ENHANCED COMMERCIAL ENZYME PRODUCTION BY *PENICILLIUM NOTATUM* IBGE 03

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ABSTRACT: *In this work optimization parameters for maximum production of invertase by Penicillium notatum IBGE 03 in submerged fermentation were studied. Various agricultural based by-products (sunflower waste, cotton stalk, rice husk, date syrup and molasses) were used as sources of carbon. Optimal conditions for maximum production of invertase (6.41 U/mL) by P. notatum were observed when the strain was grown on culture medium (CM1) containing yeast extract as a source of nitrogen, molasses as a source of carbon after 48 h of incubation at initial pH 6.5, temperature 40° C, inoculum size of 6x10^6 conidia in 50 mL of culture medium and agitation rate of 200 rev/min. The strain was proved thermo (up to 60° C) and pH (up to 9.0) stable so it might be a potential strain for industrial utilization.*

Key words: fermentation, *Penicillium notatum*, commercial enzyme, optimization, submerged fermentation

1. INTRODUCTION

The modern biotechnological setup due to increasing demand of enzymes has motivated the need for enlarged survey of microorganisms surviving and producing enzyme in extreme conditions [1]. For the production of large quantities of enzymes filamentous fungi have biotechnological importance [2].

Invertase (Enzyme Code 3.2.1.26), splits sucrose into glucose and fructose. It is one of the most widely used enzymes by food industry in making chocolate covered cherries. This enzyme is also used in paper industry and to make artificial honey in which it contributes to anti-bacterial properties [2, 3].

In the present work specific interest has been focused on agriculture based by-products like sunflower, cotton stalk, rice husk, date syrup and molasses because they are usually related with pollution. Being the cost effective sources of carbon agricultural wastes have a potential for conversion into useful products [1]. In this work the secretion of invertase by *Penicillium notatum* IBGE 03 in submerged fermentation with all optimised parameters are being reported because no work is done on the strain to optimize all parameters for the production of invertase.

2. MATERILS AND METHODS

P. notatum IBGE 03 was obtained from the Institute of Biotechnology & Genetic Engineering, University of Sindh and the culture was maintained as followed by Dahot [4].

2.1 Optimization of Parameters

All experiments were done in such a way that the parameter optimized in one experiment was fixed in the following experiments.

1.1.1. Culture Medium

First of all the most suitable culture medium was determined. Composition of various culture media were (in g/L)

CM1: Dextrose 10, Peptone 5, Epsom salt 5, KH_2PO_4 5, Common salt 2.5 and ferrous sulphate hepta hydrate 0.01 [5].

CM2: Yeast extract 10, peptone 20 and sucrose 20 [6].

CM3: Yeast extract 20, peptone 40, sucrose 20, KH_2PO4 2 and Epsom salt 1 [7].

CM4: $NaNO_3$ 3, KCl 0.5, Epsom salt 0.5, ferrous sulphate hepta hydrate 0.01, K_2HPO_4 1, Sucrose 30 [8].

CM5: Sucrose 40, Corn steep liquor 30, $NaNO_3$ 3, KH_2PO_4 0.5, Epsom salt 0.05, $CaCO_3$ 2.5 [9].

2.1.2 Incubation Time

After the determination of the most suitable culture medium, optimum incubation time period was determined. It was done by growing the strain on CM1 at various interval of time from 24-240 hours.

2.1.3 Carbon Sources

After the optimization of incubation time the most suitable carbon source was determined. It was done by replacing the glucose (control) of CM1 by various wastes including sunflower waste, cotton stalk, rice husk which were hydrolysed by 0.3 *N* H_2SO_4 and 0.6 *N* H_2SO_4. Date syrup and molasses were used 0.5 % and 1 % in place of glucose (control).

2.1.4 Nitrogen Sources

After the determination of the most suitable carbon source various nitrogen sources were checked for optimum production of invertase. It was done by replacing peptone of CM1 by Corn steep, Casein, Potassium Nitrate Albumin Ammonium Sulphate Urea and Yeast Extract.

2.1.5 Incubation Temperature

The most suitable culture medium CM1 was tested on varying temperature from 30-70^o C to determine the most suitable incubation temperature.

2.1.6 Initial pH of Medium

The initial pH of a medium has an effect on growth and productivity of microorganism. A range of pH between 4.0-9.0 was checked for optimum production.

2.1.7 Inoculum Size

Productivity was also checked in terms of No. of conidia/mL in 50 mL of optimised culture medium in order to obtain the optimized inoculum size of culture medium. The No. of conidia/mL was counted by haemocytometer.

2.1.8 Agitation Rate

Effect of Agitation rate was also checked for optimization at 50, 100, 150, 200, 250 and 300 rev. /minute in orbital shaking incubator.

2.1.9 Determination of Enzyme Activity

Invertase activity was determined by BernFeld method [10]. One unit of invertase activity is the amount of enzyme which releases 1 µg of reducing sugar at 37° C per minute.

3. RESULTS AND DISCUSSIONS

3.1. Effect of culture media

Effects of various culture media on invertase production by *P. notatum* IBGE 03 after 24 h, at temperature 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min are presented (Fig 3.1). The strain was grown on five different culture media *i.e.* CM1, CM2, CM3, CM4 and CM5. It was capable of growing well on all types of culture media but production of invertase was maximum (1.62 U/mL) on culture medium CM1, which was selected for the next study.

3.2. Effect of incubation time period

The effects of incubation time periods on invertase production by *P. notatum* IBGE 03 in CM1 at temperature 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min are given (Fig 3.2). Invertase activity was measured at regular interval of 24 h and it was found that the maximum activity (2.54 U/mL) was observed after 48 h of incubation. On prolonged incubation enzyme activity was decreased, which might be due to denaturing of enzyme or synthesis of inhibiting metabolite [1]. Incubation time period of 48 h was reported for invertase production by *Aspergillus fumigatus* and *Aspergillus flavus* [3].

3.3. Effect of carbon sources

The effects of various carbon sources on invertase production by *P. notatum* IBGE 03 after 48 h in CM1 at temperature 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min are exhibited (Fig 3.3). It was observed that invertase activities were lower in case of 0.3*N* sulphuric acid hydrolysed agriculture waste (1.49, 1.27 and 1.13 U/mL for cotton stalk, sunflower waste and rice husk respectively) and 0.5 % of molasses and date syrup (1.87 and 1.69 U/mL respectively). Invertase activities were closed to control, glucose (2.54 U/mL) when 0.6*N* sulphuric acid hydrolysed agriculture waste (2.65, 2.39 and 2.51 U/mL for cotton stalk, sunflower waste and rice husk respectively) and enzyme activities were higher when 1 % of molasses (3.47 U/mL) and date syrup (2.76 U/mL) were used. Agricultural wastes in Pakistan are usually disposed of by environment non-friendly manner. So there may be two objectives which can be achieved by using agricultural wastes as sources of carbon. On one hand they can be used as raw materials for the production of valuable enzymes and other useful products while on the other hand pollution problem can be reduced. A number of nonconventional carbon sources such as starch, oilcakes, cassava starch, potato, corn and tapioca have been used in submerged fermentation for various enzymes production [12, 13, 14, 15].

3.4. Effect of nitrogen sources

The effects of various nitrogen sources on invertase production by *P. notatum* IBGE 03 after 48 h in CM1 containing molasses as carbon source at temperature 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min are presented (Fig 3.4). Various nitrogen sources (corn steep liquor, casein, potassium nitrate, albumin, ammonium sulphate, urea and yeast extract) were used in 0.25 and 0.50 % in place of peptone (control having enzyme activity 3.46 U/mL). The strain showed the capability of utilizing well all types (except urea) of nitrogen sources but yeast extract was found to be the best (2.87 U/mL in 0.25 % and 4.07 U/mL in 0.50 %). Yeast extract was also reported as the best nitrogen source for *Candida utilis, Saccharomyces cerevisiae* [6] and *Aspergillus ochraceus* [11].Very low values (0.17 and 0.08 U/mL) of invertase activities were observed when urea was used as nitrogen source. It might be due to denaturing effect of urea on invertase [17].

3.5. Effect of temperature

The effects of incubation temperatures on invertase production by ***P. notatum*** IBGE 03 after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, at initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min are exhibited (Fig 3.5). The fermentation medium was incubated at a range of temperatures 20-70° C. Invertase activity was the highest (4.64 U/mL) about 40° C. Similar optimum temperature was reported for *Penecillium lilacinum* by Ahmed *et al*. [2]. The strain showed thermo stability up to 60° C (0.23 U/mL), which is a requirement for industrial use of a microorganism [1].

3.6. Effect of initial pH

The effects of initial pH of fermentation medium on invertase production by *P. notatum* IBGE 03 after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, temperature 40° C, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min are plotted (Fig 3.6). A range of pH (4.0 to 9.0) was studied and found that initial pH of 6.5 would be the best for maximum enzyme production (5.23 U/mL). After and before the pH enzyme activities were decreased. Similar optimum pH for invertase production was reported by Dworschack & Wickerham form *Candida utilis* and *Saccharomyces crevisiae* [6].

3.7. Effect of inoculum Size

The effects of inoculum sizes on invertase production by *P. notatum* IBGE 03 after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, temperature 40° C, at initial pH 6.5 and agitation rate 50 rev/min are presented (Fig 3.7). Flasks were added with $4x10^6$-$8x10^6$ conidia and

maximum invertase activity (5.79 U/mL) was observed when $6x10^6$ conidia were added to the medium. Researchers had used inoculum size in varying percentages [1, 2, 4, 11]. Large inoculum size causes overgrowth and nutritional imbalanced resulting less production of enzyme [1, 4, 11].

3.8. Effect of agitation rate

The effects of agitation rates on invertase production by *P. notatum* IBGE 03 after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, temperature 40° C, at initial pH 6.5 and inoculum size $6x10^6$ conidia are shown (Fig 3.8). The fermentation medium was agitated at 50, 100, 150, 200, 250 and 300 rev/min. Invertase activity was maximum (6.41 U/mL) at 200 rev/min. Literature survey exposed that researchers have reported various agitation rates for different enzymes production by different microorganisms [3, 4, 19, 20, 23, 25].

4. CONCLUSION

Optimal conditions for the production of invertase (6.41 U/mL) by *Penicillium notatum* IBGE 03 were observed when the strain was grown on culture medium CM1 containing yeast extract as a source of nitrogen, molasses as a source of carbon after 48 h of incubation at initial pH 6.5, temperature 40° C, inoculum size of $6x10^6$ conidia in 50 mL of culture medium and agitation rate of 200 rev/min. The strain showed enzyme activity up to pH 9.0 (0.24 U/mL) and temperature 60° C (0.23 U/mL) which is basic requirement of a microorganism for its industrial use.

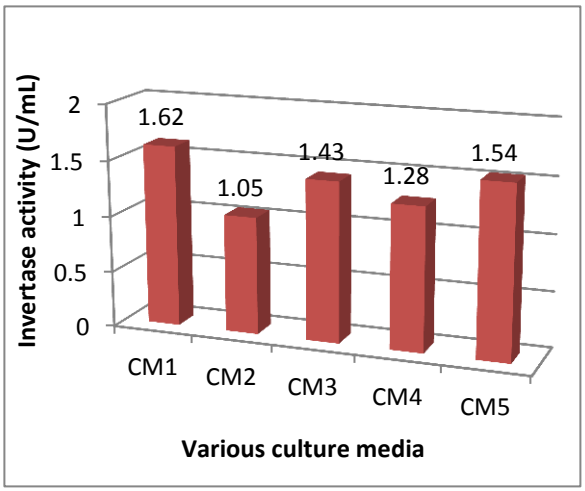

Fig 3.1: Effects of various culture media on invertase production by *Penicillium notatum* after 24 h, at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

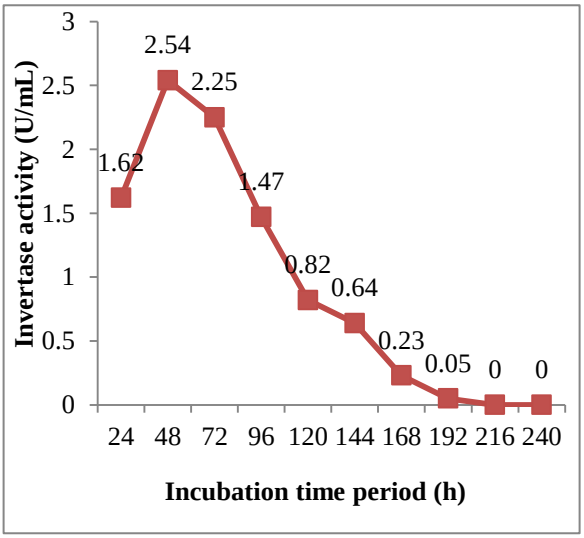

Fig 3.2: Effects of incubation time periods on invertase production by *P. notatum* in CM1 at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50

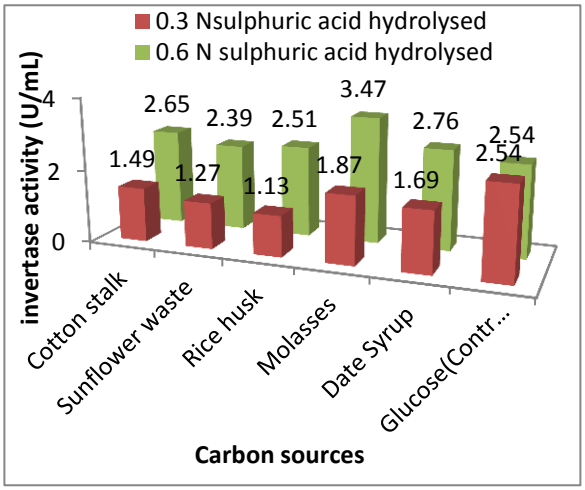

Fig 3.3: Effects of various carbon sources on invertase production by *P. notatum* after 48 h in CM1 at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

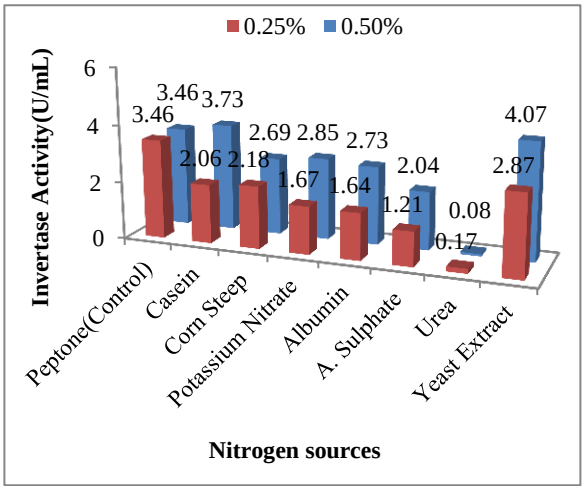

Fig 3.4: Effects of various nitrogen sources on invertase production by *P. notatum* after 48 h in CM1 containing molasses as carbon source at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

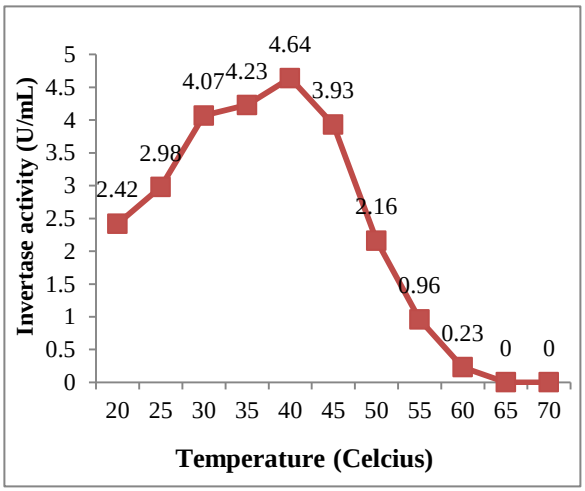

Fig 3.5: Effects of incubation temperatures on invertase production by *P. notatum* after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, at initial pH 6.0, inoculum size 4x10^6 conidia and agitation rate 50 rev/min.

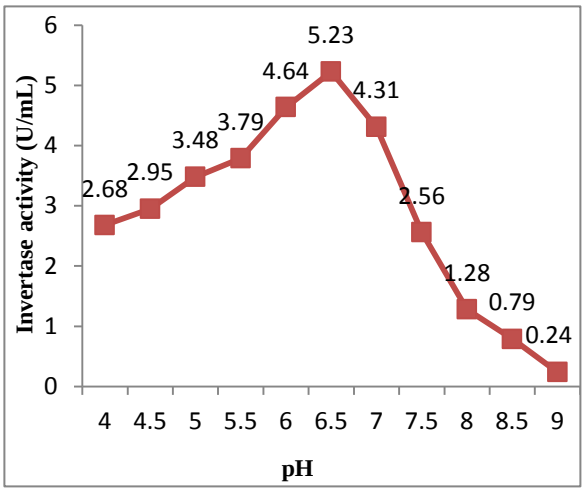

Fig 3.6: Effects of initial pH of fermentation medium on invertase production by *P. notatum* after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source at 40° C, inoculum size 4x10^6 conidia and agitation rate 50 rev/min.

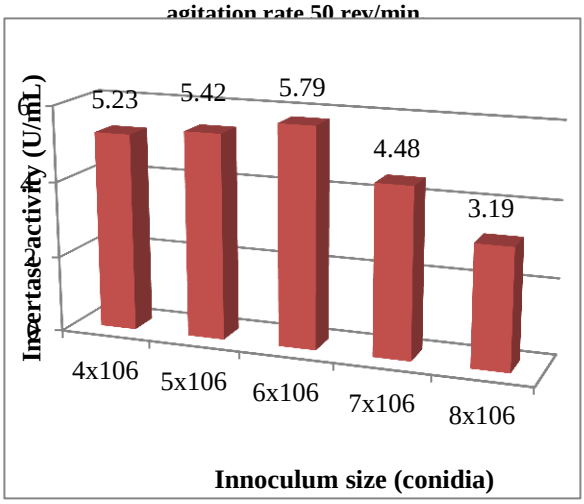

Fig 3.7: Effects of inoculum sizes on invertase production by *P. notatum* after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, at 40° C, initial pH 6.5 and agitation rate 50 rev/min.

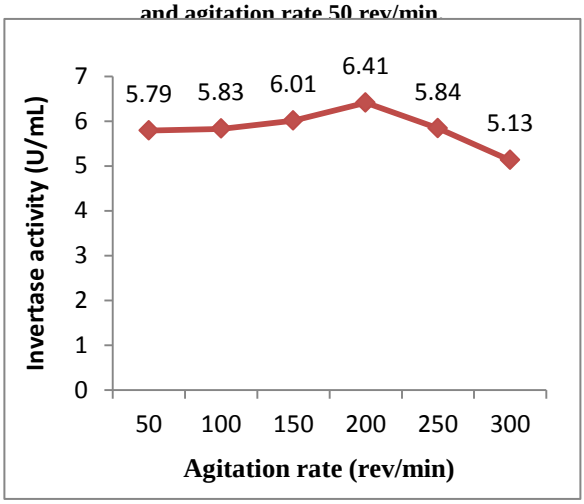

Fig 3.8: Effects of agitation rates on invertase production by *P. notatum* after 48 h in CM1 containing molasses as carbon source, yeast extract nitrogen source, at 40° C, initial pH 6.5 and inoculum size 6x10^6

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