RESEARCH PAPER | Microbiology | Volume : 3 | Issue : 6 | June 2013 | ISSN - 2249-555X

Screening and cultural studies of Aspergillus niger for production of Glucose Oxidase

KEYWORDS: Glucose oxidase, Aspergillus niger strain-F-405-2, pH, Temperature

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ABSTRACT *From total 17 isolated fungi from varied microbial ecosystem the fungal strain Aspergillus niger strain-F-405-2 (Gene Bank Accession No.JQ388756.1) was selected based on highest glucose oxidase activity (4.86U/ml). The growth parameters of Aspergillus niger strain-F-405-2 were optimised for increased production of Glucose-oxidase. Enzyme activity was found to be enhanced at pH 5.0 (8.96 U/ml) and at temperature of 400C (8.69U/ml). Further during periodic monitoring of growth the highest enzyme activity was observed in logarithmic phase of growth after 48 hours of incubation and was found to be 1.78U/ml. In the presence of glucose as carbon source supplemented with sucrose the highest glucose activity was observed (6.31U/ml). Similarly enhanced glucose oxidase activity was observed in presence of ammonium nitrate supplemented with proteose peptone as source (6.2U/ml)*

Introduction:
Glucose oxidase (GOx, D-glucose: oxygen, 1-oxidoreductase, EC 1.1.3.4) is a flavo-protein which catalyses the oxidation of D-glucose to glucono ∂ lactone with the concomitant reduction of molecular oxygen to hydrogen peroxide. Glucose oxidase is used in food industries as a safe, natural, nontoxic preservative (to remove glucose and oxygen from food and beverages). In addition, Glucose oxidase is also one of the most important enzymes in clinical analysis and in biosensor technology.

The success of Aspergillus niger for industrial production of biotechnological products is largely due to the metabolic versatility of this strain. Glucose oxidase is normally produced by the controlled fermentation by number of organisms, such as Aspergillus niger, Aspergillus flavus, Penicillium variabile, Penicillium adametzii, Penicillium amagasakiene, Pleurotus ostreatus & Aureobasidium pullulans.

In this study an effort has been made to screen Aspergillus niger strain from extremophilic condition for Glucose oxidase production. The culture conditions were optimized for enhanced Glucose oxidase production with regard to various parameters like pH, temperature, incubation period, sugars and nitrogen sources.

Materials and Methods:
Screening:
Screening of 17 fungal isolates capable of growing at temperature 50^{0}C was done on the basis of glucose oxidase activity. Spore suspension of all isolates was inoculated at 2% inoculums level into 50 ml of production medium containing (Gm/100ml) molasses 2, glucose 2.0 , Proteose peptone 0.1 ,$CaCo_3$ 0.05 ,KH_2PO_4 0.5, NH_4No_3 0.2, $MgSo_4$ 0.02, $NH_4(So_4)_2$ 0.4, Trisodium citrate 0.5 and yeast extract 0.2 .he pH was adjusted to 5.5 using 0.1M HCl. The medium was sterilized at 100^{0}c for 35 minutes. All flasks were incubated on rotary incubator shaker (150rpm) adjusted to 50^{0}C for 48 hrs. All flasks were subjected for filtration using whatmann filter paper to collect filtrate as source of extracellular glucose oxidase. The glucose oxidase activity was determined by O-dianisidine method.

Glucose Oxidase assay
In colorimetric assay a coupled peroxide O-dianisidine system is used. The H_2O_2 liberated is split into H_2O and the oxygen is directly coupled to the dye O-dianisidine which turns to a brownish red colour.

Assay procedure
Pipetted out the following addition into a cuvette: O-dianisidine-2.4ml, Glucose-0.50ml, Peroxidase-0.10ml, mixed and allowed to equilibrate to 30^{0}c to this addition 0.1ml of enzyme added and mixed it well. Incubate it at temperature: 30^{0}c for 20 minutes. Thus by measuring the intensity at 436 nm of colour developed the enzyme is assayed (WWW. Faizyme.com/assagluohtm).

Enzyme Unit
One unit of glucose oxidase catalyzes the conversion of one micromole of glucose to gluconic acid, consuming one micromole of oxygen and liberates one micromole of oxygen and liberates one micromoles of hydrogen peroxide as per Assay Procedure (WWW.Faizyme.com/assagluohtm).

Optimisation of Cultural Conditions
a .Effect of pH
The optimum pH for production of glucose oxidase was determined by setting the fermentation using production medium set at various pH using standard buffers. The production was set by incubating the flasks on rotary incubator shaker (150rpm) adjusted to 40^{0}C for 48 hrs. After incubation the said enzyme was separated from growth by filtration and glucose oxidase activity was determined at various pH.

b. Effect of temperature
The optimum temperature for production of glucose oxidase was determined by setting the fermentation using production medium set at pH 5.0 using citrate buffer. The production was set by incubating the flasks on rotary incubator shaker (150rpm) adjusted at various temperatures in the range of 30 to 60^{0}C for 48 hrs. After incubation the said enzyme was separated from growth by filtration and glucose oxidase activity was determined at various temperatures.

c. Periodic monitoring for production of glucose oxidase.
The production medium set at pH 5.0 was inoculated with spore suspension of Aspergillus niger strain-F-405-2 was incubated on rotary incubator shaker (150rpm) adjusted to 40^{0}C. Every after twelve hours of incubation 5 ml aliquots were removed to determine glucose oxidase activity using filtrate. The production of glucose oxidase was monitored upto 5 days of incubation.

d. Effect of Carbon source:
To study the effect of supplementary carbon sources, the production medium containing glucose was supplemented with different other carbon sources (2%) viz. sucrose, starch,

Volume : 3 | Issue : 6 | June 2013 | ISSN - 2249-555X

cellulose and sodium succinate. To this medium spore suspension of Aspergillus niger strain-F-405-2 was inoculated at 2% level and all flasks were incubated at 40^{0}C for 48 hrs. Enzyme assay was carried out to determine the efficient supplementary carbon source.

e. Effect of nitrogen source:
To study the effect of supplementary nitrogen sources, the fermentation medium containing glucose was supplemented with different nitrogen sources (0.2%) viz. urea, proteose peptone, casein enzyme hydrolysate, soya meal, NH_4Cl, Ca $(No_3)_2 4H_2O$, sodium nitrate. To this medium (each 50 ml), Aspergillus niger strain-F-405-2 was inoculated at 2% level and all flasks were incubated at 40^{0}C for 48 hours. Enzyme assay was carried out to determine the efficient supplementary nitrogen source.

Result and Discussion:
Amongst 17 fungal isolates, the strain of Aspergillus niger strain-F-405-2 was shown highest glucose oxidase activity (4.86U/ml) at temperature of 40^{0}c at pH 5.0(Figure: 1). This strain was further subjected for optimization of cultural parameters. The production and enzyme activity was observed in pH range of 5 to 9 but maximum glucose oxidase activity was observed at pH 5.0(Table: 1). Our findings were similar to the work that has been carried out by Rogalski (1998) while working with glucose oxidase of Aspergillus niger G-13 Mutant (pH 5.0 to 5.5). Similar to pH the selected strain has shown production of glucose oxidase upto temperature of 60^{0}C. As the enzyme is protein in nature maximum production and activity was seen at temperature of 40^{0}c (Table 1). Our enzyme was found to be more thermo stable than the glucose oxidase studied by Shazia Khurshid in 2005 while working with Aspergillus niger (Optimum temperature 30^{0}c). During periodic monitoring it was observed that in logarithamic phase (48 hrs) the production was found to be enhanced with highest enzyme activity (Table: 1). It is observed that production of glucose oxidase increased with increase in fermentation period from 12-48 hrs , reached maximum at 48 hrs. These results are in accord with Willis (1996) who worked with Aspergillus niger, produces higher glucose oxidase after 48 hrs of fermentation while Hamid et al (2003) obtained highest glucose oxidase yield from Aspergillus niger after 36 hrs. of fermentation.

The production medium supplemented with sucrose has been found to be effective in supporting glucose for high yield of glucose oxidase (Table 2). Whereas El-Enshay in 1997 has found that fructose has induced production of glucose oxidase in Aspergillus niger Similarly production medium containing ammonium nitrate as nitrogen source supplemented with organic nitrogen as a proteose peptone gave maximum production (Table: 2). Maximum Glucose Oxidase activity was observed in presence of Protease peptone as supplemented nitrogen source, (Table: 2) which was also observed by Sandeep (2007).

Based on these fundamental studies we can formulate fermentation medium which is economical using statistical design for enhanced production of this important industrial enzyme as glucose oxidase.

Fig: 1 Glucose oxidase production by Various Fungi

Table: 1 Optimization of Cultural parameter for GOx production

pH	Glucose-oxidase activity (U/ml)	Temperature (^{0}c)	Glucose-oxidase activity (U/ml)	Incubation period (hours)	Glucose-oxidase activity (U/ml)
5.0	8.96	30	8.62	12	0.3706
6.0	8.69	40	8.69	24	0.4724
7.0	7.96	50	8.54	36	1.217
8.0	7.2	60	8.38	48	1.7843
9.0	7.06			60	0.702
				72	0.800
				84	0.3788

Table: 2 Optimization of Fermentation parameter for GOx production

Carbon sources (2%)	Glucose-oxidase activity (U/ml)	Nitrogen sources(---)	Glucose-oxidase activity (U/ml)
Glucose + Sucrose	6.34	Ammoniun Nitrate + Urea	6.12
Glucose + Strach	6.27	Ammoniun Nitrate + Protease Peptone	6.22
Glucose + Cellulose	6.035	Ammoniun Nitrate + Caseain Enzyme Hydrolysate	6.1
Glucose + Sodium succinate	6.154	Ammoniun Nitrate + Soya meal	6.2
		Ammoniun Nitrate + NH_4Cl	6.07
		Ammoniun Nitrate + $Ca(NO_3)_2.4H_2O$	6.12
		Ammoniun Nitrate + Sodium Nitrate	6.12

Acknowledgement:
The Authors acknowledges Director, Govt. Institute of Science, Aurangabad.

Vasantrao Naik Mahavidyalaya Botany Research Centre, Aurangabad.

Xceris Labs Ltd. Sydney House, Premchand Nagar Road, Ahmadabad.

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