



## **Production of Invertase from Actinomycetes Isolated from Soils in Eliogbolo and Oyigbo, Rivers State using Submerged Fermentation Technique**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author TOO designed the study, wrote the protocol and the first draft of the manuscript. Authors OKA and ECW managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The use of commercial sources of invertase for the production of High fructose syrup and other pharmaceutical formulations has been a problem due to its high ash content and cost implication, therefore there is a need to explore bacterial species that can produce invertase. In this study, actinomycetes strains were isolated from soils from a botanical garden, sugar cane sites and garden egg at Eliogbolo and Oyigbo respectively. Yeast extract agar; Czapeks Dox agar and Arginine glycerol agar were used for the isolation of actinomycetes. The study showed that Czapeks Dox agar gave the highest population count followed by Yeast extract and Arginine glycerol agar. The dominated genus of actinomycetes observed were species of *Streptomyces* designated as OZMU 1;

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OZMU 2; OZMU 3 and OZMU 4. The results showed that the reducing sugar content of the *Streptomyces* sp. had similar reducing sugar liberation and consumption behavior without any lag phase, with OZMU 1 concentration peak at 12 h (0.12 g/l), and OZMU 3 (0.15 g/l). But OZMU 2 indicated a lag phase from 6-12h, increased from 12-24 h (0.18 g/l), but activity declined dramatically at 36 h and 48 h. The reducing sugar content for *Streptomyces* sp. OZMU 4 revealed longer lag phase, which was almost constant throughout the investigation period from 6-36 h. But at 48 h (0.06 g/l) a decline in the reducing sugar content was noticed compared to the other isolates, indicating that all the available reducing sugar were completely consumed by the other strains. The research work revealed the ability of indigenous actinomycetes strains to produce invertase enzyme which has high conversion efficiency and can be used in various pharmaceutical and nutraceutical industries.

**Keywords:** *Actinomycetes; invertase; reducing sugar content; submerged fermentation technique.*

## 1. INTRODUCTION

Invertase is an enzyme of importance in confectionery, beverage, bakery and other pharmaceutical formulations for the preparation of invert sugar and high fructose syrup (HFS) from sucrose [1]. Invertase from microbial sources are less expensive to produce and do not have high ash content. Microbial invertase can be used for the manufacture of calf feed and food for honeybees [2].

Submerged Fermentation (SmF)/Liquid Fermentation (LF) utilizes free flowing liquid substrates, such as molasses and broths. The bioactive compounds are secreted into the fermentation broth. It is best suited for microorganisms such as bacteria that require high moisture content and substrates are constantly replaced or supplemented with nutrients since they are rapidly used up. Another advantage of this technique is that purification of products is easier. SmF is primarily used in the extraction of secondary metabolites that need to be used in liquid form especially in case of bacterial enzyme production, due to the requirement of higher water potential [3]. Secondary metabolites range from several antibiotics to peptides, enzymes, and growth factors [4,5,6] herbicides, pesticides and anti-parasitic agent [7]. More than 75% of the Industrial enzymes are produced using SmF, one of the major reasons being that SmF supports the utilization of genetically modified organisms to a greater extent than Solid State Fermentation (SSF). Another reason why SmF is widely used is the lack of paraphernalia regarding the production of various enzymes using SSF. The production of invertase has also been reported for *Aspergillus* sp. (Claudia, et al. 2009) *Aureobasidium pullulans* [8], *Fusarium solani* (Bhatti, et al. 2006), *Bifidobacterium infantis* [9],

*Azotobacter chroococcum* [10], *Saccharomyces cerevisiae* GCB-K5 (Shagiq and ul-Haq, 2002), *Thermomyces laniginosus* (Uma, et al. 2010). The present study screened the ability of actinomycetes genera isolated from soils in Eliogbolo and Oyigbo, Rivers State to produce invertase enzyme using submerged fermentation technique

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Actinomycetes

Three different media were used for isolation. The media included; Yeast extract agar (YEA), Czapek's Dox agar (CDA) [11], and Arginine glycerol agar (AGA) (Arnold and Nadine, 1986).

Using YEA and CDA, one gram of each soil sample was weighed and dispensed into 9 ml of physiological saline in sterile test tubes. The soil sample was shaken vigorously to obtain a suspension then serially diluted tenfold to  $10^{-7}$ . Then, 1 ml aliquot of  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-7}$  dilutions was dispensed into Petri plates after which, cooled (50°C) but molten YEA or CDA containing nystatin (50 µg/ml) was poured into the plates. After swirling the plates to mix the inoculum with the media, the plates were incubated at 28°C in an incubator (Mettler 854, Schwabach, England) for 5-7 days.

For AGA, 1 gram of soil sample was initially treated with calcium carbonate ( $\text{CaCO}_3$ ) to reduce the number of vegetative cells and promote the growth of Actinomycetes. The pre-treated soil sample was incubated for 10 days at 28°C in an inverted Petri dish containing filter paper saturated with water on the inside of lid. After incubation, the pre-treated soil sample was dispensed into sterile test tubes containing 9 ml of diluent and shaken vigorously to obtain a

suspension. The suspension was diluted tenfold up to  $10^{-7}$ . Then, 1ml aliquot of  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-7}$  dilutions were dispensed into Petri plates then, cooled ( $50^{\circ}\text{C}$ ) but molten AGA without nystatin was poured into the plates. After swirling the plates to mix the inoculum with the media, the plates were incubated at  $28^{\circ}\text{C}$  in an incubator (Memmert 854, Schwabach, England) for 10 days. Distinct colonies were selected by cultural morphology and purified by subculturing onto sterile CDA plates and incubated at  $28^{\circ}\text{C}$  in an incubator (Memmert 854, Schwabach, England) for 5-7 days. Pure cultures were maintained on CDA slants at  $28^{\circ}\text{C}$ .

## 2.2 Identification of Isolates

Identification of the isolates was based on cultural morphology, microscopic examination, and biochemical tests according to Bergey's Manual of Systemic Bacteriology [12].

## 2.3 Screening for Invertase Producing Actinomycetes

Actinomycetes, were isolated from soil sample and were identified based on their ability to use sucrose as sole carbon source (Nakamura, et al. 1997). Purified culture of each isolate was transferred to agar slants for growth at  $37^{\circ}\text{C}$  for 24 hours, subcultured fortnightly and stored at  $4^{\circ}\text{C}$  for routine use. The medium used for isolation, maintenance and enzyme production was Czapeks Dox medium ( $\text{NaNO}_3$  3.0 g,  $\text{K}_2\text{HPO}_4$  1.0 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 g, KCl 0.5 g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.01 g, Sucrose 30 g, Agar 15 g per litre ( for solid medium), pH 5.0).

## 2.4 Invertase Assay

Hundred millilitre (100 ml) of Czapek's Dox broth (CDB) in a 500 ml Erlenmeyer flask, was inoculated with the test organisms and incubated in a rotary shaker 1 (Stuart S150, B and T, Scarle company, England) set at  $37^{\circ}\text{C}$  and speed of 150 rpm for 48 hrs. At 6 hours interval, about 5 ml samples were withdrawn, centrifuged at speed of 10,000 rpm for 20 mins at  $4^{\circ}\text{C}$ , the supernatant was then stored at  $-20^{\circ}\text{C}$  for further analysis [13]. Invertase activity was assayed by measuring the amount of reducing sugar released from sucrose. The assay mixture contained 0.1 ml enzyme extract (the invertase produced by each strain, contained in the liquid medium), 0.9 ml (1.1% w/v) sucrose in 0.1 M sodium acetate buffer (pH 5.5). The mixture was incubated at  $60^{\circ}\text{C}$  for 1 hour and reaction stopped by adding 1 ml of Nelson's reagent.

## 2.5 Determination of Reducing Sugar Content

After each incubation period (6, 12, 24, 36 and 48 h) in the shaker, 1 ml of the sample was transferred into sterilized test tubes using a sterile pipette then reducing sugar was determined spectrophotometrically (Spectronic Unicam, England) at 540 nm by Nelson's method [14,11]. One unit of invertase was defined as the amount of enzyme, which liberated  $1\mu\text{mol}$  of product per min per ml under the assay condition. A calibration curve was drawn with fructose (10 – 100 mg/ml) [11].

## 3. RESULTS

Morphological and biochemical characterization showed that the isolates were actinomycetes strains of the genus *Streptomyces* and were coded as *Streptomyces* sp. OZMU 1, OZMU 2, OZMU 3 and OZMU4 (Figs.1 and 2). The population of the *Streptomyces* from different collection sites showed that the botanical garden had the highest population of OZMU 1, OZMU 3, and OZMU 4 while OZMU 2 was not isolated from that site. High population of species OZMU 1 and OZMU 2, were obtained from Eliogbolo sugarcane dumpsite, OZMU 3 and OZMU 4 species, were not isolated from Eliogbolo while OZMU 2 specie was not isolated from Oyigbo sugarcane dumpsite. The lowest population was obtained from garden egg and maize farmlands. Furthermore, OZMU 3 and OZMU4 species were not isolated from the garden egg farmland while OZMU 1 was not isolated from the maize farmland.

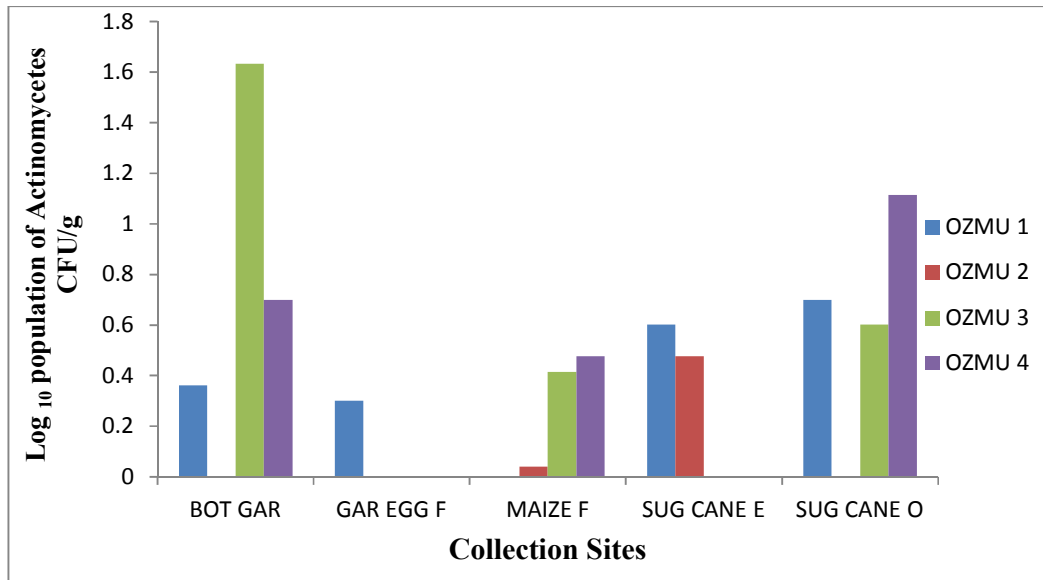
Isolation on CDA, YEA, and AGA showed the highest population count was obtained from CDA plates for all the isolates followed by YEA, though *Streptomyces* sp. OZMU 4 did not grow on it and *Streptomyces* sp. OZMU 3 did not grow on AGA which had the lowest population count (Fig. 2).

The table shows grams reaction and biochemical characterization for the identification of the isolates.

The investigation on invertase activity of the *Streptomyces* sp. OZMU 1 to OZMU 4 at different time interval showed the ability of the strains to produce invertase with varied activity profile. *Streptomyces* sp. OZMU 2 had its maximum activity within 6h of cultivation ( $88\text{ IU}^{-1}$ ) after which it declined at 12 h then a

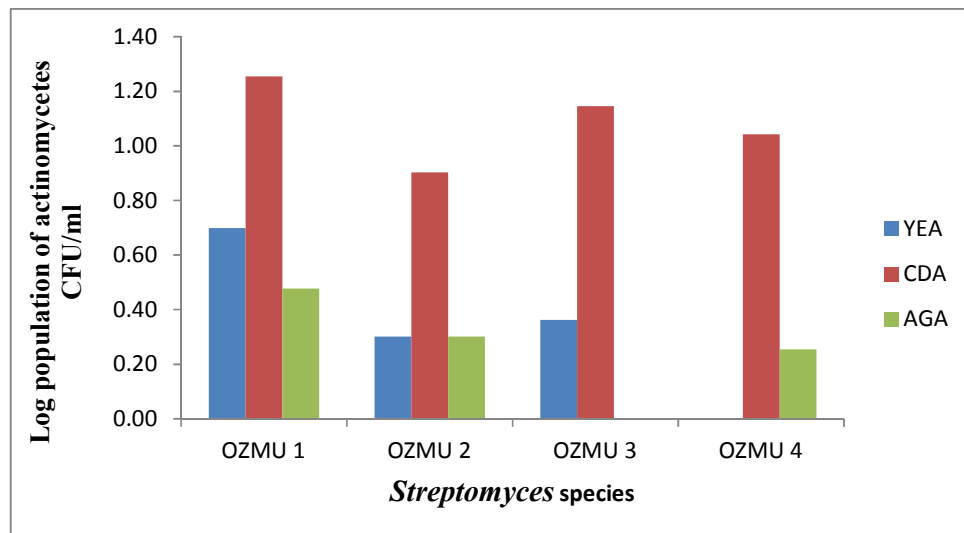
rise in activity again at 24 h and fall at 36 and 48 h. *Streptomyces* sp. OZMU 3, had its highest activity at 24 h of cultivation ( $86 \text{ IU}^{-1}$ ) followed by a decline at 36 and 48 h. *Streptomyces* sp. OZMU 3 and OZMU 4 had their maximum activity of  $86 \text{ IU}^{-1}$  and  $77 \text{ IU}^{-1}$  respectively within 24 h of cultivation. This was followed with subsequent decrease at 36 and 48 h (Fig. 3).

The result of reducing sugar (fructose) content indicated that *Streptomyces* strains OZMU 1 and OZMU 3 had high sugar peaks after 12 h of cultivation, while *Streptomyces* sp. OZMU 2 was at 24 h of cultivation. *Streptomyces* sp. OZMU4 had reducing sugar content which remained relatively constant. However, all the species exhausted all the available reducing sugar by 48 h (Fig. 4).



**Fig. 1. Population of *Streptomyces* sp. recovered from collection sites**

Key: (BOT GAR – Botanical Garden, GAR EGG F – Garden Egg Farm, MAIZE F – Maize Farm, SUG O – Sugar cane site Oyigbo, SUG E – Sugar cane site Eliogbolo)

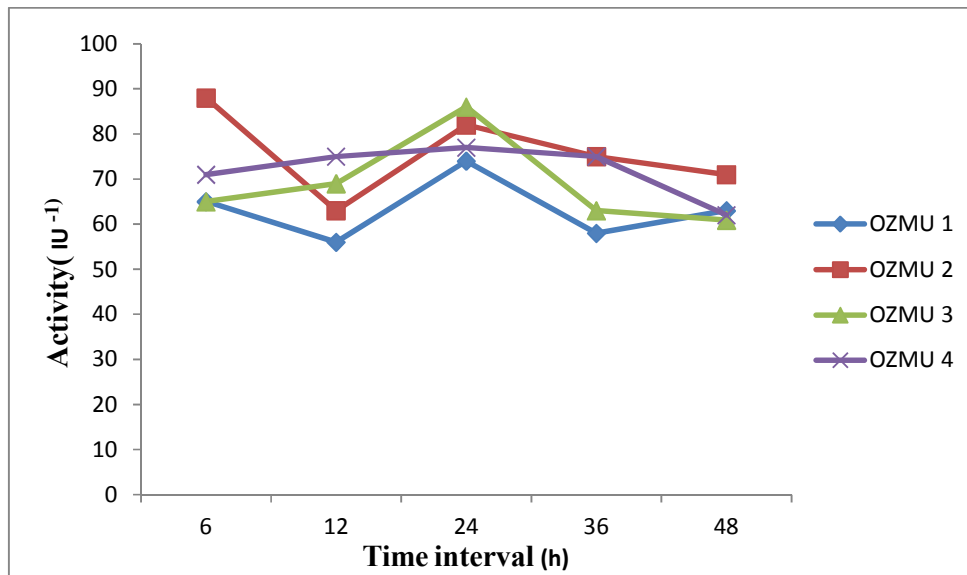


**Fig. 2. Cultivation of *Streptomyces* sp. on different culture media**

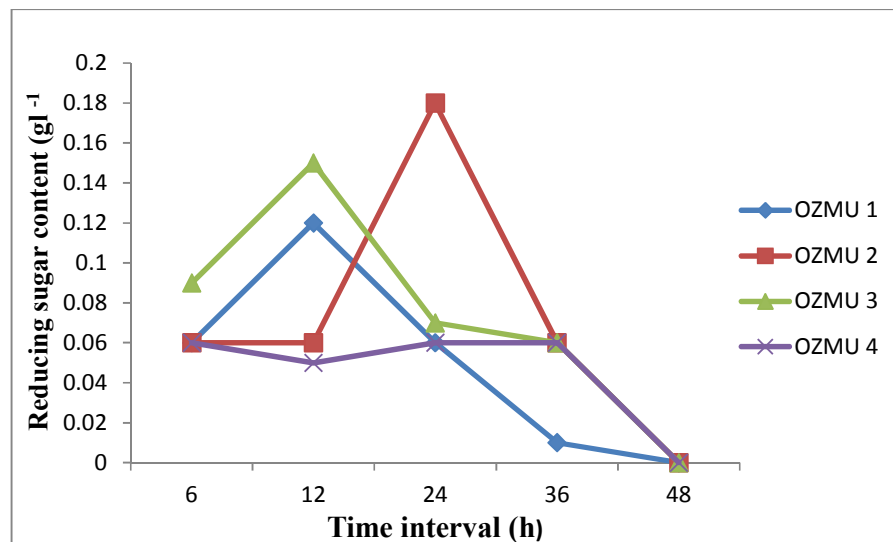
Key: (YEA-yeast extract agar, CDA- Czapek's Dox agar, AGA- arginine glycerol agar)

Table 1. Identification of isolates

Isolates	Grams RXN	Colour of colony	Form of colony	Pigment on media	Aerial mycelium	Reversa pigment	Spore formation	Citrate	Sucrose	Glucose	Lactose	Maltose	Starch hydrolysis	Urea hydrolysis	MR	VP	Catalase	Indole	Organisms
OZMU 1	+	Orange white	Folded & compact	Light brown	Present	Dark brown	RF	+	+	+	-	-	+	-	+	-	-	-	<i>Streptomyces</i> spp
OZMU 2	+	Yellow brown	Leathery & folded	White	Present	Light brown	RT	+	+	-	-	+	-	+	+	-	-	-	<i>Streptomyces</i> spp
OZMU 3	+	White flat	Flat dotted	White	Present	Sand yellow	RF	+	+	-	+	-	-	+	+	-	-	-	<i>Streptomyces</i> spp
OZMU 4	+	Greenish white	Flat dotted	White	present	Greenish	RF	+	+	+	+	+	+	+	+	-	-	-	<i>Streptomyces</i> spp



**Fig. 3. Time course of invertase production by *Streptomyces* strains in submerged shake culture (CDB at 37°C, pH 5.5)**  
KEY: CDB Czapek's Dox Broth



**Fig. 4. Reducing sugar content during invertase production by *Streptomyces* at different time interval**

#### 4. DISCUSSION

The present study investigated the ability of local strains of actinomycetes to produce invertase enzyme. This was simultaneously compared using three different media for the isolation. Morphological and biochemical characterization of the isolates showed that all the four isolates that produced invertase belonged to the genus *Streptomyces*. A total of eight actinomycetes isolates were recovered. Preliminary screening

for invertase production showed that four of the isolates produced invertase and the other four did not. The investigation revealed that the isolates designated as *Streptomyces* OZMU 1, OZMU 2, OZMU 3, and OZMU 4 isolated from sugarcane dumpsites and the University of Port Harcourt botanical garden had the highest counts. This can be attributed to the presence of sugarcane deposits on the site (9) *Streptomyces* sp. OZMU 2 was not isolated from the botanical garden soil while *Streptomyces* sp. OZMU 3 and

OZMU 4, were not isolated from Eliogbolo sugarcane dumpsite. Also *Streptomyces* sp. OZMU 2 was not isolated from Oyigbo sugarcane dumpsite. High population counts from botanical garden and the sugarcane dumpsites may be attributed to the presence of decomposing organic matter. Oudouch, et al. [15], reported that actinomycetes population was more in areas with high decomposing organic matter rich in carbonaceous and humus material. Gurmores, et al. [16], reported the production of invertase from agro industrial residues by *Aspergillus ochraceus* with higher production in sugar cane baggasse.

Isolation of actinomycetes on three different media showed that the preferred media for the isolation is Czapek's Dox agar because it supported the highest population of actinomycetes followed by Yeast extract agar and lastly Arginine glycerol agar which supported the lowest population. The additional observation was that *Streptomyces* sp. OZMU 4 did not grow on Yeast extract agar while *Streptomyces* sp. OZMU 3 did not grow on Arginine glycerol agar.

Assaying for the production of invertase by the four species isolated showed that, all produced invertase with different activity profile. *Streptomyces* strain OZMU 2 had the highest activity which occurred within 6 h of cultivation ( $88 \text{ IU}^{-1}$ ). This short incubation period could be advantageous for batch production of invertase in industries. The level of invertase production is higher than the reported quantity of invertase produced by *A. niger* ( $20 \text{ IU}^{-1}$ ) after 60 h of incubation [17] and *Penicillium* sp. TN-40 and TN-85 ( $40 \text{ IU}^{-1}$ ) after 5 days (Nakamura, et al. 1997). Comparing our findings with other researchers showed that the findings by Kaur and Sharma [11] revealed invertase activity of *Streptomyces* sp. ALK C5 and ALK C8 were  $113.3 \text{ IU}^{-1}$  and  $350 \text{ IU}^{-1}$  obtained within 6 h and 24 h of cultivation respectively. Uma, et al. (2010) observed higher invertase activity for *Aspergillus flavus* (3% inoculum) at 48, 72 and 96 h of cultivation ( $16.4$ ,  $19.6$ , and  $25.8 \text{ IU ml}^{-1}$  respectively), Shafiq, et al. [18], noted higher activity of invertase for *Saccharomyces cerevisiae* 48 h after inoculation ( $8.35 \text{ U ml}^{-1}$ ) *Streptomyces* sp. OZMU1, OZMU 3 and OZMU 4 showed maximum activity at 24 h of cultivation ( $74$ ,  $86$  and  $77 \text{ IU}^{-1}$  respectively). Therefore, the production of invertase varies depending on the physiological characteristics of the strains of actinomycetes. Investigating the reducing sugar content of the media at different time intervals

revealed that *Streptomyces* sp. OZMU 1 and OZMU 3 had similar reducing sugar liberation and consumption behavior. Their reducing sugar concentration peak was at 12 h ( $0.12 \text{ g/l}$ ) and ( $0.15 \text{ g/l}$ ), respectively while *Streptomyces* sp. OZMU 2 was at 24 h. Activity declined dramatically at 24 h and 36 h. The decline showed carbon source consumption by all the species. The reducing sugar content for *Streptomyces* sp. OZMU 4 was almost constant throughout the investigation period, though by 48 h all the available reducing sugar was completely consumed by all the species. This finding agrees with the report of (1).

## 5. CONCLUSION

The study showed that *Streptomyces* species were capable of producing invertase within a short period of time, a trait suitable for industrial processes. Thus purification and further studies on these organisms should be carried out to ascertain their commercial potentials.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Gehlawat JK. New technology for invert sugar and high fructose syrups from sugarcane Indian Journal of Chemistry Technology. 2001;8:28-32.
2. Vandamme EJ, Derycke DG. Microbial invertases: fermentation process, properties and applications. Advanced Applied Microbiology. 1983;29:139–176.
3. Chahal DS. Foundations of biochemical engineering kinetics and thermodynamics in biological systems. In Blanch, H.W, Papoutsakis, E.T., and G Stephanopoulos (Eds.), ACS Symposium Series, 207, American Chemical Society; 1983.
4. Balakrishnan K, Pandey A. Production of biologically active secondary metabolites in solid state fermentation. Journal of Scientific and Industrial Research. 1996;55:365-372.
5. Machado CM, Oishi BO, Pandey A, Soccol CR. Kinetics of *Gibberella fujikori* growth and gibberellic acid production by solid state fermentation in a packed-bed column bioreactor. Biotechnology Progress. 2004;20:1449-1453.
6. Robinson T, Singh D, Nigam P. Solid-state fermentation: a promising microbial



- Technology for secondary metabolite production. *Applied Microbiology and Biotechnology*. 2001;55:284-289.
7. Mustafa O, Tamer AU, Cem A. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *African Journal of Biotechnology*. 2004;3(9):441-446.
8. Yoshikawa J, Amachi S, Shinoyama H, Fujii T. Multiple  $\beta$ -fructoduranosidases by *Aureobasidium pullulans* DSM 2404 and their roles in the fructooligosaccharide production. *FEMS Microbiology*. 2006;265:159-163.
9. Warchol M, Perrin S, Grill JP, Schneider, F. Characterization of a purified  $\beta$ -fructofuranosidase from *Bifidobacterium infantis* ATCC 15697. *Letter Applied Microbiology*. 2002;15697:462-467,35.
10. De la Vega M, Cejudo F, Paneque A. Purification and properties extracellular invertase from *Azotobacter chroococcum*. *Enzyme Microbial Technol*. 1991;13:267-271.
11. Kaur N, Sharma AD. Production, optimization and characterization of extracellular Invertase by an actinomycete strain. *Journal of Scientific and Industrial Research*. 2005;64:515-519.
12. Goodfellow M. Classification of actinomycetes. In: *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins, London. 2002;2333–2339.
13. Gogoi BK, Pillai KR, Nigram JN, Bezbaruah RL. Extracellular  $\alpha$  - amylase and Invertase from amyolytic yeast *Saccharomycopsis fibuligera*. *Indian Journal of Microbiology*. 1998;38:15-19.
14. Anjali H, Sharma AD. Production and partial characterization of invertase from actinomycetes strains. *Asian Journal of Microbiology Biotechnology Environmental Science*. 2005;7(2):205-208.
15. Ouhdouch Y, Barakate M, Finance C. Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. *European Journal of Soil Biology*. 2001;37:69-74.
16. Guimaraes LHS, Terenzi HF, Polizeli MLTM, Jorge JA. Production and characterization of a thermostable extracellular  $\beta$ -D-fructofuranosidase produced by *Aspergillus ochraceus* with agroindustrial residues as carbon sources. *Enzyme Microbial Technology*. 2007;42:52-57
17. Gomez SJR, Augur C, Gonzalez G. Invertase production by *Aspergillus niger* in submerged and solid state fermentation. *Biotechnology Letter*. 2000;22:1255-1258.
18. Shafiq K, S Ali, I Haq. Time course study for yeast invertase production by submerged fermentation. *J. Bacteriology*. 2003;3:984-988.

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