:

In the screening process 100 ml GYE and Nutrient broths were taken in each 250 ml Earlyen Mayer flasks and sterilized at 121 ^{o}C at 15 lb. pressure for 30 minutes in autoclave. The sterilized mediums were inoculated by 1.0 ml suspensions of 0.5 OD at 600 nm prepared from overnight grown yeast and bacterial isolates on slant. All the flasks were kept on the rotary shaker at 150 rpm at 30°c for 24 – 72 hrs.

Enzyme Preparation:

At the interval of every 24 hrs. 10 ml of the broth was removed in a sterile test tube under aseptic condition. The sample was centrifuged at 10000 rpm for 10 minutes and supernatant was collected as a crude enzyme. From this crude enzyme invertase production was measured using DNSAmethod.

Invertase activity:

Invertase activity was determined using the method of Sumner and Howells with slight modification by incubating 0.5 ml of enzyme solution with 0.5 ml of sucrose (1 gm. %) and 1.0 ml 0.1M acetate buffer (pH 5.0) which was incubated at 55 °C for 10 minute [Sumner, J.B *et al*, 1935]. To stop

the reaction, 1ml of the dinitro salicylic acid (DNS) reagent was added and heated for 10 minutes in boiling water bath, cooled to room temperature and diluted with 9.0 ml of distilled water. Finally, the absorbance was read at 540 nm in spectrophotometer [Shall S *et al,* 1971].

Unit of Invertase:

One unit of Invertase activity (IU) is defined as the amount of enzyme which liberates 1 µ mole of reducing sugar/minute under standard assay conditions [Reena C *et al,* 2016].

Optimization of Invertase production at shake flask:

Many factors that influence invertase production from isolated yeast culture had been studied, which includes various carbon sources, concentration of carbon source, various nitrogen sources, initial pH of the medium, inoculum size.

Optimization of carbon source:

Various carbon sources including Fructose, Glucose, Sucrose, Mannitol and Soluble starch were added in production medium (GYE broth) at 2.0 gm. % to assess the effect of carbon source on invertase production. The flasks were inoculated with 1% of the inoculum and incubated at 30°C for 24 h with shaking at 150 rpm. After 24 hours of incubation samples were centrifuged at 10000 rpm for 10 min. The cell-free supernatant was used as the source of crude invertase enzyme. The enzyme activity was checked by DNSA method [Reena C *et al,* 2016].

Optimization of effect of concentration of glucose on Invertase production:

Different concentration of Glucose such as 2 gm.%,4gm%,6gm%,8gm% and 10gm% were added to production medium (GYE broth) to test their ability to induce invertase production at shake flask level. The flasks were inoculated with 1% of the inoculum and incubated at 30°C for 24 h with shaking at 150 rpm. After 24 hours of incubation samples were centrifuged at 10000 rpm for 10 min. The cell-free supernatant was used as the crude invertase enzyme. The samples were used for DNSA test [Reena C *et al,* 2016, P. Suresh *et al,* 2012].

Optimization of effect of Nitrogen on Invertase production:

Effect of different nitrogen sources including yeast extract, peptone, 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 1% of the inoculum and incubated at 30°C for 24 hours under shaking conditions at 150 rpm. After 24 hours of incubation samples were centrifuged at 10000 rpm for 10 min. The cell-free supernatant was used as the source of crude invertase enzyme. The samples were used for DNSA test [Reena C *et al,* 2016].

Optimization of 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 4-8 using 1 N sterile NaOH and 1N sterile HCL after sterilization. Then each flask was inoculated with 1% of the inoculum and incubated at 30°C for 24 hours under shaking conditions at 150 rpm. After 24 hours of incubation samples were centrifuged at 10000 rpm for 10 min. The cell-free supernatant was used as the source of crude invertase enzyme [Reena C *et al,* 2016]

Optimization of effect of Inoculum Size on Invertase production:

Different concentration of inoculum level such as 0.5, 1, 1.5, 2, and 2.5 ml were tested for their ability to induce invertase production in the production medium [K. Shafiq*et al* 2003]. Each flask was incubated at 30°C for 24 hours under shaking conditions at 150 rpm. After 24 hours of incubation samples were centrifuged at 10000 rpm for 10 min. The cell-free supernatant was used as the source of crude invertase enzyme [Reena C *et al,* 2016].

Results and Discussion

Isolation and screening of invertase producing organisms

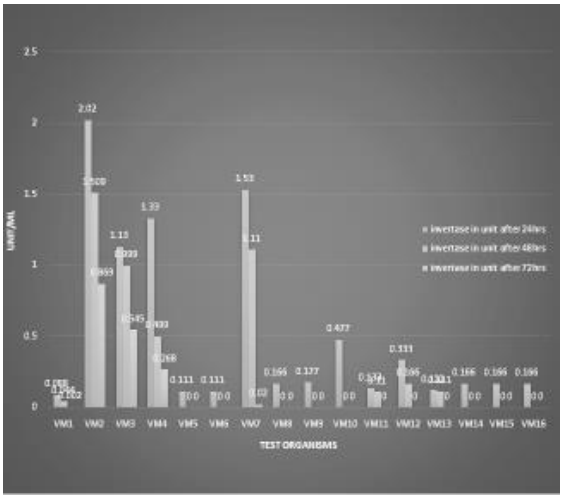
The smples were collected from Gandhinagar Gujarat, India used for isolation of invertase producing yeast. Appropriate dilution of the samples were plated on GYE agar plates and incubated at 30 °C for 72 hours. Total 16 Organisms were isolated in pure form.

Morphological characteristics of isolates:

No of Isolates	Colony characteristics	Motility	Gram's staining and morphology	Invertase Production after 24hrs of incubation in Units/ml	Invertase Production after 48hrs of incubation in Units/ml
VM1	Creamy white, umbonate, raised, dry colonies	Motile	Gram positive short rods	0.088	0.044
VM2	Creamy white, umbonate, raised, dry colonies	Motile	Gram positive short rods	2.02	1.509
VM3	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	1.13	0.999
VM4	Creamy white, umbonate, raised, dry colonies	Motile	Gram positive short rods	1.33	0.499
VM5	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	0.111	0.00
VM6	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	0.111	0.00
VM7	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	1.53	1.11

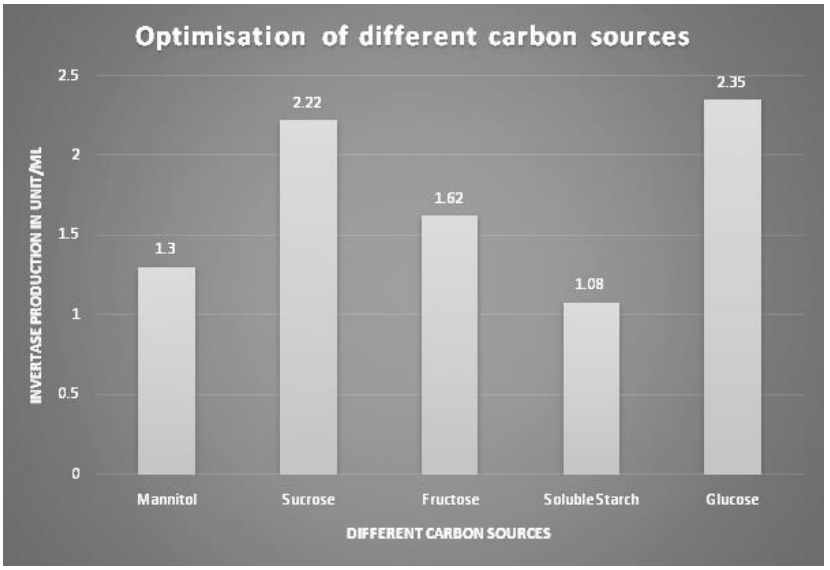
VM8	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	0.166	0.00
VM9	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	0.177	0.00
VM10	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	0.477	0.00
VM11	Creamy white, umbonate, raised, dry colonies	Motile	Gram positive short rods	0.133	0.111
VM-12	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive short rods	0.333	0.166
VM13	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive cocci	0.122	0.111
VM14	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive cocci	0.166	0.00
VM15	Creamy white, umbonate, raised, dry colonies	Non-motile	Gram positive cocci	0.166	0.00
VM16	Creamy white, umbonate, raised, dry colonies	Motile	Gram positive cocci	0.166	0.00

Invertase production by isolated cultures by submerged fermentation at shake flask level:



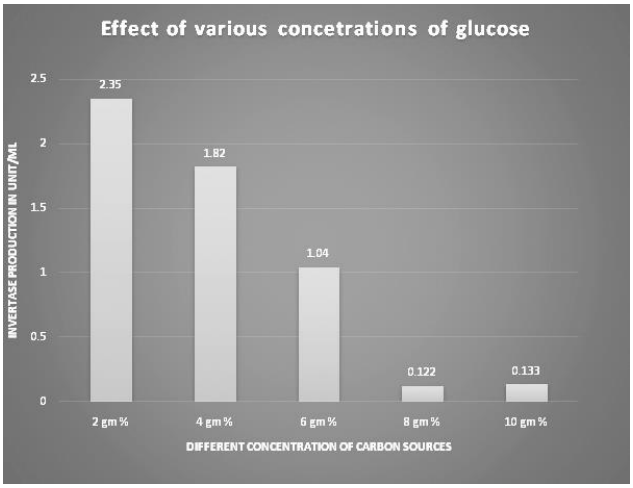
All the isolates are screened for invertase production and from these isolates VM2, VM3, VM4 and VM7 found to be the higher invertase producers. These four isolates were screened for further work. After 48 hours Invertase production was decreased so, for further work 24 hours of incubation wasconsidered.

Optimisation of different carbon sources for Invertase production using VM2 strain:



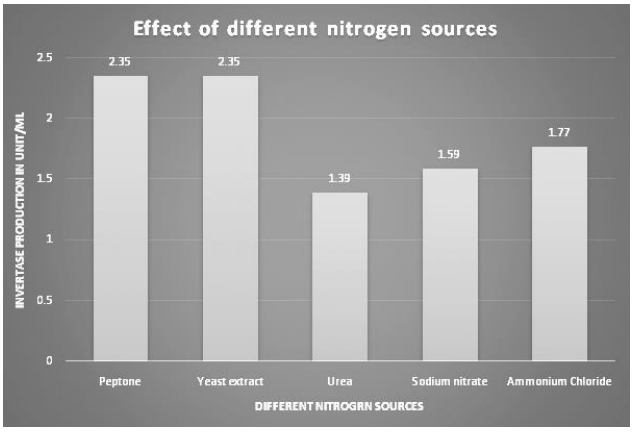
The effect of different carbon sources on invertase production by isolated yeast culture (VM2) after 24 hours of incubation at 30°C was checked. As shown in figure-3 the maximum invertase production of 2.35 U/ml was recorded when 2.0 gm % of Glucose was added in place of sucrose to GYE medium.

Effect of various Concentrations of glucose on Invertase Production:



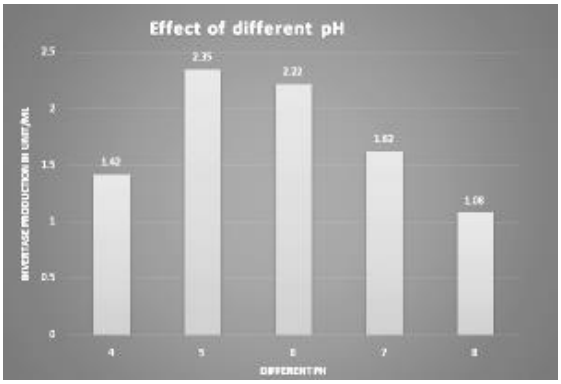
The effect of various concentrations of carbon source on invertase production was studied at shake flask level. 2.35 U/ml of invertase production was observed by yeast (VM2) after 24 hours of incubation at 30°C. As shown in figure-3 the maximum invertase production was recorded in 2.0 gm% Glucose which was 2.35 U/ml.

Effect of different Nitrogen sources on Invertase production:



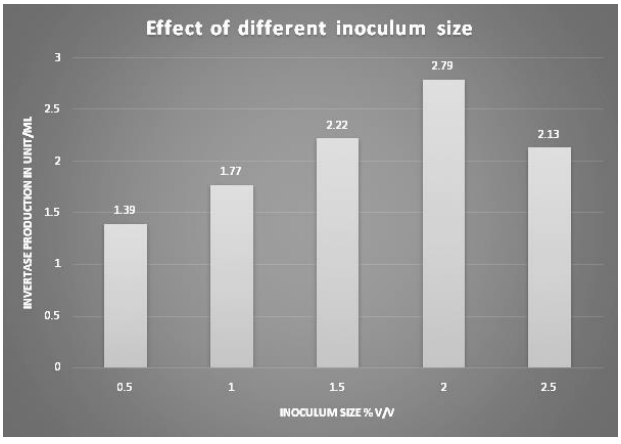
The effect of different nitrogen sources on invertase production by VM-2 after 24 hours of incubation at 30 °C showed maximum amount of enzyme production which was 2.35 Unit/ml in yeast extract,and the same amount of invertase was produced (2.35 U/ml) when peptone was used in the medium, when 2.0 gm % of Glucose was added to GYE medium.

Effect of pH on Invertase production:



In order to determine optimum pH for invertase production. The invertase production was assessed at pH range 4 .0 to 8.0. Maximum amount of invertase production of 2.35 U/ml was achieved at pH 5.0, when 2.0 gm % of Glucose and 0.5 gm % of peptone were added to GYE medium.

Figure-7. Effect of Inoculum size on Invertase production:



The initial inoculum level in the invertase media is a critical factor in fermentation process. The maximum invertase production (2.79 IU/ml) was observed at 2 % V/V of inoculum level when 2.0

gm % of Glucose and 0.5 gm % peptone were added to GYE medium at 5.0 pH.

Discussion

Invertase production by *Pichiakudriavzevii* in GYE medium after the incubation of 24 hrs was 2.02 U/ml but upon further incubation after 48 hrs of fermentation the estimated invertase was 1.509 U/ml. No increment of invertase production was noted after 24 hrs hence all further experiments were designed to terminate at 24 hrs of incubation.

The different carbon sources were investigated for invertase production by *Pichiakudriavzevii*. Marginal increased amount of invertase production of 2.35 U/ml was recorded in Glucose compared to sucrose 2.22 U/ml, this may be due to glucose being easily utilizable source of carbon and energy developed higher growth and cell mass which in turn produced little more invertase compared to sucrose. In the similar experiment k .Mehta obtained 13.7μM invertase using fructose compared to 4.3 μM invertase when sucrose was used as carbon source produced by *Aspergillusniger* even lactose yielded 12 μM invertase in the experiment [K. MEHTA *et al*, 2014]

The different concentration of optimized carbon source glucose were tested for maximum invertase production by *Pichiakudriavzevii*. Maximum of 2.35 U/ml of invertase was produced at 2.0 gm % glucose concentration. The invertase production reduced with increased in glucose concentration finally 1.22 U/ml of invertase was produced at 8.0 gm % glucose concentration. In their experiment T. Shankar *et al* could produce 0.4 U/ml invertase at 2.0 gm % concentration of sucrose. The production was decreased upon increased in concentration of sucrose which was 0.03 U /ml at 3.5 gm % concentration of sucrose [T. Shankar Reena C *et al*, 2016].

The different Nitrogen sources were investigated for invertase production by *Pichiakudriavzevii.* Invertase production after 24 hours of incubation period at 30°C showed maximum 2.35 U/ml Invertase production in yeast extract containing medium and also 2.35 U/ml invertase production in peptone containing medium and minimum amount 1.39 U/ml invertase production in urea supplemented medium. Similar results were obtained by T.shivkumar*et.al,* in their experiment they could produce 0.24 U/ml invertase using *sachharomycescerevices* MTCC170 in yeast extract containing medium and 0.23 U/ml invertase production in peptone containing medium but only

0.04 U/ml invertase was produced in Urea containing medium [T.Sivakumar1 *et al*, 2013].

The effect of initial pH on invertase production by *Pichiakudriavzevii* is investigated in current study. Maximum 2.35 U/ml invertase production was obtained when early pH of the medium was 5.0 and minimum 1.08 U/ml invertase production was recorded in pH 8.0 by*Pichiakudriavzevii*. The result indicate that the yeast is acidophilic. P. Costaglioli, *et al*, 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 developed pH was due to blocked enzyme secretion from the yeast cells [P. Costaglioli*et al*, 1997].

In the present investigation effect of various concentrations of inoculum were tested on invertase production by *Pichiakudriavzevii*. Maximum invertase production of 2.79 U/ml by *Pichiakudriavzevii* was registered at 2.0 % v/v inoculum level and minimum amount of invertase production of 1.39 U/ml was registered at 0.5 % v/v inoculum level. I. Ul-Haq in his experiment explained that the inoculum size was further increased, the production of the enzyme gradually decreased due to the fact that at high level of inoculum size yeast grow fast by consuming the essential nutrients at the initial stages and rapid accumulation of by-product into the fermentation medium is observed [I. Ul-Haq*et al*,2005].

Conclusion

This study indicates the potential of fruits as efficient sources of invertase producing yeast. Glucose and yeast extract as well as peptone found to be best sources of carbon and nitrogen. pH- 5.0 and 2.0 % v/v inoculum size found to bethe best optimized condition for higher invertase production.

References

A.C.P. Alegre, M.D.L.T.D.M. Polizeli, H.F.T, J.A. Jorge, L.H.S. Guimaraes. Production of thermostableinvertases by aspergilluscaespitosus under submerged or solid state fermentation using agro industrial residues as carbon source, Brazilian journal of microbiology, (2009), 40, 612-622.

F. Veana, C.N. Aguilar, R. Rodriguez-Herrera. Kinetic studies of invertase production by xerophilicAspergillus and Penicillium strains under submerged culture,

MicologiaAplicadaInternacional, 2011, 23 (2), 1534-258.

Ikram-ul-Haq, MirzaAhsenBaig, SikanderAli, Effect of cultivation conditions on invertase production by hyperproducing Saccharomyces cerevisiae isolates. App. Environ. Microbiol,(2005), 21, 487-497.

k. mehta and j.s. duhan production of invertase from aspergillusniger using fruit peel waste as a substrate, International Journal of Pharma and Bio Sciences ISSN 0975-6299, Int J Pharm Bio Sci 2014 April; 5 (2): (B) 353 - 360.

K. Shafiq, I. Ul-Haq, S. Ali. Predicting vegetative inoculums performance to maximize invertase production by Saccharomyces cerevisiae in submerged fermentation, Journal of Food Technology, 2003b, 1 (4), 160-163.

K. Shafiq, S. Ali and I. Ul-Haq. Temperature optima for Invertase secretion by yeast in synthetic medium, Pak. J. Life Soc. Sci, 2004, 2 (1), 21-23.

M. C. Silveira, E. M. Oliveira, E, Carvajal, E, P, Bon. Nitrogen regulation of Saccharomyces cerevisiaeinvertase.Role of the URE2 gene, Appl. Biochem.Biotechnol, 84-86: 247-254, 2000.

P. Costaglioli, E. Meilhoc, I. Janatova, R. Klein, J. Masson. Secretion of invertase from Schwanniomycesoccidentalis, Biotechnol.Lett, 19: 623-627, 1997.

Plant invertases: structure, function and regulation of a diverse enzyme family Vasileiosfotopoulus. J Biol Res. 2005;4:127-137.

P. Suresh, Kamble, C. B. Jyotsna. Effect of nitrogen sources on the production of invertase by yeast Saccharomyces cerevisiae 3090, International Journal of Applied Biology and Pharmaceutical Technology, Vol. 2:539-550, 2012.

Reena C. Chauhan, Poonam B Chauhan, MayurGahlout KBS Commerce and NatarajProfessioal Science College, Vapi, Gujarat, India. Isolation Screening and Optimization of Invertase Production under Submerged Fermentation, Volume III, Issue V, May 2016, ISSN 2321 – 2705.

Romero-Gomez S, Augur C, Viniegra-Gonzalez G. Invertase production by Aspergillusný´ger in submerged and solid-state fermentation. BiotechnolLett. 2000;22:1255-1258.

SamarthKulshrestha[a]PrasidhiTyagi[b]VinitaSindhi[a]KameshwarSharmaYadavilli[c]. Invertase and its applications – A brief review.Volume 7, Issue 9, September 2013, Pages 792-797.

Shall S, Baseer A, Waheed A. The Mechanism of Action of Yeast Invertase. Biochem J. 1971 March;122(1):19e20.

Sumner, J.B and Howell, S.F. 1935. A method for determination of saccharase activity. *J. Biol. Chem*.108: 51-54.

T. Shankar Reena C. Chauhan, Poonam B Chauhan, MayurGahlout. Isolation Screening and Optimization of Invertase Production under Submerged Fermentation. Volume III, Issue V, May 2016, IJRSI, ISSN 2321 – 2705.

T.Sivakumar1, M. Ravikumar2, M.Prakash1 and V. Shanmugaraju3, Production of extracelluarinvertase from Saccharomyces cerevisiae strain isolated from grapes, ISSN: 2347-3215 Volume 1 Number 2 (2013) pp. 72-83.

Uma C, Gomathi D, Multhulakshmi C, Gopalkrishana VK. Production, puriûcation and characterization of invertase by Aspergillusûavus using fruit peel waste as substrate. AdvanBiol Res. 2010; 4(1):31-36.

❑