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PRODUCTION OF INVERTASE FROM *ASPERGILLUS NIGER* USING FRUIT PEEL WASTE AS A SUBSTRATE

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ABSTRACT

Aim of present study was production, purification and characterization of invertase by *Aspergillus niger* (isolated from soil) using fruit peel waste as a substrate. Screening of various fungi grown on potato dextrose-agar (PDA) was done using liquid medium of 50% sucrose-Cazpek. After incubation at 28˚C for 10 days, the invertase activity was determined in the culture filtrate using Fehling's solution. The positive result was indicated by brown and green precipitate. Sugar generated by the hydrolysis of sucrose was measured by DNSA method. *A. niger* produced maximum invertase on 4^{th} day of incubation under optimized culture conditions i.e. pH 5.0, temperature 30°C, inoculum size 10^6 -10^8 spores/ml in Czapek Dox using fruit peel waste as a substrate by submerged fermentation (SmF). Invertase enzyme was also produced by *A. niger* from other carbon source (lactose, fructose, sucrose etc.) as substrate. Out of these fructose showed maximum production of invertase. Fruit peel may be proved a good economic source for invertase production on an industrial scale.

KEY WORDS: ***Aspergillus niger*, fruit peel waste, submerged fermentation**

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INTRODUCTION

Invertase also known as β-fructofuranosidase catalyzed the terminal non-reducing residue of β-fructofuranoside [1]. This enzyme acts on 1, 4 glycoside linkage of sucrose and splits it into D-glucose and D-fructose [2].The resulting mixture of fructose and glucose is called invert sugar syrup. Invert sugar is sometimes referred to as artificial honey since its compositions and properties are nearly same. It is one of the most widely used enzymes in food industry where fructose is preferred than sucrose especially in the preparation of jams and candies, because it is sweeter and does not crystallize easily [3]. A wide range of microorganisms were reported to secrete invertase, it includes yeast *Saccharomyces cerevisiae* and *S. carlsbergensis* [4] and some fungi, *Penicillium* spp*, Neurospora* spp, *Aspergillus* spp [5]. The inverted sugar is incorporated more easily in industrial preparations and has more added value than sucrose [6]. Processing of fruits produces two types of waste: a solid waste of peel/skin, seeds, stones, etc, and a liquid waste of juice and wash waters. In some fruits the discarded portion can be very high (e.g. mango 30-50 %, banana 20 %, pineapple 40-50 % and orange 30-50 %). Therefore, there is often a serious waste disposal problem, which can lead to problems with flies and rats around the processing room, if not correctly dealt with. So there should be plans to use this solid fruit wastes for obtaining economically useful products like enzymes, oils, pectin, wine/vinegar etc. Even though the Brazilian, sucrose production is largest in the world, but the production of inverted sugar is not sufficient for the Brazilian demand. However, due to the sucrose low market value, the research on methods to produce inverted sugar from sugarcane, sucrose has increased in interest. The objective of the present research was production; purification and characterization of invertase by *Aspergillus niger* using fruit peel waste as a substrate.

MATERIALS AND METHODS

Isolation of microorganism

The soil samples were collected from sugarcane fields from different villages i.e. Hasan (Karnal, Haryana) and Mansa (Panjab, Haryana). Fungal strain was isolated by dilution spread-plate techniques. Strain was identified as *Aspergillus niger* based on morphological and biochemical properties. Strain was maintained on Czapek-Dox agar at 4˚C.

Screening of microorganisms for invertase activities

Aspergillus niger was incubated at 28˚C for 10 days and invertase activity was determined in the culture filtrate using Fehling's solution. The positive test was indicated by brown and green precipitate.

Processing of the substrate

The fruit peel waste (chiku, pineapple and banana) were obtained from the kitchen waste, washed, shade dried, grinded and stored in the polyethylene bags at room temperature. This fruit peel waste powder was used as substrate.

Submerged-state fermentation (SmF) and extraction of enzyme

The medium used for enzyme production under submerged fermentation consists of (g/l): sucrose 20.0, yeast extract 10.0, $(NH_4)_2SO_4$ 1.0, KH_2PO_4 3.5, $MgSO_4$ 0.75, pH - 5.0. Cultivation was carried out in 250 ml Erlenmeyer flasks each containing 50 ml of sterile medium. The flasks were then inoculated with 10^6 spores and incubated for 7 days at 30 ºC in an incubator shaker at 125 rpm. After fermentation, the supernatant was harvested by centrifugation at 10,000 rpm for 10 minute at 4°C and used as crude enzyme extract. The mycelial mass was collected by filtration and its dry weight was determined.

Estimation of invertase

Invertase activity in the culture filtrate was measured by using the method [7] given by Sumner and Howells, incubating 0.1 ml of enzyme solution with 0.9 ml of sucrose in 0.03 M acetate buffer (pH 5.0). To stop the reaction, 1.0 ml of dinitrosalicylic acid reagent was added and heated for 5 min in a boiling water bath. Finally the absorbance was read at 540 nm with the help of spectrophotometer. One unit of invertase (IU) is defined as the amount

of enzyme which liberates 1.0 micromoles of glucose/minute/ml under the assay condition. According to the International Union of Biochemistry, one enzyme international unit has been defined as the amount of enzyme that will catalyze the hydrolysis of 1.0 micromole of substrate per minute, at reaction conditions. In describing the activity of invertase, 1.0 IU corresponds to the amount of enzyme required to release 1.0 micromole of reducing sugar in one minute.

1 µg/ml = O.D + 0.0674/ 0.0009 = x µg/ml

1 micromole (µM) = x/Mol. Wt. of substrate.

Optimization of parameters for invertase production

The optimum pH and temperature for invertase activity were determined by carrying out the enzymatic assay at various pH ranging from 3.0-8.0 and temperature ranging from 20-60ºC, respectively.

Statistical analysis

All the experiments were carried out twice with two replicates in each treatment. Results were analyzed for mean and SD.

RESULTS AND DISCUSSION

Production of invertase under different fermentation conditions

A. niger secretes invertase enzyme into the culture medium and thus has an interesting biotechnological potential. To determine improved culture conditions of *A. niger,* extensive studies were undertaken to optimize invertase production by varying process conditions like substrate type, pH and temperature of the medium, carbon source concentration (sucrose) and metal ion source.

Table 1

Production of extra cellular invertase by A. niger at standard conditions using submerged fermentation.

Fungus	Invertase activity (µM)	Biomass (g)
A. niger	4.3±0.21	0.343 ± 0.03

Invertase production was 4.3±0.21 (µM) at standard conditions i.e. pH 5 and temp 30°C (Table 1). Invertase production was also affected by biomass i.e. larger biomass result in large amount of invertase production.

Effect of metal ion on production of extracellular invertase by A. niger

The invertase activity was determined under various salts such as KCL, $FeSO_4$, $MgSO_4$ and $HgCl_2$. *A. niger* exhibited maximum (6.8± 0.29 µM) with K^+ ions and minimum invertase activity (i.e. 4.3±0.17 µM) when Hg^{2+} was used as metal ion source (Fig. 1). These results were supported by the results of Antosova *et al.,* and Kaur and Sharma [8, 2] where Mn^{2+}, K^+ and Co^{2+} ions elicited a 1.4-1.9 fold increase in the activity of the enzyme, whereas it shown 36-61% inhibition at low concentrations of Hg^{2+} and Zn^{2+} during the purification and kinetic characterization of a fructosyltransferase from *Aspergillus aculeatus*.

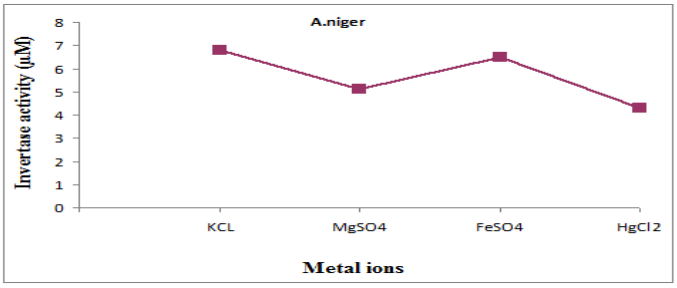

Figure 1

Effect of metal ions on the production of invertase by A. niger in submerged fermentation.

Effect of substrate on production of extracellular invertase by A. niger

Different substrates, i.e. sucrose, fructose, lactose and fruit peel were used to study their effect on growth and production of invertase from *A. niger*. Maximum invertase activity i.e. 13.7±0.64 µM was obtained when fructose was used as a carbon source while invertase production was least i.e. 4.3±0.18 µM when sucrose was used as a carbon source (Fig 2). The best carbon source was in order: fructose > lactose > fruit peel (2 pineapple: 2 banana: 1 chikku) > sucrose as shown in Figure 2.The rate of sucrose hydrolysis decreased by increasing substrate concentrations which may be due to substrate inhibition. The K value was similar to that obtained with the invertase from *A. niger* [9] and seemed to be higher than those prepared from *A. ochraceus* [10] and *A. niveus* [11], while it was lower than those from *R. glutinis* [12] and *S. cerevisiae* [13].

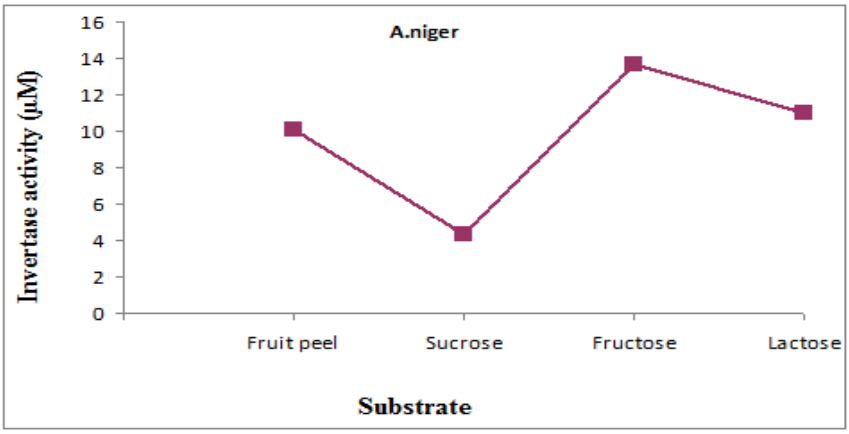

Figure 2

Effect of substrates on the production of invertase by A. niger in submerged fermentation

Effect of temperature on production of extracellular invertase by A. niger

The effect of temperature was determined by incubating the reaction mixture at different temperatures ranging from 20-60°C. Maximum invertase production i.e.12.9±0.84 µM was observed at 30 °C while minimum production i.e. 3.5±0.14 µM was observed at 60°C (Fig. 3). The enzyme activity decreased with increase in temperature due to denaturation of proteins at high temperature.

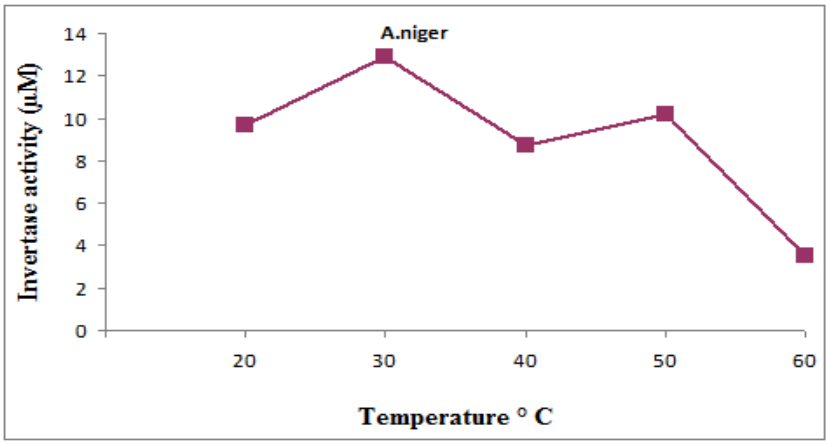

Figure 3

Effect of temperature on the production of invertase by A. niger in submerged fermentation.

Davies[14] found similar results during invertase formation in *Saccharomyces fragilis.* This result was also similar to that reported by Nguyen *et al.,*[15] found that the optimum temperature of *A. niger* invertase was 50° C.

Effect of pH on production of extracellular invertase by A. niger

A pH range from 3.0- 8.0 was studied for the determination of optimum pH for invertase production. Highest enzyme production (7.10 ±0.57 µM) was observed at pH-5.0 with *A. niger*. With the increase in pH, the invertase production decreases in case of *A. niger* (Fig. 4). Enzyme activity increased at pH values 5.0 to 7.0 possibly due to the loss of an inhibitor. Irreversible inactivation of the enzyme occurred at pH values of 7.5 or greater [16].

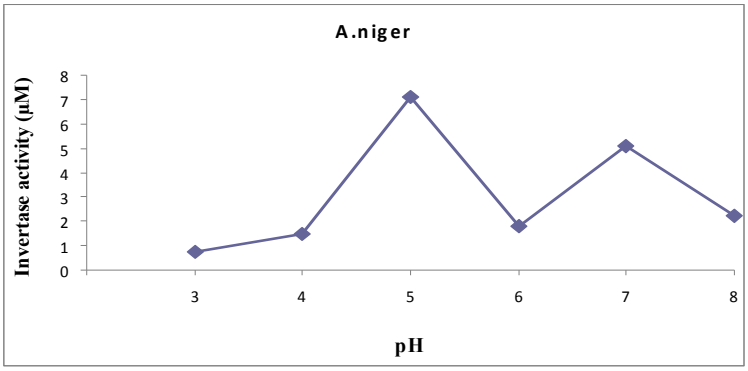

Figure 4
Effect of pH on the production of invertase by A. niger in submerged fermentation.

Characterization of invertase isolated from A. niger

Effect of temperature on stability of invertase enzyme

Temperature is one of the important parameters that determine the success of submerged fermentation. In order to study the stability of the enzyme with respect to temperature, the invertase was pre-incubated at temperatures ranging from 20-50 °C for 30 min. The optimum activity for invertase (i.e. 13.70±0.67 µM) was found at 40° C (Fig. 5). The enzyme was unstable at temperature above 70°C. Grabski *et al*., [17] reported invertase from *S. roisecleroticus* to have the temperature optima at 60° C.

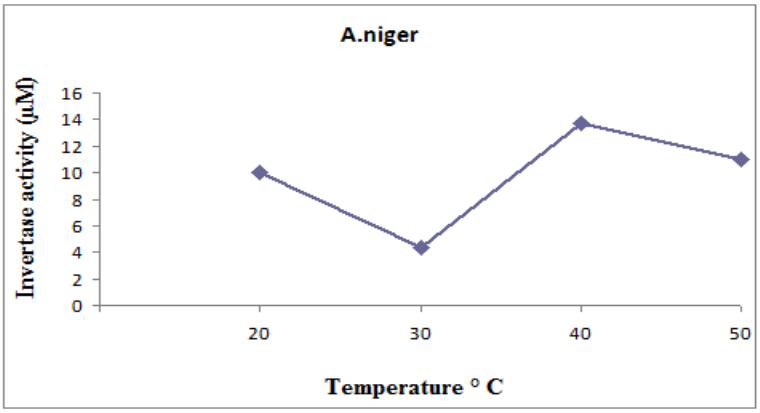

Figure 5
Effect of temperature on stability of invertase produced by A. niger

Effect of pH on stability of invertase enzyme

To determine the optimum pH for enzyme activity, the samples were assayed at various pH viz. 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 by DNS method after exposing the samples for 30 min at 25°C [18, 19,] using sucrose as substrate and sodium acetate as buffer.

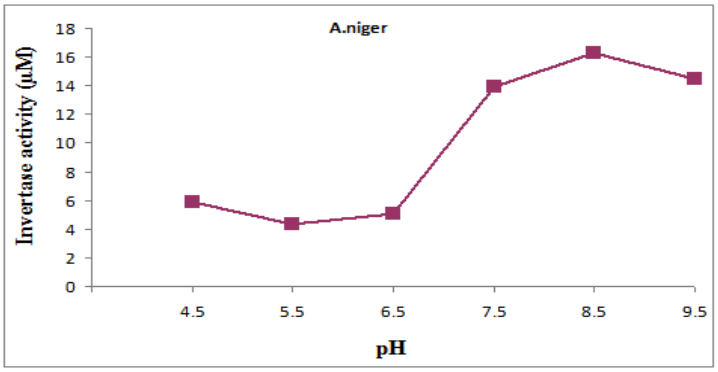

Figure 6
Effect of pH on invertase activity produced by A. niger.

Maximum invertase activity i.e. 16.25±0.60 µM was found at pH-8.5 in *A. niger* (Fig. 6). Seong *et al.,* and Rustiguel *et al.,* [20, 21] observed similar results during purification and characterization of neutral and alkaline invertase from carrot. The purified enzymes had sharp pH profiles with maximal activities at pH 6.8 and 8.0, corresponding to the activity maxima observed with the enzyme mixture.

Effect of sucrose concentration on invertase activity produced by A. niger

Maximum invertase enzyme activity i.e. 4.3± 0.32 µM was observed at 1.0% sucrose concentrations (Fig.7). Further increase in sucrose concentration the enzyme activity was decreased in *A. niger.* These results were supported by Karen and Fernando [16], Poonawalla *et al.,* [5] Aranda *et al.,* [3].

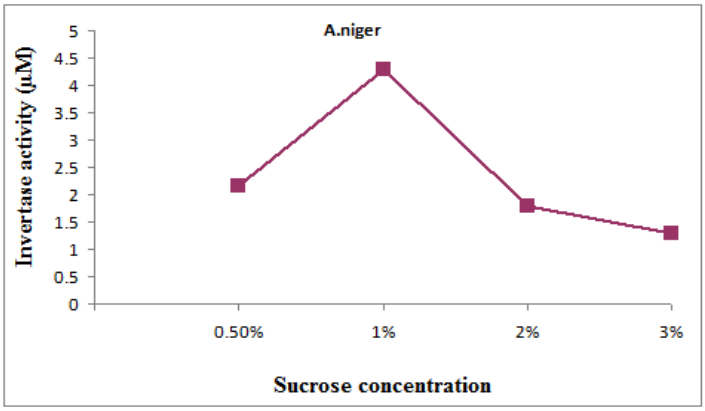

Figure 7
Effect of sucrose on invertase activity produced by A. niger.

CONCLUSION

Invertase enzyme was produced by *A. niger* using fruit peel wastes or other carbon source (lactose, fructose, sucrose etc.) as substrate. Fructose showed maximum production of invertase. Fruit peel also produced a considerable amount of invertase. Maximum production of invertase was reported when KCl or $FeSO_4$ was used as an ion source. Optimum temperature and pH for production of enzyme was 30°C and 5.0, respectively. Enzyme was found to be stable upto 40 °C for 30 min, however further increase in

temperature to 50°C reduced the stability upto 7%. Invertase was stable upto pH 8 and maximum enzyme activity was observed at 1% sucrose concentration. It is concluded that this organism may be exploited for bulk production of invertase using fruit peel a waste product of packed juice industry and kitchen waste, which is inexpensive and abundantly available in our country.

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