December 2007 Volume 1 Issue 3

Trade Science Inc.

BioTechnology
An Indian Journal

FULL PAPER
BTAIJ, 1(3), 2007 [117-121]

Enhancement Of Glucose Oxidsae Production From *Aspergillus Niger* MTCC-281

Jagdish Singh[1]*, Neelam Verma[1], S.S.Marwaha[3]
[1]Department of Biotechnology, Punjabi University Patiala - 147 002 Punjab, (INDIA)
[3]Biotechnology Division Punjab State Council for Science and Technology, Sector -26, Chandigarh, (INDIA)
E-mail : jagdish122@rediffmail.com
Received: 16th July, 2007 ; Accepted: 21st July, 2007

ABSTRACT

Effect of polysaccharide sodium alginate, oligosaccharides oligoman nuronate(OM), oligoguluronate and n-hexadecane on glucose oxidase (GOx) production from *Aspergillus niger* were studied. There was 39% increase when sodium alginate(500mg/l) at zero hours was supplemented in the medium. With OM blocks(10%) obtained from hydrolysis of sodium alginate with 0.1, 0.2, 0.3 and 1.0 M HCL respectively there was 41, 42, 42, 41.05 % increase as compare to the control. Almost similar results were obtained with the OG blocks. Small increase(24.5%) was observed with 5% n-hexadecane. Maximum enhancement(48.35%) was achieved when n-hexadecane was supplemented in combination with sodium alginate(500mg/l) at zero hours of fermentation. No significant influence was observed on fungal biomass production with enhancer.

KEYWORDS

Aspergillus niger;
Glucose oxidase;
Sodium alginate;
Oligosaccghradies;
N-hexadecane.

INTRODUCTION

Glucose oxidase(EC 1.1.3.4, β-D-glucose oxygen 1-oxidoreductase) is a flavoprotein which catalyses the oxidation of, β-D- glucose by molecular oxygen to D-glucolactone and H_2O_2. It removes hydrogen from glucose and gets reduced, the reduced form of the GOx is then reoxidised by molecular oxygen, and the produced hydrogen peroxide is decomposed by catalase to water and oxygen. The D-glucolactone hydrolyses spontaneously to gluconic acid[1,2,3,4,5].

β-D-Glucose+Enzyme-FAD→Enzyme-$FADH_2$+D-glucono-1, 5-lactone Enzyme-$FADH_2$+O_2→Enzyme-FAD+H_2O_2

Enzyme is produced on industrial scale from *Aspergillus* and *Penicillium* genus. GOx is an intracellular and extracellular enzyme[6] It is widely used for the determination of glucose, desugaring the egg products, paper test strip for diabetic patients and removing oxygen from certain foods and beverages[3,7,8,9,10]. Oligosaccharides have been reported to act as a class of elicitors for fungal[11], bacterial[12], plant[13,14] and animal[15] cell cultures in the last decades. In this article effort was made to enhance the GOx production from *A. niger* using sodium alginate, oligosaccharides, and n-hexadecane.

MATERIAL AND METHODS

Microorganism

FULL PAPER

A.niger (MTCC 281) was used in this study. Culture was maintained on potato dextrose agar at 4-6^{0}C and sub cultured after every 20 days.

Pre culture

Spores of fungus *A.niger*(7.5×10^5/ml) were grown in 250ml Erlenmeyer flask containing 50ml of the medium, the composition was(g/l): $(NH_4)_2HPO_4$ 0.4; KH_2PO_4, 0.2; $MgSO_4$, 0.2; peptone, 10; sucrose, 70 and pH 5.5. This culture was incubated for 24 hrs in rotary shaker at 200 rpm at 30^{0}C.

Culture conditions

50ml of the medium containing (g/l): sucrose 75, peptone 15, $(NH_4)_2HPO_4$ 2, $MgSO_4$ 2, $NaNO_3$ 2.0, KCl 0.5, $CaCO_3$ 20.0 and pH 5.5-6.0 was inoculated with the germinated pre culture spores (15%) of 24 hrs age and culture was incubated in orbital shaker(250 rpm) at 30^{0}C

Enzyme assay

GOx activity was determined spectrophotometrically by Ciucu and Petroescu[16], as modified by Markwell et al.[17] method by the reduction of benzoquinone to hydroquine. The hydroquinone estimation was carried out at 290nm(ε=2.31mM^{-1}cm^{-1}) on UV-VIS spectrophotometer. One unit(U) of GOx activity was defined as amount of enzyme, which reduces 1.0μM of benzoquinone ml^{-1} minute^{-1}

Intra cellular enzyme determination

5gm fungal cell biomass (wet weight) was suspended in 10ml sodium citrate buffer pH 5.75(50mM). The cell paste was placed in Mortar and incubated at 4^{0}C for half an hour. Washed river sand(3gm) was added in the biomass paste and grinded with mortar for 10-15minutes. After crushing, biomass was filtered and enzyme activity was determined in the filtrate.

Effect of sodium alginate on GOx production

Different concentrations of the sodium alginate (50-1000mg/l) were added at different time intervals(0, 24 and 36hrs) in the fermentation medium and effect on the GOx production was observed.

Effect of oligosaccharides blocks on GOx production

To study the effect of oligosaccharides i.e. oligomannuronate(OM) and oligoguluronate(OG) on the GOx production, sodium alginate was hydrolyzed into OM and OG fragments, by the methods of Ariyo et al.[11], with slight modification. OM and OG blocks obtained from different molar concentration of HCl(0.1, 0.2, 0.3 and 1.0 M) were added at various concentrations(5, 10 and 15%) with respect to different time intervals(0, 24 and 36hrs) in the fermentation medium and effect on the GOx production was observed.

Effect of n-hexadecane on GOx fermentation

n-Hexadecane was added at various rates(2.5, 5.0, 7.5 and 10.0%) in the fermentation medium at zero hrs and its effect on the GOx production was observed. In other set of experiments, hydrocarbon n-hexadecane (5%) was also supplemented in the fermentation medium along with sodium alginate(500mg/l), OM(10%) or OG(10%) to study the effect of hydrocarbons in combination with polysaccharides and oligosaccharides on GOx production.

RESULTS AND DISCUSSION

Effect of sodium alginate on GOx production

Sodium alginate at various rates(50-1000mg/l) was supplemented in the fermentation medium at different time intervals(0,24,36hrs). As the concentration was increased(50–500mg/L) there was increase in GOx production and reached maximum(39%) as compared to the control after 48hrs of fermentation with sodium alginate 500mg/l but on further increase in concentration there was slight fall in the enzyme activity. Sodium alginate was most effective when added at zero hrs as compared to the 24 and 36 hrs of fermentation(TABLE 1 and figure 1). Addition of sodium alginate to culture of *A. niger* did not effect mycelia growth.

Effect of OM blocks on GOx the production

Different percentage of OM blocks(5,10and 15) at different time intervals(0,24 and 36 hrs) was supplemented in the fermentation medium, as the concentration was increased from 5-10% there was increase in GOx production, but above that there was slight fall in enzyme activity. There was maximum 41, 42, 42 and 41.05% increase as compared to the control when OM blocks(10%) obtained from hydrolysis of sodium algi-

FULL PAPER

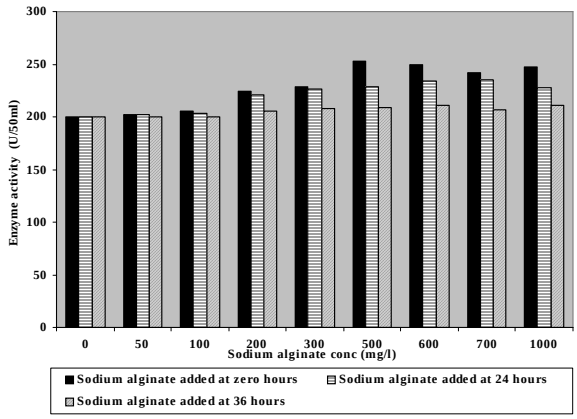

Figure 1 : Effect of sodium alginate and time of addition on GOx production by *A.niger*(values are the means of at least two indepent experiments)

TABLE 1 : Effect of sodium alginate on the production of GOx

Sodium alginate (mg/l)	Time (hrs)*	Enzyme activity (U/50ml)		
		Extracellular	Intracellular	Total
0	-	68.0±2	132.0±2	200.0±2
50	0	70.0±2	132.0±2	202.0±2
-	24	68.7±5	133.0±2	201.7±2
-	36	68.0±2	132.0±2	200.0±2
100	0	75.0±2	138.0±2	205.0±2
-	24	70.0±2	133.0±2	203.0±2
-	36	68.0±2	132.0±2	200.0±2
200	0	82.6±2	142.0±2	224.4±2
-	24	81.3±2	140.0±2	221.3±2
-	36	70.3±2	135.0±2	205.3±2
300	0	86.4±2	142.0±2	228.4±2
-	24	80.0±2	146.0±2	226.0±2
	36	72.3±2	135.0±2	207.3±2
500	0	95.3±2	183.0±2	278.3±2
	24	86.4±2	142.0±2	228.4±2
	36	74.3±2	135.0±2	209.3±2
600	0	93.3±2	177.0±2	269.3±2
	24	86.0±2	148.0±2	234.0±2
	36	74.3±2	137.0±2	211.3±2
700	0	95.3±2	167.0±2	262.3±2
	24	87.5±2	148.0±2	235.5±2
	36	76.4±2	130.0±2	206.4±2
1000	0	85.7±2	161.3±2	257.0±2
	24	75.7±2	141.3±2	227.0±2
	36	72.3±2	139.0±2	211.3±2

***Time of addition of sodium alginate (hrs) in fermentation media**

nate with 0.1, 0.2, 0.3, and 1.0 M HCl, respectively was supplemented in the medium at zero hrs of fermentation(TABLE 2), results depicting the effect of other concentrations of OM on the GOx production are not shown). In all the experiments most of the enhancement was noticed when OM blocks was added at zero hrs of fermentation. There was not significant difference in the fungal biomass between the control and supplemented cultures regardless of the concentration and time of addition of supplements.

TABLE 2 : Effect of OM blocks[a] on the production of GOx from *A.niger*

Conc. of HCL (M)[b]	OM blocks (%)[c]	Enzyme activity (U/50 ml)			% Increase as compare to the control
		Extracellular	Intracellular	Total	
-	0	70.0±2	130.0±2	200.0±2	-
0.1	10	104.0±2	178.0±2	282.0±2	41.0
0.2	10	104.0±2	180.0±2	284±2	42 .0
0.3	10	107.0±2	179.2±2	286.2±2	42 .0
1.0	10	105.0±2	177.1±2	282.1±2	41.05

[a]Results depicting the effect of other concentrations on the GOx production are not shown, [b]Concentration of HCL used for the digestion of sodium alginate, [c]OG blocks were added at zero hours in fermentation media.

TABLE 3 : Effect of OG blocks on the production of GOx from *A.niger*

Conc. of HCL (M)**	OG blocks (%)	Enzyme activity (U/50 ml)			% Increase as compare to the control
		Extracellular	Intracellular	Total	
-	Control	70±2	130.0±2	200.0±2	--
0.1	10	104.0±2	180.1±2	282.1±2	42.0
0.2	10	109.3±2	177.2±2	286.5±2	43.0
0.3	10	106.8±2	178.3±2	285.1±2	42.5
1.0	10	105.8±2	177.1±2	282.9±2	42.0

[a]Results depicting the effect of other concentrations are not shown, [b]Concentration of HCL used for the digestion of sodium alginate, [c]OG blocks were added at zero hours in fermentation media

Effect of OG blocks on GOx production

From the results(TABLE 3), results depicting the effect of other concentrations on the GOx are not shown) it was observed there was maximum 42.0, 43.0, 42.5 and 42.0% increase as compared to the control with OM blocks(10%) obtained from hydrolysis of sodium alginate with 0.1, 0.2, 0.3 and 1.0M HCl, respectively was supplemented in the medium at zero hrs of fermentation. Although at 15% concentration there was slight fall in GOx production as compare to the 10% concentration but the activity was more as compare to the control. In all the experiments most of the enhancement was noticed when OG blocks was added at zero hrs of fermentation No significant difference was observed on mycelia biomass(FDW) between culture supplemented with OG blocks and control regardless of the concentration and time of addition of the supplements.

FULL PAPER

TABLE 4 : Effect of n-hexadecane on GOx fermentation from *A.niger*

n-Hexadecane (%)	Enzyme activity (U/50 ml)			% Increase
	Extracellular	Intracellular	Total	
0	70±2	130±2	200±2	--
2.5	72±2	149±2	221±2	11.5
5.0	81±2	168±2	249±2	24.5
7.5	77±2	160±2	237±2	18.5
10	75±2	154±2	229±2	14.5

TABLE 5 : Effect of n-hexadecane in combination with sodium alginate, OM or OG blocks on the GOx production

Enhancer	Enzyme activity (U/50 ml)			% Increase as compare to control
	Extracellular	Intracellular	Total	
Control	70.0±2	130.0±2	200±2	00.00
Hexadecane[a]	81.0±2	168.0±2	249±2	24.90
Elicitor[b]	98.0±2	180.0±2	278±2	39.00
Hexadecane[a]+ Elicitor[b]	104.7±2	193.2±2	296.7±2	48.35
Elicitor[c]	104.7±2	178.0±2	284±2	42.00
Hexadecane[a]+ Elicitor[c]	115.0±2	179.3±2	294.3±2	47.15
Elicitor[d]	106.8±2	177.3±2	284.1±2	42.05
Hexadecane [a]+ Elicitor [d]	103.0±2	190.7±2	293.7±2	46.85

Hexadecane[a]=n-hexadecane(5%) Elicitor[b]=Sodium alginate (500 mg/l); Elicitor[c]Oligomannuronate(10%); Elicitor[d]=oligogulu ronate blocks(10%)

Effect of n-hexadecane on the GOx production in combination with sodium alginate, OM or OG blocks

n-dodecane, n-hexadecane and soybean oil has positive influence on the formation of extra and intracellular GOx[18]. As the concentration of n-hexadecane was increased from 0-5% there was increase in GOx production and reached maximum(24%) as compared to the control with 5% n-hexadecane (TABLE 4) and above that there was slight fall in GOx production. There was 48.5, 47.5 and 46.8% increase as compared to the control (without any enhancer) when 5% n-hexadecane was supplemented in the medium in combination with sodium alginate (500mg/l), OM(10%) or OG(10%) blocks respectively at zero hrs of fermentation (TABLE 5). There was no significant difference on mycelia biomass between control and culture supplemented with hydrocarbon n-hexadecane.

DISCUSSION

Microorganism shows the physiological and morphological responses to a range of physical and chemical factors known as elicitors. These responses have been considered as defense mechanism to ensure the survival and competitiveness. Carbohydrates are main class of compounds used as defined elicitors. Physiological changes brought about on plant and microbial cultures include the expression of novel metabolites and over production of already known products.

Oligosaccharides OM and OG obtained from sodium alginate have been reported for the enhancement of Penicillin G from *Penicillium chrysogenum*[11] and GOx from the *P.variables*[19]. There are reports which elaborates the effect of oligosaccharides on the physiological activities[12,15,20,21,22] of living organisms but less reports are available on the elicitation effect of sodium alginate.

In this paper the effect of sodium alginate, oligosaccharides and n-hexadecane on GOx enhancement from *A.niger* was investigated.

Sodium alginate from the microbial origin enhances the production of secondary metabolites in the plant system, but there was no elicitation when sodium alginate of plant origin was used[13]. Elicitation effect of sodium alginate was reported for the enhancement of GOx production on from *P.varibile*[18]. Addition of enzymatically depolymerised alginate to the culture of *Bifidobacteria spp.* promoted the cell growth. Depoly merised alginate are not used as a carbon source for the growth of bacteria, so it is very likely that alginate acts as elicitors for the enhancement of biomass[12]. It is likely that alginate function as activator of defensive system in fungus *A.niger and P.variabile* so there is enhancement of GOx production .

Enhancement of GOx production remains almost constant with oligosaccharides OM and OG blocks obtained from the hydrolysis with different molar concentration of HCl.This phenomenon shows that different molar HCl used for the hydrolysis of alginate does not affect the degree of polymerization of OM and OG blocks. Enhancement of GOx production from *P.varibile*[18] and Penicillin G from *P. chrysogenum*[11] has been reported. It is likely that OM and OG binds to receptor present on the cell membrane which gives the signal for the enhanced production of GOx in fungus. Enhanced GOx activity in fungus can be the function of other mechanism of biocontrol for the living organism[23,24,25] in the presence of alginate and oligosaccharides thus increasing the competitiveness of such fungi.

Addition of hydrocarbon in the combination with antifoaming agent(antofoam reduces the resistance in oxygen transfer rate) in the fermentation broth has positive influence in the formation of GOx[18] and there was 110% increase as compare to the control. In our study we applied n-hexadecane without the antifoaming agent so there was only 24% increase as compare to the control because there was less oxygen transfer rate due to oxygen transfer resistance. But in combination with alginate, OM and OG blocks there was 46-48% increase which is due to the above-explained mechanism.

REFERENCES

[1] Q.H.Gibson, B.E.P.Swoboda, V.Massey; Biol. Chem., **239**, 3927-3934 **(1964)**.
[2] F.R.Duke, M.Weibel, D.S.Phage, V.C.Bulgrin, J. Luthy; J.Amer.Chem.Soc., **91**, 3904-3909 **(1969)**.
[3] S.A.Barker, J.A.Shirley; 'Microbial Enzymes and Bioconversion', San Francisco, London New York, Toronto and Sydney, Academic Press, 173-226 **(1980)**.
[4] T.Doppner, W.Hartmeir; Starch/Strake, **36**, 283-287 **(1984)**.
[5] A.Crueger, W.Crueger; 'Elsevier Applied Sciense', Publishers, London and New York, 177-226 **(1990)**.
[6] G.E.Ward; 'Production of gluconic acid, glucose oxidase, fructose, Sorbose', In Microbial Technology, New York, Reinhold Publishing Corporation, 200-221 **(1967)**.
[7] J.H.Pazur; Meth.Enzymol., **9**, 82-87 **(1966)**.
[8] G.Richter; 'Industrial Enzymology: The Application of Enzymes in Industry', New York, The Nature Press, 428-436 **(1983)**.
[9] W.H.Pitcher; 'Immobilized enzymes for food processing', New York, CRC Press, 95-99 **(1980)**.
[10] M.Kamalrookh, S.F.D'Souza; J.Food.Scien. Technol., **31(2)**, 153-155 **(1994)**.
[11] B.T.Ariyo, C.Bucke, T.Keshavartz; Biotechnol. Bioeng., **53**, 17-20 **(1997)**.
[12] H.Akiyama, T.Endo, R.Nakakita, K.Murata, Y. Yenemoto, K.Okayama; Biosci.Biotechnol. Biochem., **56**, 355-356 **(1992)**.
[13] H.Dornenburg, D.Knorr; J.Food.Agri.Chem., **42**, 1048-1052 **(1994)**.
[14] S.C.Fry, S.Aldington, P.R.Hetherington, J.Aitken; Plant.Physiol., **103**, 1-5 **(1993)**.
[15] M.Otteeirei, G.Skjak-Braek, O.Smidsrod, T.Espevick; J.Immune.Ther., **10**, 286-291 **(1991)**.
[16] A.Ciucu, C.Patroescu; Anal.Lett., **17**, 1417-1427 **(1984)**.
[17] J.Markwell, L.G.Frakes, E.C.Brott, J.Osterman, F.W.Wagner; Appl.Microb.Biotechnol., **30**, 166-169 **(1989)**.
[18] L.T.Hsing, L.T.Liang; J.Fermentation and Bioeng., **78(4)**, 293-303 **(1984)**.
[19] M.Petruccioli, F.Federici, C.Bucke, T.keshavarz; Enzy.Microb.Tech., **24**, 397-401 **(1999)**.
[20] K.Murata, T.Inose, T.Hisano, S.Abe, Y.Yonemoto, T.Yamshita, M.Takagi, K.Sagakuchi, A.Kimura, T.Imanakae; J.Fer.Bioeng., **76**, 427-437 **(1993)**.
[21] M.Natsume, Y.Y.Kamo, M.Hiaryama, T.Adachi; Carbohydrates.Res., **258**, 187-197 **(1994)**.
[22] R.Curitis, P.Wang, A.Humphrey; Enz.Microb. Technol., **17**, 554-557 **(1995)**.
[23] B.Halliwell; Cell.Biol.In.Rep., **2**, 113-118 **(1978)**.
[24] M.Tiina, M.Sandholm; Int.J.Food.Microb., **5**, 165-174 **(1989)**.
[25] W.Yoo, A.G.Rand; J.Food Sci., **60**, 868-879 **(1995)**.