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Glucose Isomerase Production by *Penicillium fellutanum* Isolated from Mangrove Sediment

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Abstract: Effects of pH, temperature, mineral salts, incubation time, sources and concentrations of carbon and nitrogen were tested in submerged fermentation process in production of glucose isomerase by *Penicillium fellutanum* isolated from coastal mangrove soil. The production medium prepared in distilled water, supplemented with 0.1% mineral salts, 1.5% xylose (carbon source), 1% yeast extract (nitrogen source), maintained with pH of 6.5 and incubated at 30°C for 120 h was found optimal for production of glucose isomerase.

Key words: Glucose Isomerase (GI), *Penicillium fellutanum,* mangroves, rhizosphere soil, *Rhizophora*

Introduction

With ever increasing demand for sugar and its rising price, considerable effort has been made during the past decade to find alternative sweetener. The production of sweetener from corn starch by microbial saccharides is an important application of enzyme technology in the food industry (Mermelstein, 1975; Aunstrup *et al*., 1979). The present study for high fructose corn syrup production involves several separate enzymatic steps, including liquification by amylase, saccharification by gluco-amylase and isomeraization by glucose isomerase. Of these, Glucose isomerase is an important one, which is an intracellular enzyme found in microbes which can utilise xylose as a carbon source for growth (Chen, 1980a). Physiological function of the enzyme is isomerization of D-xylose to D-xylulose *in vivo* and conversion of D-glucose to D-fructose *in vitro* (Bok *et al*., 1984). Activity of the enzyme also depends on reaction conditions (i.e., temperature, pH and metal cofactor) (Antrim *et al*., 1979; Buke, 1980). The enzyme is commercially used after extracting from many microorganisms of only terrestrial origin, but not from marine sources (Buke, 1977). Therefore, in the present study, the enzyme was attempted in a fungus isolated from coastal mangroves for maximizing its production under optimal conditions.

Materials and Methods

Microorganism

The fungus, *Penicillium fellutanum* Biourge., was isolated from rhizosphere soil of a mangrove species, *Rhizophora annamalayana* Kathir., by plating method using Sabouraud Glucose Agar medium supplemented with an antibiotic (Chloromphenicol 0.1 g L^{-1}) (Boukhout and Robert, 2003).

Chemicals

All analytical reagents and media components were purchased from Hi-Media (Mumbai, India) and Sigma chemicals (St. Louis, USA).

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Growth Media

For isolation of *Penicillium* sp., Sabouraud Glucose Agar medium containing glucose 20 g, peptone 10 g, agar 20 g, aged seawater 500 mL and distilled water 500 mL was used.

Production Medium

Production medium was composed 1% glucose, 1% yeast extract, 0.1% $MgSO_4$, 0.1% $CoCl_2$, aged seawater (500 mL) and distilled water (500 mL). The pH was adjusted to 6.5 and the media were sterilized in an autoclave for 15 min at 121°C. The media were inoculated with a loop-full of spore suspension *of P. fellutanum* and then incubated at 30°C in an orbital shaker set at 100 rpm for 96 h. At the end of the fermentation, the cell mass was disrupted to extract the intracellular enzyme and centrifuged. The supernatant was assayed for glucose isomerase activity.

Enzyme Assay

The enzyme reaction mixture contained 0.5 mL of 0.02 M sodium phosphate buffer (pH 7.0), 0.2 mL of enzyme extract. The final volume of the enzyme reaction mixture was made up to 2 mL with distilled water. The mixture was incubated at 70°C for 1 h and the reaction was stopped by adding 2 mL of 0.5 M perchloric acid. The fructose produced was determined by the method of Sadasivam and Manickam (1996). One unit of glucose isomerase activity was defined as the amount of the enzyme that produced 1 μg of D-fructose per min under assay condition described.

Optimization of Culture Conditions

The factors such as pH, temperature, mineral salts (KH_2PO_4, $NaNO_3$ and $CaCO_3$) and various sources of carbon and nitrogen that influence production of glucose isomerase were optimized by varying parameters one at a time. The experiments were conducted in 200 mL Erlenmeyer flask containing production medium. After sterilization by autoclaving, the flasks were cooled and inoculated with culture and maintained under various operational conditions separately such as pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5), temperature (20, 30, 40°C), incubation period (24, 48, 72, 96, 120, 144 h), carbon sources (glucose, xylose, maltose, lactose and sucrose, each at 1.5%), nitrogen source (peptone, beef extract, yeast extract, tryptone and casein, each at 1%) and salinity (0, 40, 50, 60, 70, 80, 90, 100% sea water). After 96 h (expect for incubation period effect), the culture filtrate was assayed in triplicate samples for glucose isomerase activity.

Statistical Analysis

Statistical analysis was done by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Production medium inoculated with *Penicillium fellutanum* and incubated for 96 h, exhibited the enzyme activity as 19 U mL^{-1} at pH 6.5 and 30°C (Table 1). The activity was significantly higher at pH 30°C than 20 and 40°C (Table 1), when the culture was incubated at 120 h the maximum production of 27 U mL^{-1} was detected. There was a 4-fold increase in activity at 120 h of incubation as compared to 24 h (Table 1).

Among the carbon sources, xylose was the best source to enhance enzyme production of 27 U mL^{-1}, which was 11% higher than maltose (Table 2). Among the nitrogen sources, yeast extract showed maximum level of production 22 U mL^{-1} (Table 2), which was about 12% higher than casein.

Xylose at 1.5% was found optimal in the production medium, exhibiting an enzyme activity of 32 U mL^{-1} (Table 3), which was 17% higher than 2.5%. Yeast extract at 1.0% showed maximum

Table 1: Effect of various physical parameters

Physical parameters		GI activity (U* mL^{-1})
pH	5.5	09±1.73[a]
	6.0	16±2.30[bc]
	6.5	19±1.15[c]
	7.0	17±1.75[c]
	7.5	11±2.60[ab]
Temperature (°C)	20.0	11±2.02[a]
	30.0	19±1.15[b]
	40.0	15±1.15[ab]
Incubation period (h)	24.0	07±0.81[a]
	48.0	11±1.47[b]
	72.0	15±0.52[c]
	96.0	19±1.15[df]
	120.0	27±0.61[e]
	144.0	21±1.75[f]

*One unit of Glucose Isomerase (GI) activity was expressed as the amount of enzyme which converts 1.0 µg of glucose 30 min^{-1} at 30°C, Values are mean±standard error from 3 replicates in each group, Values not sharing a common superscript letter(s) differ significantly at $p<0.05$ (DMRT)

Table 2: Effect of various chemical parameters

Chemical parameters		GI activity (U* mL^{-1}) mean±S.E
Carbon sources (1%)	Xylose	27±1.73[a]
	Glucose	19±1.15[bc]
	Sucrose	25±2.88[ab]
	Maltose	16±1.18[c]
Nitrogen sources (1%)	Casein	10±1.73[a]
	Beef extract	13±1.15[ab]
	Peptone	19±1.15[bc]
	Yeast extract	22±1.50[c]
	Tryptone	15±2.71[ab]
Salinity (% of sea water)	0	42±1.15[a]
	20	36±2.28[b]
	40	27±1.73[c]
	50	19±1.15[de]
	60	16±1.40[e]
	80	13±1.63[ef]
	100	09±1.58[f]

*One unit of Glucose Isomerase (GI) activity was expressed as the amount of enzyme which converts 1.0 µg of glucose 30 min^{-1} at 30°C, Values are mean±standard error from 3 replicates in each group, Values not sharing a common superscript letter(s) differ significantly at $p<0.05$ (DMRT)

Table 3: Effect of various concentrations of carbon (xylose), nitrogen (yeast extract) and mineral salts

Concentration (%)		GI activity (U* mL^{-1}) mean±SE
Xylose	0.5	21±1.20[a]
	1.0	27±1.73[b]
	1.5	32±1.15[b]
	2.0	24±0.48[a]
	2.5	15±2.02[c]
yeast extract	0.5	16±1.45[ac]
	1.0	22±1.50[b]
	1.5	19±1.15[bc]
	2.0	16±1.53[ac]
	2.5	13±1.42[a]
Mineral salts ($MgSO_4$ and $CoCl_2$)	0.1	19±1.15[a]
	0.2	25±0.61[b]
	0.3	18±1.42[a]
	0.4	15±1.45[a]
	0.5	11±1.15[c]

*One unit of Glucose Isomerase (GI) activity was expressed as the amount of enzyme which converts 1.0 µg of glucose 30 min^{-1} at 30°C, Values are mean±standard error from 3 replicates in each group, Values not sharing a common superscript letter(s) differ significantly at $p<0.05$ (DMRT)

activity (21 U mL^{-1} Table 3), which was 8% higher than 2.5%. Among the mineral salts, 0.2% exhibited higher activity (25 U mL^{-1}, Table 3), 14% more than 0.5% did. The activity was about 5 fold high in absence of seawater, as compared to 100% seawater (Table 2).

To our best knowledge, these findings represent the first reported studies on the general physiochemical properties and the regulation of glucose isomerase from a coastal microorganism. The media optimization is an important aspect to be considered in the development of fermentation technology. Among physical parameters, pH of the growth medium plays an important role by inducing morphological changes in microbes and in enzyme secretion. The pH change observed during the growth of microbes also affects product stability in the medium (Gupta *et al*., 2003). The pH optima for glucose isomerase is generally between 7.0 and 8.5 (Chen, 1980b). Although the apparent pH optima for *Penicillium fellutanum* Glucose Isomerase (GI) fall in this range, the enzyme was stable and active at pH 6.5. Temperature optimum for GI was found to be at a range of 70-80°C for the thermophilic microbes (Bok *et al*., 1984; Chou *et al*., 1976) and in the present study since it is a coastal isolate the optimal temperature was recorded at 30°C. The influence of temperature on GI production is related to the growth of microbes. The incubation period varies with enzyme productions (Smitt *et al*., 1996). Short incubation period offers potential for inexpensive production of enzymes (Sonjoy *et al*., 1995). In the present study the GI activity increased steadily and reached maximum at 120 h of incubation (Table 2), as against a short duration of 24 h in *Streptomyces* (Bok *et al*., 1984).

Among carbon sources, most reports have suggested that xylon and xylose exhibit higher activity (Chen *et al*., 1979; Lee *et al*., 1990) which is similar to the present study. Xylose exhibits the higher activity which is 31% higher than maltose. Among the organic nitrogen sources, yeast extract exhibits higher activity. In this study, the presence of mineral salts exhibit higher activity (Table 3) which is supporting earlier studies (Chen and Anderson, 1979; Chen *et al*., 1979). Even though the fungal strain was isolated from coastal soil, it produced less concentration of GI when production medium was prepared with 100% seawater (Table 2). Hence, it can be a terrestrial species facultatively halophilic in nature. Conditions optimal for production of GI by *Penicillium fellutanaum* was developed in the study.

Conclusions

The nature of culture conditions and composition of media for optimal production of glucose isomerase by *Penicillium. fellutanum* has been developed in this study. *P. fellutanum* was isolated from coastal mangrove rhizosphere soil and it produced less concentration of enzyme before optimization of physico-chemical parameters. The production medium without addition of seawater and supplemented with 0.1% mineral salts, 1.5% xylose (carbon source), 1% yeast extract (nitrogen source), maintained with pH of 6.5 and incubated at 30°C for 120 h was found optimal for production of GI. Even though the fungal strain was isolated from coastal soil, it produced less concentration of enzyme when production medium prepared with 100% sea water (09±1.58). Hence, it can be a terrestrial species facultatively halophilic in nature.

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References

Antrim, R.L., W. Colliala and B.J. Schnyder, 1979. Glucose Isomerase Production of High Frctose Syrups. Applied Biochemistry and Bioengineering. Wingard, L.B. (Ed.), Academic Press, Inc., New York, pp: 97-155.

Aunstrup, K., O. Andersen, E.A. Falch and T.K. Nielsen, 1979. Production of Microbial Enzymes. In: Microbial Technology, Peppler, H.J. and D. Perlman (Eds.), 2nd Edn., Vol. 1, Academic Press, Inc., New York, pp: 281-309.

Bok, S.H., Seidman and P.W. Wopat, 1984. Selective isolation of acidophilic *Streptomyces* strains for glucose isomerase production. Applied Environ. Microbiol., 47: 1213-1215.

Boukhout, T. and V. Robert, 2003. Yeast in Food. Wood Publishing Ltd. Cambridge, England, pp: 95-113.

Buke, C., 1977. Industrial Glucose Isomerase. In: Topics in Enzyme and Fermentation Biotechnology, Wiseman, A. (Ed.), Ellis Horwood Limited, Chichester, England, pp: 147-171.

Buke, C., 1980. Enzymes in fructose manufacture, In: Enzymes and food processing. Birch, G.G., N. Blackbrough, J.K. Parker (Edn.). Applied Science Publishers, London, pp: 51-72.

Chen, W., 1980a. Glucose isomerase. A review. Proc. Biochem., 15: 30-35.

Chen, W., 1980b. Glucose isomerase. A review. Proc. Biochem., 15: 36-41.

Chen, W.P., A.W. Anderson and Y.W. Han, 1979. Production of Glucose isomerase by *Strepyomyces flavogriseus*, Applied Environ. Microbiol., 37: 324-331.

Chen, W.P. and A.W. Andeson, 1979. Purification, Immobilization and some properties of Glucose isomerase from *Strepyomyces flavogriseus*, Applied Environ. Microbiol., 38: 1111-1119.

Chou, C.C., M.R. Ladisch and G.T. Tsao, 1976. Studies on glucose isomerase from a *Streptomyces* species. Applied Environ. Microbiol., 32: 489-493.

Gupta, R., P. Gigras, H. Mohapatra, V.K. Goswami and B. Chauhan, 2003. Microbial α-amylases: A biotechnological perspective. Proc. Biochem., 38: 1599-1616.

Lee, C., B.C. Saha and J.G. Zeicus, 1990. Characterisation of *Thermoanaerobacter* glucose isomerase in relation to saccharides synthesis and development of single step processes for sweetener production. Applied Environ. Microbiol., 56: 2895-2901.

Mermelstein, N.H., 1975. Immobilised enzymes produce high- fructose corn syrup. Food Technol., 29: 20-26.

Sadasivam, S. and A. Manickam, 1996. Biochemical Methods. 2nd Edn., New Age International (P) Ltd, New Delhi, pp: 256.

Smitt, J.P., J. Rinzema, H. Tramper, M. Van and W. Knol, 1996. Solid state fermentation of wheat bran by *Trichoderma reesei* QMQ 414. Applied Microbiol. Biotechnol., 46: 489-496.

Sonjoy, S.B. K. Bex and H. Houston, 1995. Cellulase activity of *Trichoderma reesei* (RUT-C 30) on municipal solid waste. Applied Biochem. Biotechnol., 15:145-153.