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Effect of Medium Composition on Glucose Oxidase Production by *Penicillium fellutanum* Isolated from Mangrove Rhizosphere Soil

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Abstract: The 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 oxidase by *Penicillium fellutanum* isolated from coastal mangrove soil. The production medium without addition of seawater and with provision of 1.5% lactose as carbon source, 1% beef extract as nitrogen source, 0.03% mineral salts; incubated for 96 h; maintained with pH of 6.5 and at 30°C, was found optimal for production of glucose oxidase.

Key words: Glucose oxidase (GOD), *Penicillium fellutanum*, mangroves, rhizosphere soil, *Rhizophora*

INTRODUCTION

Glucose oxidase (GOD: EC. 1. 1. 3. 4) belongs to class oxidoreductase and is also called as glucose aerodrehydrogenase (Sidney and Northon, 1955) which catalyses the oxidation of glucose, producing gluconic acid and hydrogen peroxide. It was described in 1928 by Muller in fungal cultures of *Aspergillus niger* (Barker and Shirley, 1980) and later detected in diverse sources such as insects, honey, some algae and many micro-fungi and it is now obtained on an industrial basis from fungi such as *Penicillium amagasakience*, *P. Variabile* and *A. Niger* (Pettruccioli and Federici, 1993; Petruccioli *et al*., 1993a, 1994; 1995a, b). Glucose oxidase has practical applications in food industries and clinical analysis (Richter, 1983) and a number of other potential uses have been proposed (Shaw *et al*., 1986; Jiang and Ooraikul, 1988; Schmid and Karube, 1988; Dondero *et al*., 1993). They are only few papers concerned with optimization of cultural conditions for simultaneous production of the enzyme by fungi, confined to terrestrial strains (Caridis *et al*., 1995; Petruccioli *et al*., 1994, 1995a,b) however, to our knowledge, no investigation has been performed in fungi from coastal mangroves for production and optimization of glucose oxidase.

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 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 *P. fellutanum* 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 of sucrose (1.5 g), KH_2PO_4 (0.25 g), $NaNO_3$ (4 g), $CaCO_3$ (0.25 g), 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 120 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 oxidase activity.

Enzyme Assay

An enzyme assay mixture was prepared by adding 0.2 mL of reducing sugar solution (2.5 g L^{-1}), 0.2 mL of crude enzyme preparation and 1 mL of citrate phosphate buffer with sodium nitrate 0.02 (g L^{-1}) to inhibit catalase activity. The reaction mixture was incubated at 30°C for 30 min. The reaction was stopped by keeping the tube in boiling water. To measure residual sugar, 2 mL dinitrosalicyclic acid reagent were added to above tube and boiled for 5 min followed by cooling to room temperature and diluting to 24 mL. The absorbance was read at 575 nm and glucose concentration was determined from the standard curve. One unit of glucose oxidase activity is expressed as the amount of enzyme which converts 1.0 μg of glucose into gluconic acid per 30 min at 30°C.

Optimization of Culture Conditions

The factors such as pH, temperature, mineral salts (KH_2PO_4, $NaNO_3$ and $CaCo_3$), various sources of carbon and nitrogen that influence production of glucose oxidase 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 h), carbon sources (glucose, fructose, maltose, lactose and sucrose each at 1.5%), nitrogen source (peptone, beef extract, yeast extract, meat extract and casein each at 1%) and salinity (0, 20, 40, 60, 80, 100% sea water). After 120 h (expect for incubation period effect), the culture filtrate was assayed in triplicate samples for glucose oxidase activity.

Statistical Analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncans Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Production medium inoculated with *P. fellutanum* and incubated for 120 h, exhibited the enzyme activity as 125 U mL^{-1} at pH 6.5 and 30°C (Table 1). The activity was about four fold higher at pH 30°C than 20°C (Table 1). when the culture was incubated at 96 h, the maximum production of 175 U mL^{-1} was detected. There was a 10 fold increase in activity at 96 h of incubation as compared to 24 h (Table 1).

Among the carbon sources, lactose was the best source to enhance enzyme production of 488 U mL^{-1}, which was 31% higher than sucrose (Table 2). Among the nitrogen sources, beef extract showed maximum level of production (625 U mL^{-1}, Table 2), which was about 6% higher than casein.

Table 1: Effect of various physical parameters

Physical parameters		GOD activity (U* mL^{-1})
pH	5.0	30±2.88^{a}
	5.5	70±3.46^{a}
	6.0	105±1.15^{bc}
	6.5	125±2.88^{c}
	7.0	115±2.30^{bc}
	7.5	90±1.15^{b}
	20	40±1.15^{a}
Temperature (°C)	30	125±2.88^{b}
	40	85±1.73^{C}
	24	20±1.15^{a}
	48	90±2.30^{b}
Incubation period (h)	72	130±4.61^{c}
	96	175±2.30^{d}
	120	125±2.88^{c}

*One unit of glucose oxidase (GOD) activity was expressed as the amount of enzyme which converts 1.0 μg of glucose per 30 min 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		GOD activity (U* mL^{-1}) Mean±SE
	Lactose	490±9.8^{a}
	Glucose	440±5.77^{b}
Carbon sources (1.5%)	Sucrose	175±2.88^{e}
	Fructose	225±4.61^{d}
	Maltose	395±3.46^{g}
	Casein	560±2.88^{a}
	Yeast extract	590±3.46^{b}
Nitrogen sources (1%)	Beef extract	625±2.88^{c}
	Peptone	585±3.46^{b}
	Meat extract	570±5.77^{ab}
	0	710±4.61^{a}
	20	660±2.88^{b}
Salinity (% of sea water)	40	640±4.04^{c}
	50	625±2.88^{d}
	60	620±1.55^{d}
	80	540±1.73^{e}
	100	360±3.46^{f}

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)

Lactose at 1.5% was an optimal in production medium, exhibiting an enzyme activity of 625 U mL^{-1} (Table 3). which was 35% higher than 0.5% lactose. Beef extract at 1.0% showed maximum activity (625 U mL^{-1} Table 3). which was 18% higher than 0.2%. Among the mineral salts, 0.03% exhibited higher activity (625 U mL^{-1} Table 3). 6% more than 0.01%. The activity was about 2 fold high in absence of seawater, as compared to 100% seawater (Table 2).

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). Most of the earlier studies revealed the optimum pH range 7.0 for the growth of *Aspergillus* strains and enzyme production (Kona *et al*., 2001; Miron *et al*., 2002). However, *P. fellutanum* released maximum GOD only in pH about 6.5. Temperature optimum for GOD was found to be 30-35°C for the mesophilic fungi (Fiedurek and Gromada, 1997; Kona *et al*., 2001; Miron *et al*., 2002 and the present study also recorded 30°C as optimal, which agrees with earlier findings. The influence of temperature on GOD 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

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

Concentration (%)		GOD Activity (U* mL^{-1}) mean±SE
	0.5	320±4.61[a]
	1.0	490±5.77[b]
Lactose	1.5	625±2.80[c]
	2.0	590±4.61[d]
	2.5	515±8.08[e]
	0.2	440±6.92[a]
Beef extract	0.4	500±13.86[b]
	0.6	550±11.54[c]
	0.8	590±11.54[d]
	1.0	625±2.80[d]
	1.2	580±6.92[d]
	0.01	560±2.88[a]
	0.02	580±6.92[ab]
Mineral salts (KH_2PO_4,	0.03	625±2.88[c]
$NaNO_3$ and $CaCO_3$)	0.04	590±8.7[b]
	0.05	570±11.52[ab]

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)

of enzymes (Sonjoy *et al*., 1995). In the present study the GOD activity increased steadily and reached maximum at 96h of incubation (Table 2). as against a short duration of 36 h in *Aspergillus niger* (Haflz *et al*., 2003).

Among carbon sources, most reports have suggested that glucose and sucrose exhibit higher activity (Fiedurek and Gromada, 1996; Kona *et al*., 2001) in contrast; the present study shows lactose exhibited the higher activity which was 31% higher than sucrose. Organic nitrogen sources like beef extract, yeas extract, peptone, casein, malt extract, have not been beneficial for the production of GOD (Kona *et al*., 2001), but in our studies they enhance the production of GOD in which beef extract exhibited higher activity. In this study, higher concentration of mineral salts exhibit lower activity (Table 3) which is supporting earlier studies (Kona *et al*., 2001). Even though the fungal strain was isolated from coastal soil, it produced less. Concentration of GA when production medium prepared with 100% sea water (Table 2). Hence, it can be a terrestrial species facultatively halophilic in nature. Conditions optimal for production of GOD by *P. fellutanaum* was developed in the study.

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