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Invertase Production by Irradiated *Aspergillus niger* OSH5 Using Agricultural Wastes as Carbon Source

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors OEAA and IAH managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of this study was to isolate microbial species having the ability for production of invertase enzyme and to study some optimal culture conditions for maximum invertase production by an isolate of *Aspergillus niger* OSH5 which showed the highest invertase activity among the screened isolates. The enhancement effect of gamma irradiation on invertase activity was also investigated.
Place and Duration of Study: Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, between July, 2013 and January, 2014.
Methodology: Seven microbial isolates were screened for invertase production. The most active isolate *Aspergillus niger* OSH5 was subjected to gamma irradiation mutagenesis to enhance invertase production. The optimal culture conditions including shaking speeds, temperature, incubation period, and pH were tested for maximum invertase production. Moreover, production of invertase using solid-state fermentation was also studied.
Results: Among the seven isolates, *A. niger* OSH5 was found the most active on invertase

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production. The most effective carbon source in intracellular invertase production (14.8±1.27 u/ml) was sucrose at a concentration of 3% (w/v) using shaking speed of 150 rpm at 25ºC and pH 6.5 after incubation for 6 days. Among the screened agro-industrial wastes that were used as solid substrates for invertase production, wheat bran at a concentration of 14% (w/v) was the most conductive substrate for extracellular invertase production (15.9±2.44 u/g).

Conclusion: The obtained results indicate the efficient use of low cost fermentation for invertase production by an *A. niger* isolate. Moreover, these findings indicate the efficient use of gamma irradiation as a tool for invertase production enhancement.

Keywords: Invertase; gamma irradiation; solid-state fermentation; agricultural wastes.

1. INTRODUCTION

The enzyme known as invertase (E.C. 3.2.1. 26-B-D-fructofuranosidase) catalyzes the sucrose hydrolysis producing as equimolar mixture of glucose and fructose named inverted sugar. The sugar cane is one of the most important sucrose sources, containing up to 20% sucrose [1]. Invertases are intracellular as well as extracellular enzymes [2]. The enzyme has wide range of commercial applications e.g. fermentation of cane molasses into ethanol, manufacture of calf feed, honey bees and confectionery and also in food industry, where fructose is preferred than sucrose especially in the preparation of Jams and Candies, because it is sweeter and does not crystalline easily [3]. Invertase has appreciably gained importance in recent years due to its various biotechnological applications in confectionary, beverage, bakery and other pharmaceutical formulations for the preparation of invert sugar and high fructose syrup from sucrose.

Invertase is classified in the GH32 family of glycoside hydrolases that includes over 370 members [4]. Fructose is more satiating and it is up to 1.8 times sweeter than sucrose. Fructose is also ideal for use in diabetic foods as it has very little effect on blood glucose and only a negligible effect on the secretion of insulin [5].

Production of invert sugar by acid hydrolysis method is highly uneconomical, because of low conversion efficiencies (65-70%) heaving no sweetening capacity. Therefore, microbial invertases which cleave sucrose by a single step reaction, offer an attractive alternative [6]. The enzymatic activity of invertase has been characterized mainly in plants and microorganisms. Microorganisms reported for invertase production include *Saccharomyces cerevisiae* [7], *Aspergillus niger* [8] and *Candida utilis* [9], etc. The production of extracellular invertase under solid state fermentation (SSF) using wheat bran was around 5.5 fold higher than that obtained in submerged fermentation (SbmF) [10]. SSF is characterized by development of microorganism in a low aqueous content on a non soluble material that can act as physical support and in sometimes also as nutrient sources [11,12]. The enzymatic production in SSF has advantages over SbmF as higher productivity fermentation, absence of contaminant organisms, concentrated product formation and use of agro-industrial residues as substrate [13]. The aim of this work was to study the production of extracellular and intracellular invertase by gamma irradiated isolate of *Aspergillus niger* under SbmF and SSF using agro-industrial wastes as substrate.

2. MATERIALS AND METHODS

2.1 Bee Honey Samples Used in Microbial Isolation

Samples of bee honey were purchased from a local market at Cairo, Egypt in May, 2012. The samples were opened at room temperature for 7 days to give favorable conditions for infection by moulds and bacteria. After which they were used for bacterial and fungal isolation.

2.2 Organism and Inoculum Preparation

By dilution plate method, fungi were isolated on Czapek's agar medium (CDAM) (g/L; Sucrose 30, $NaNO_3$ 3, KH_2PO_4 0.5, KCl 0.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.01 and agar 20) and bacteria were isolated on nutrient agar medium (g/L; peptone 5.0, sodium chloride 5.0, beef extract 2.0, yeast extract 1.0 and agar 20) from infected bee honey. The Czapek's broth and nutrient broth were autoclaved at 121ºC for 20 min at 1.5 atm. The invertase production was screened using Czapek's broth medium for fungal cultures and using nutrient broth medium for the bacterial isolates. The most active isolate was selected on the basis of its potentiality for invertase activity, as subsequently mentioned. It was identified

according to species-based macroscopic and microscopic morphological criteria according to Raper and Fennel [14] and Samson et al. [15]. All fungal isolates were subcultured on Czapek's agar medium. Meanwhile, bacterial isolates were subcultured on nutrient agar medium. The isolates were reserved at 4ºC. Spore suspension was prepared by flooding of the slants with sterile water containing 0.1% Tween 20 and gently scrapping off the spores with sterile glass rod. The spores were quantified by using haemocytometer. Spore suspension of 10^5 spores/ ml was prepared from 4 days old slant culture for invertase production.

2.3 Gamma Irradiation Mutagenesis

Spore suspension of 10^5 spores /ml from 4 days old slant culture was exposed to gamma radiation (^{60}Co) using Russian gamma radiation cell at the Nuclear Research Center, Cairo, Egypt. The dose rate at the time of radiation treatment was 3.2 KGy/h. The spore suspension was exposed to 0.00, 0.25, 0.50, 1.00, 2.00, 4.00 and 6.00 KGy. This exposure range was selected on the basis of preliminary experiments which showed that the growth of organism is still evident at 6 KGy. An aliquot of 1 ml of the irradiated spore suspension was inoculated in 250 ml Erlenmeyer flasks containing 50 ml of Czapek's Dox broth medium (pH 6.5). The cultures were incubated at 25ºC, with agitation (100 rpm) for 2, 4 and 6 days.

2.4 Culture Conditions

The effects of some cultural conditions, including different agitation rates (0, 30, 60, 90, 120, 150 and 180 rpm), incubation periods (1-10 days), different incubation temperatures (20-40ºC) and different initial pH-values (5.0-8.0) on the production of invertase by the irradiated *A. niger* OSH5 strain (at 2.0 KGy, the best gamma irradiation dose) were tried during the optimization study.

2.5 Preparation of Agricultural Wastes

Wheat straw, wheat bran, sugar cane bagasse, rice straw, rice bran and oatmeal were dried at 70ºC for 48 hr, ground by mixer for 15 min, sieved and used as substrate for invertase production.

2.6 Effects of Different Carbon Sources

In this experiment, 11 compounds (sucrose, glucose, maltose, lactose, starch, wheat straw, wheat bran, sugar cane bagasse, rice straw, rice bran and oatmeal) were tested as carbon sources at two concentrations: 1% (w/v) and 3% (w/v). Sucrose of the Czapek's broth (pH 6.5) was replaced by one of the respective carbon sources. The Czapek's broth supplemented individually with the tested carbon sources was sterilized by autoclaving at 121ºC for 15 min at 1.5 atm. Spore suspension (10^5 spores/ml irradiated at 2.0 KGy, the best gamma irradiation dose) was inoculated in 250 ml Erlenmeyer flasks containing 50 ml of Czapek's broth medium supplemented with one of the mentioned carbon sources. The cultures were incubated at 25ºC and 150 rpm for 6 days. At the end of incubation period, the cultures were filtered and the invertase activity was determined as subsequently described.

2.7 Invertase Production Using SSF

Wheat bran as the best carbon source was used as a solid substrate at different concentrations (5-14%, w/v). Spore suspension (10^5 spores/ml) was inoculated in sterilized 250 ml Erlenmeyer flasks containing wheat bran humidified with tap water, distilled water or salt solution as moistening agents. The salt solution was composed of (%, w/v): ammonium nitrate 0.5, potassium dihydrogen orthophosphate 0.2, sodium chloride 0.1, and magnesium sulphate 0.1. The flasks were incubated at 25ºC and pH 6.5 for 6 days [16].

2.8 Determination of Dry Cell Weight

In SbmF, the fungal culture flasks were filtered through pre-weighted Whatman No.1 filter papers. The cells were then oven dried at 70ºC to a constant weight before measuring dry weight. In SSF, the fungal biomass was extracted from the cultures and analyzed according to Asha-Augustine et al. [17].

2.9 Extraction of Extracellular and Intracellular Invertase

The cultures resulted by SbmF after incubation for 6 days were harvested by vacuum filtration and the filtrate (30 ml, extracellular crude extract) was used for enzymatic activity. The mycelia were disrupted in a porcelain mortar by grinding for 15 min with acid-washed sea sand at 4ºC, extracted in distilled water and centrifuged (20,000 $\times$g) for 10 minutes, the supernatant was called intracellular crude extract and also used for enzymatic activity.

For SSF cultures, 50 ml of distilled water were added to 8 g fermented substrate and submitted agitation using a magnetic stirrer for 30 min at 4ºC. After this, the suspension was vacuum filtered, the obtained filtrate was centrifuged at (20,000 ×g) to remove the residues and the supernatant was used to determine the extracellular invertase activity (as mentioned in the subsequent paragraph) and the protein content was determined by the method of Lowry et al. [19] using Bovine Serum albumin (BSA) as a standard.

2.10 Enzyme Assay

Invertase activity was determined using the method of Sumner and Howell's [18] with slight modification by incubating 0.5 ml of enzyme solution with 0.5 ml of 1.0% sucrose in sodium acetate buffer 100 mM, pH 4.5. The reaction was carried out at different time intervals at 30ºC and stopped by adding 1.0 ml of dinitrosalicylic acid (DNS) and heated for 5 minutes in a boiling water bath. The absorbance was read at 540 nm [20]. One unit of invertase activity was defined as the amount of enzyme that releases 1 mg of glucose per min. under the assay conditions. The values of enzyme activity were expressed as u/ml culture filtrate for SbmF or u/g dry substrate for SSF.

2.11 Statistical Analysis

All tests were performed in triplicates and results were expressed as the mean ± standard deviation (SD). Statistical significance was evaluated using analysis of variance (ANOVA, SPSS software version 22) test followed by the least significant difference (LSD) test at 0.05 level.

3. RESULTS AND DISCUSSION

3.1 Isolation of the Most Potent Microbe

In total, two bacterial and 5 mould isolates were obtained from the contaminated bee honey. The screening results for invertase producing potential by the 7 isolates were recorded in Table 1, where the highest level of extracellular invertase activity (9.7 u/ml) and biomass dry weight (1.7 g/100 ml) at the end of 6 days incubation were produced by isolate No. 5 which selected as the most potent and identified as *A. niger*. Identification of the fungus to species level was performed according to the microscopic and macroscopic criteria, as previously reported [15,16]. On Czapek's agar the colonies consist of a compact fairly loose white mycelium that bears abundant erect and crowded conidial structures, typically black, covering the entire colonies except for a narrow growing margin; reverse usually colorless; exudate lacking. Conidial heads were typically large and black, at first globose, then radiate; conidiphore variable with walls smooth; vesicles globose; sterigmata into series, brownish in color; conidia typically globose, appearing brown with walls irregularly roughned [15,16]. It was followed by isolate No. 4 which exhibited invertase activity 4.9 u/ml and dry weight 0.76 g/100 ml.

3.2 Effect of the Gamma Irradiation

Data presented in Table 2 illustrated that gamma radiation have no effect on extracellular invertase production and biomass dry weight produced by *A. niger* OSH5 up to 0.5 KGy as exposure dose, which exhibit 10.2 u/ml with dry weight 1.8g/100 ml at the end of 6 days incubation, while slightly increase obtained by increasing exposure doses until reached to 12.2 u/ml enzyme activity with 2.2g/100ml dry weight at exposure dose of 2.0 KGy. At 2.0 KGy, significant differences ($p \leq 0.05$) in the values of enzyme activity were obtained as compared to other treatments after 2 and 6 days of incubation. Meanwhile, after 4 days of incubation, significant differences were obtained as compared to other treatments except 2 KGy. So, in the subsequent experimental series irradiation dose of 2.0 KGy will be used. After this dose, the fungal production was sharply dropped and exhibit 2.4 u/ml invertase activity and 0.41 g/100 ml biomass dry weight as similar to Ribeiro et al. [21] who reported that the maximum level of ochratoxin A produced by *Aspergillus flavus* and *A. ochraceus* was achieved at 2.0 KGy exposure dose. Younis and Ahmed [22] reported that gamma radiation exhibit a slightly increase of chitinase produced by *Trichoderma harzianum* at dose of 0.8, 1.0, 2.0 and 4.0 Gy. Furthermore, Ismaiel et al. [23] used gamma radiation at a dose of 0.75 KGy for production enhancement of mycophenolic acid by *Penicillium roqueforti* strains. Irradiation by gamma rays may cause some mutations to cells through the DNA repair mechanisms within the cells [21].

3.3 Effect of Static and Shake Cultures

The invertase activity was around 1.5 fold higher under shaking condition at 30 rpm than that under static condition (4.9 u/ml for static and 7.4 for 30 rpm) at the end of 6 days incubation period as shown in Table 3, which also illustrated that the invertase activity was increased by

increasing shaking speed up to 150 rpm (10.8 u/ml). After this speed, the enzyme activity was maintained at the same level up to 180 rpm (10.6 u/ml).

3.4 Effect of Incubation Period

To determine the optimum incubation period for invertase enzyme production, fermentation flasks were incubated for different time duration, (1-10 days). Enzyme activity was analyzed at every day time intervals. As shown in Table 4, the maximum rate of enzyme production obtained after three days of incubation achieving net activity 3.0 u/ml, and then the enzyme production rate was declined, which might be on the basis of consumption of nutrients. Similar trend was noticed by Shafiq et al. [24] who reported that the optimum production of invertase by *S. cerevisiae* was found after 48 hours. The specific activity reached maximum (2.5 u/mg protein/ml) at the end of 6 days incubation time, then gradually decreased to 2.3 with net enzyme activity 0.00 u/ml at the end of 10 days. These results are in accordance with the observations made by Alagarsamy et al. [25].

3.5 Effect of Incubation Temperature

The results showed that the maximum invertase activity was produced at 25ºC as observed in Fig. 1a, which shows the optimum production temperature of invertase was found to be 25ºC (2.8 u/ml) and dry weight of biomass was 0.56 g/100 ml. The activity and biomass were decreased after the third day. Similar results occurred at the end of 6th day as shown in Fig. 1b, where the growth occurred at all the temperatures but the productivity was maximum only at 25ºC as reported by Malathi et al. [26] and Parnthaman et al. [27].

3.6 Effect of Initial pH

The effect of initial pH of medium on the invertase production was presented Fig. 2.The optimum pH for invertase production by *Aspergillus niger* OSH5 was as pH 6.5, and this findings are in accordance with earlier reports for invertase production (pH 6.7) by *S. cerevisiae* [28] and *Candida utilis* [9].

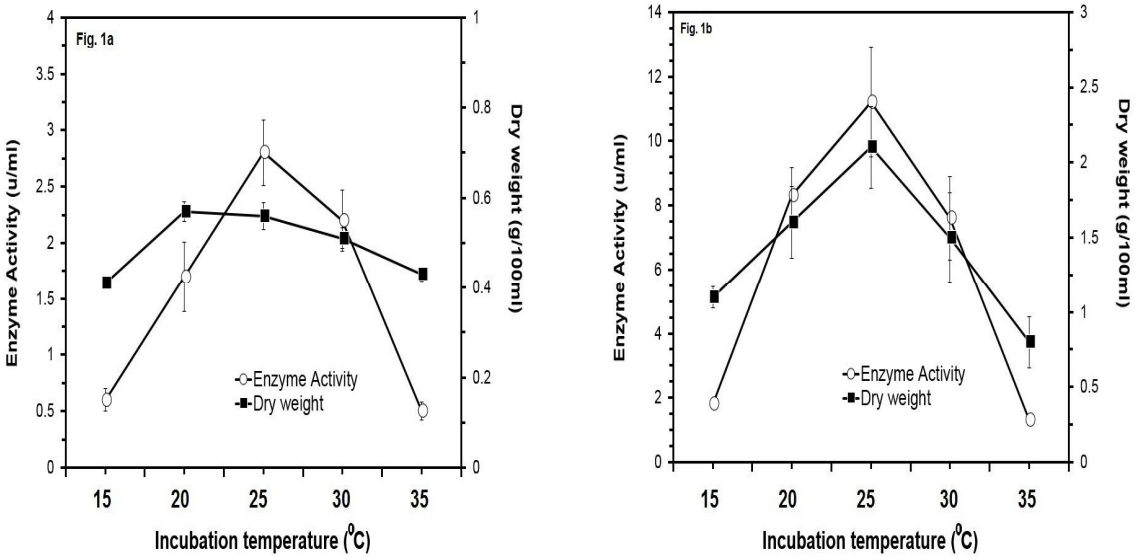

Fig. 1. Effect of different incubation temperatures on invertase production and biomass dry weight. The gamma-irradiated (at 2.0 KGy) *A. niger* OSH5 was grown on Czapek's broth medium at pH 6.5, 25ºC and 150 rpm incubated for 3 days (a) and 6 days (b).

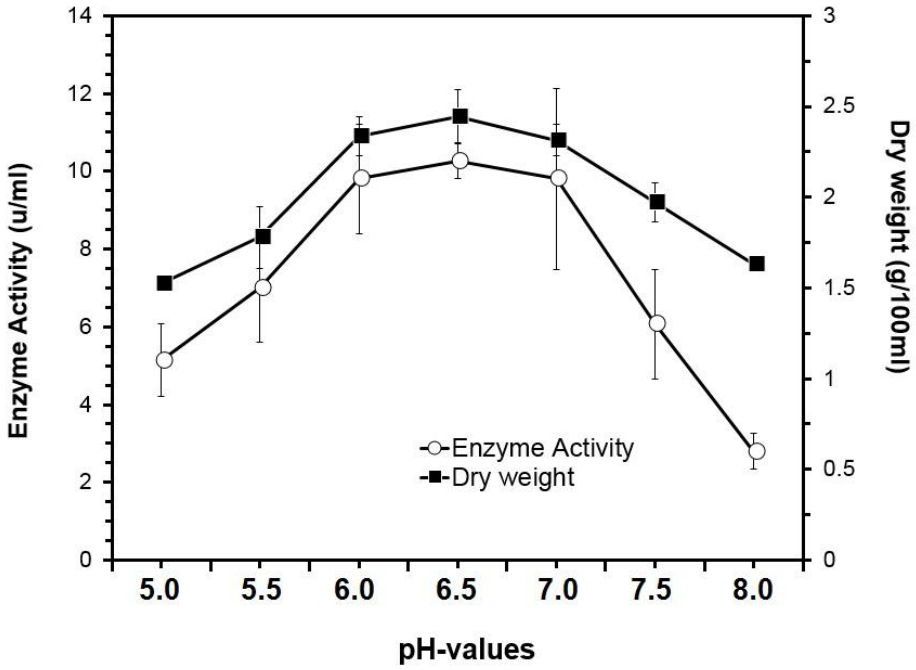

Fig. 2. Effect of different initial pH-values on invertase production and biomass dry weight. The gamma-irradiated (at 2.0 KGy) *A. niger* OSH5 was grown on Czapek's broth medium at pH 6.5, 25ºC and 150 rpm incubated for 6 days.

3.7 Effect of Different Carbon Sources

The highest levels of extracellular invertase activity in SbmF were obtained when the fungus was cultured in Czapek's broth with 3% sucrose (11.7 u/ml), wheat bran (9.7 u/ml), and oat meal (7.7 u/ml) as carbon sources, respectively (Table 5). The best production for the intracellular invertase occurred also in medium with 3% of sucrose (14.8 u/ml) followed by 1.0% glucose (11.8 u/ml). Other agro-industrial wastes at 3% (w/v) used as carbon sources in Czapek's broth medium (Table 5) could support invartase production but in lower amounts; rice bran 5.1 u/ml; sugar cane bagasse 2.0 u/ml and wheat straw 0.9 u/ml.

3.8 Influence of Different Wheat Bran Concentrations

SSF was found to support extracellular invartase production in higher amounts than SubmF. When the irradiated *A. niger* OSH5 was grown under SSF using 5%, 8%, 11% and 14% wheat bran as solid substrate, it was found that the greater concentration of the wheat bran was used, the higher production of both extracellular and intracellular invertase was obtained. The maximum production of extracellular invertase (15.9 u/g) was obtained in the SSF with 14% (w/v) wheat bran humidified with salt solution (Table 6), while the intracellular invertase production was 1.4 u/g at the same conditions. Regarding to the availability of the moistening agent for both extracellular and intracellular invertase activity, salt solution was superior to distilled water and tap water. Salt solution was used previously in several reports as a moistening agent [12,30]. Aronda et al. [12] reported that the higher production of invertase in SSF than SubmF can be attributed to difference in the induction and repression of enzyme synthesis. Water affects the physical properties of the solid substrate mainly by causing swelling of the substrate and facilitates effective absorption of the nutrient from the substrate for growth and metabolic activity [29]. Moisture content below the optimum level might reduced the nutrient solubility of the substrate and affects the initial growth of fungal spores which could disrupt the fungal growth and enzyme activity [30,31]. Low moisture content not only lessened substrate swelling but also reduced nutrient solubility and caused higher water tension which also result poor fungal growth [32].

Table 1. Quantitative determination of extracellular invertase production and biomass dry weight by the microbial isolates

Isolate code no.	Microbial group	2 days		4 days		6 days	
		Enzyme activity (u/ml)	Dry weight (g/100 ml)	Enzyme activity (u/ml)	Dry weight (g/100 ml)	Enzyme activity (u/ml)	Dry weight (g/100 ml)
OSH1	Fungus	0.6 ± 0.02^{d}	0.23 ± 0.06^{b}	1.8 ± 0.09^{d}	0.48 ± 0.17^{d}	2.2 ± 0.18^{g}	0.71 ± 0.21^{e}
OSH2	Fungus	0.8 ± 0.04^{c}	0.21 ± 0.02^{bc}	2.1 ± 0.12^{c}	0.49 ± 0.20^{c}	3.3 ± 0.13^{d}	0.83 ± 0.22^{b}
OSH3	Bacterium	0.8 ± 0.03^{c}	0.11 ± 0.02^{c}	2.4 ± 0.11^{b}	0.21 ± 0.17^{f}	3.1 ± 0.15^{e}	0.51 ± 0.14^{g}
OSH4	Fungus	0.9 ± 0.03^{b}	0.24 ± 0.06^{b}	0.7 ± 0.14^{f}	0.50 ± 0.41^{b}	4.9 ± 1.12^{b}	0.76 ± 0.23^{c}
OSH5	Fungus	1.7 ± 0.11^{a}	0.47 ± 0.14^{a}	5.7 ± 0.47^{a}	1.30 ± 0.20^{a}	9.7 ± 1.63^{a}	1.70 ± 0.87^{a}
OSH6	Fungus	0.9 ± 0.08^{b}	0.22 ± 0.04^{bc}	2.1 ± 0.21^{c}	0.48 ± 0.12^{d}	3.8 ± 1.33^{c}	0.73 ± 0.19^{d}
OSH7	Bacterium	0.5 ± 0.06^{e}	0.12 ± 0.03^{c}	1.2 ± 0.13^{e}	0.39 ± 0.14^{e}	2.7 ± 0.91^{f}	0.67 ± 0.23^{f}

The fungal isolates were grown on Czapek's broth medium and the bacterial isolates were grown on nutrient broth medium at 25ºC and agitation speed 100 rpm. Means with different superscript letters in the same column are considered statistically different (LSD test, $P \leq 0.05$)

Table 2. Effect of different gamma irradiation doses on extracellular invertase production and biomass dry weight

Gamma irradiation doses (KGy)	2 days		4 days		6 days	
	Enzyme activity (u/ml)	Dry wt. (g/100 ml)	Enzyme activity (u/ml)	Dry wt. (g/100 ml)	Enzyme activity (u/ml)	Dry wt. (g/100 ml)
0	1.9 ± 0.21^{d}	0.46 ± 0.16^{ab}	5.9 ± 0.51^{c}	1.4 ± 0.27^{e}	10.3 ± 1.88^{c}	1.7 ± 0.51^{d}
0.25	1.8 ± 0.22^{e}	0.47 ± 0.18^{a}	6.1 ± 0.92^{b}	1.5 ± 0.25^{d}	9.8 ± 1.71^{e}	1.8 ± 0.43^{c}
0.5	2.1 ± 0.30^{c}	0.46 ± 0.20^{ab}	6.0 ± 0.87^{c}	1.7 ± 0.23^{c}	10.2 ± 1.61^{d}	1.8 ± 0.42^{c}
1	2.4 ± 0.43^{b}	0.48 ± 0.18^{a}	6.7 ± 0.93^{a}	1.9 ± 0.31^{a}	11.1 ± 1.73^{b}	1.9 ± 0.51^{b}
2	2.7 ± 0.53^{a}	0.48 ± 0.21^{a}	6.9 ± 0.88^{a}	1.8 ± 0.43^{b}	12.2 ± 1.63^{a}	2.2 ± 0.44^{a}
4	1.4 ± 0.21^{f}	0.40 ± 0.23^{b}	5.3 ± 0.31^{d}	1.1 ± 0.11^{f}	7.1 ± 0.93^{f}	1.3 ± 0.31^{e}
6	0.6 ± 0.12^{g}	0.23 ± 0.13^{c}	1.6 ± 0.41^{e}	0.32 ± 0.10^{g}	2.4 ± 0.47^{g}	0.41 ± 0.50^{f}

A. niger OSH5 was incubated on Czapek's broth medium at pH 6.5, 25ºC and agitation rate 100 rpm. Means with different superscript letters in the same column are considered statistically different (LSD test, $P \leq 0.05$).

Table 3. Effect of different shaking conditions on extracellular invertase production and protein content

Shaking (rpm)	2 days		4 days		6 days	
	Enzyme Activity (u/ml)	Protein content (mg/ml)	Enzyme Activity (u/ml)	Protein content (mg/ml)	Enzyme Activity (u/ml)	Protein content (mg/ml)
0 (static)	0.7±0.08^{f}	0.4±0.07^{e}	2.5±0.17^{f}	1.6±0.2^{f}	4.9±0.8^{g}	2.2±0.32^{f}
30	1.6±0.23^{e}	0.7±0.13^{d}	5.1±1.11^{e}	2.8±0.17^{e}	7.4±1.14^{f}	3.4±0.41^{e}
60	1.7±0.22^{d}	0.4±0.11^{e}	5.3±1.14^{d}	3.1±0.21^{d}	8.2±1.14^{e}	3.8±0.77^{d}
90	1.9±0.36^{c}	1.2±0.22^{c}	5.7±1.17^{c}	3.3±0.36^{c}	9.7±1.15^{d}	3.9±0.71^{c}
120	2.0±0.41^{b}	1.3±0.27^{b}	6.1±1.13^{b}	3.5±0.46^{a}	10.5±1.17^{c}	4.3±0.63^{b}
150	2.0±0.50^{b}	1.4±0.52^{a}	6.2±1.13^{a}	3.4±0.33^{b}	10.8±1.18^{a}	4.4±0.66^{ab}
180	2.1±0.40^{a}	1.4±0.39^{a}	6.1±1.49^{b}	3.5±0.71^{a}	10.6±1.35^{b}	4.4±0.73^{a}

The gamma-irradiated (at 2.0 KGy) A. niger OSH5 was grown on Czapek's broth medium at pH 6.5, 25ºC. Means with different superscript letters in the same column are considered statistically different (LSD test, P ≤ 0.05)

Table 4. Effect of different incubation periods on extracellular invertase production and protein content

Incubation period (day)	Enzyme activity (u/ml	Protein content (mg/ml)	Net acivity daily (u/ml)	Specific activity (u/mg prot./ml)
1	0.0^{i}	0.2±0.04^{i}	0.0^{h}	0.0^{g}
2	1.4±0.33^{h}	1.2±0.08^{h}	1.9^{b}	1.6^{f}
3	4.9±0.41^{g}	2.7±0.12^{g}	3.0^{a}	1.8^{e}
4	6.8±0.57^{f}	3.2±0.14^{f}	1.9^{b}	2.1^{d}
5	8.6±0.59^{e}	3.6±0.26^{e}	1.8^{c}	2.4^{b}
6	10.3±0.66^{d}	4.2±0.32^{d}	1.7^{d}	2.5^{a}
7	11.7±0.61^{c}	4.8±0.43^{c}	1.4^{e}	2.4^{b}
8	12.3±0.59^{b}	5.3±0.61^{b}	0.6^{f}	2.3^{c}
9	12.4±0.93^{a}	5.3±0.76^{b}	0.1^{g}	2.3^{c}
10	12.3±0.88^{b}	5.4±0.69^{a}	0.0^{h}	2.3^{c}

The gamma-irradiated (at 2.0 KGy) A. niger OSH5 was grown on Czapek's broth medium at pH 6.5, 25ºC and 150 rpm. Means with different superscript letters in the same column are considered statistically different (LSD test, P ≤ 0.05)

Table 5. Effect of different carbon sources (with varying concentrations) on the production of extracellular and intracellular invartase

Carbon sources		Extracellular invertase		Intracellular invertase	
		Enzyme activity (u/ml)	Protein content (mg/ml)	Enzyme activity (u/ml)	Protein content (mg/ml)
Without		0.5±0.04^{p}	0.1±0.03^{n}	0.0^{p}	0.0^{n}
Sucrose	1.0%	4.3±0.73^{g}	0.8±0.14^{k}	7.1±1.14^{d}	2.2±0.45^{d}
Sucrose	3.0%	11.7±0.94^{a}	3.9±0.88^{a}	14.8±1.27^{a}	4.1±0.93^{a}
Glucose	1.0%	6.3±1.4^{e}	3.4±1.11^{b}	11.8±1.34^{b}	3.3±0.81^{b}
Glucose	3.0%	5.1±0.87^{f}	3.1±1.45^{c}	9.3±2.17^{c}	3.1±0.57^{c}
Maltose	1.0%	0.3±0.02^{r}	0.0^{o}	0.0^{p}	0.0^{n}
Maltose	3.0%	0.5±0.06^{q}	0.1±0.02^{n}	0.0^{p}	0.0^{n}
Lactose	1.0%	0.9±0.14^{l}	0.2±0.08^{m}	0.4±0.13^{o}	0.1±0.02^{m}
Lactose	3.0%	1.4±0.16^{k}	0.2±0.07^{m}	0.5±0.17^{n}	0.2±0.05^{l}
Starch	1.0%	0.6±0.09^{o}	0.1±0.04^{n}	1.4±0.12^{k}	0.3±0.08^{k}
Starch	3.0%	0.8±0.14^{m}	0.2±0.12^{m}	3.1±0.27^{f}	0.4±0.16^{j}
Wheat straw	1.0%	0.8±0.17^{m}	0.2±0.18^{m}	0.7±0.08^{l}	0.2±0.10^{l}
Wheat straw	3.0%	0.9±0.21^{l}	0.2±0.13^{m}	0.6±0.12^{m}	0.2±0.09^{l}
Wheat bran	1.0%	8.8±0.96^{c}	2.7±1.18^{e}	1.8±0.11^{j}	0.2±0.08^{l}
Wheat bran	3.0%	9.7±1.13^{b}	2.8±1.22^{d}	3.9±0.88^{e}	1.7±0.37^{e}
Rice straw	1.0%	0.7±0.18^{n}	0.2±0.06^{m}	0.0^{p}	0.0^{n}
Rice straw	3.0%	0.8±0.19^{m}	0.2±0.08^{m}	0.0^{p}	0.0^{n}
Rice bran	1.0%	3.6±0.21^{h}	1.8±0.13^{i}	1.9±0.07^{i}	0.9±0.03^{g}
Rice bran	3.0%	5.1±0.82^{f}	2.2±0.32^{h}	2.2±0.13^{h}	1.1±0.08^{f}
Oat meal	1.0%	6.3±0.91^{e}	2.4±0.47^{g}	0.4±0.03^{o}	0.2±0.03^{l}
Oat meal	3.0%	7.7±1.1^{d}	2.6±0.91^{f}	0.6±0.08^{m}	0.3±0.05^{k}
Sugar cane bagasse	1.0%	1.8±0.27^{j}	0.7±0.49^{l}	1.9±0.13^{i}	0.6±0.08^{i}
Sugar cane bagasse	3.0%	2.1±0.32^{i}	0.9±0.55^{j}	2.7±0.16^{g}	0.8±0.11^{h}

The gamma-irradiated (at 2.0 KGy) A. niger OSH5 was grown on Czapek's broth medium supplemented with one carbon source at pH 6.5, 25ºC and 150 rpm incubated for 6 days. Means with different superscript letters in the same column are considered statistically different (LSD test, P ≤ 0.05)

Table 6. Influence of different wheat bran concentrations on invertase production using SSF

Wheat bran conc.	Extracellular invertase		Intracellular invertase	
g (%, w/v)	Enzyme activity (u/g)	Protein content (mg/g)	Enzyme activity (u/g)	Protein content (mg/g)
With tap water				
5	7.4±1.71^{j}	2.3±0.55^{g}	0.6±0.04^{f}	0.3±0.07^{e}
8	8.9±1.66^{g}	2.6±0.82^{d}	0.8±0.05^{d}	0.3±0.07^{e}
11	9.8±2.11^{f}	2.6±0.87^{d}	0.8±0.07^{d}	0.4±0.09^{d}
14	11.1±1.94^{d}	2.9±1.13^{c}	0.7±0.06^{e}	0.4±0.08^{d}
With distilled water				
5	5.1±1.21^{l}	1.9±0.77^{i}	0.4±0.04^{h}	0.3±0.08^{e}
8	7.3±1.43^{k}	2.2±0.91^{h}	0.4±0.03^{h}	0.4±0.06^{d}
11	8.6±1.57^{i}	2.4±1.31^{f}	0.5±0.06^{g}	0.4±0.07^{d}
14	9.9±1.63^{e}	2.4±1.73^{f}	0.5±0.07^{g}	0.5±0.07^{c}
With salt solution				
5	8.7±1.72^{h}	2.3±0.91^{g}	0.9±0.13^{c}	0.5±0.07^{c}
8	11.8±2.31^{c}	2.5±1.17^{e}	1.2±0.11^{b}	0.5±0.06^{c}
11	14.7±2.33^{b}	3.1±1.81^{b}	1.4±0.16^{a}	0.7±0.09^{b}
14	15.9±2.44^{a}	3.3±1.97^{a}	1.4±0.15^{a}	0.8±0.12^{a}

The gamma-irradiated (at 2.0 KGy) A. niger OSH5 was incubated for 6 days in the presence of different solutions, pH 6.5, 25ºC. Means with different superscript letters in the same column are considered statistically different (LSD test, P ≤ 0.05)

4. CONCLUSION

From this study it can be concluded that invertase enzyme was produced by *A. niger* OSH5 under submerged fermentation conditions. It was also observed that exposure of *A. niger* OSH5 to gamma irradiation (at a dose of 2.0 KGy) enhanced the production of invertase. Moreover, higher concentrations of invertase were produced using solid-state fermentation conditions than submerged fermentation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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