Pak. J. Biotech. 1(1) 31-33 (2004)

BIOSYNTHESIS OF β-GLOCOSIDASE PENICILLIUM EXPANSUM

Khalid Bhatti, M. Hanif Noomrio, *M. Umar Dahot, K.D. Ujjan and M. Uris Siyal

Department of Chemistry , Shah Abdul Latif University Khairpur, Sindh, Pakistan
*Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan

ABSTRACT: Sugarcane bagasse and leaves were utilized after treatment with 0.6N NaOH and KOH as a substrate for the growth of Penicillium expansum and the production of β-glucosidase. It was observed from the results that maximum yield (4750 units/ml) of β-glucosidase was achieved at 240 hrs when 0.6N NaOH treated sugarcane bagasse was used as carbon source.

Key words: Sugarcane bagasse, leaves; Penicillium expansum, enzyme, ß-glucosidase

INTRODUCTION: A number of enzymes are associated with the degradation of cellulose, which have excellent and widely used raw material in many fermentation processes. The enzymatic degradation of native cellulose into glucose is achieved by the synergistic action of endo-cellulase (CM-cellulase), exocellulose (avicelase) and β-glucosidase [1,2]. The potent mixture of these enzymes would be desirable for the efficient saccharification of native cellulose. The hemi-cellulytic system is more complex due to the heterologous nature of hemicellulose, which is composed of various sugar units and with attached side chains and side groups [3] many filamentous fungi are good producers of cellulases and hemi-cellulases and specially the soft rot fungus Trichoderma ressei [4,5]. Trichoderma ressei has a strong cellulose degradation activity and its enzyme may be a rational choice for the industrial use but T. ressei produce only small amount of β-glucosidase despite its high production of cellobio hydrolases [6]. However, Aspergillus and penicillium spp. are able to produce a complete system of cellulases and hemicellulases but they have not been investigated to the same extent and it is still possible to identify new enzymes with interesting features from these species [7,8]. In our present studies it is reported that locally isolated Fungi *Penicillium expansum* produce high yield of β-glucosidase by using sugarcane waste as carbon source.

MATERIAL AND METHODS

1. Microorganisms: The stock culture was maintained on Czepaks agar. The sterilized slants were inoculated with *Penicillium expansum*. After inoculation the slants were incubated at 27°C to obtain luxuriant growth.

Chemicals: Cellobiose was purchased from BDH, sodium potassium tartrate from E Merck and 3,5-dinitrosalicylic acid was supplied by sigma chemicals. All other reagents used were of analytical grade.

Culture Medium: The following ingredients were used for the preparation of culture medium as reported by Burrel et al [9] without changing the chemical composition of $(NH_4)\ SO_4$ 2.5g/L; fumaric acid 2.0g/L; KH_2PO_4 1.0g/L; Mg SO_4. $7H_2O$; 0.5g/L; $(NH_4)_2\ Fe\ (SO_4)_2$. $12H_2O$; 0.2mg/L: $ZnSO_4$ $7H_2O$; 0.2mg/L; $MnSO_4$. $5H_2O$; 0.1mg/L and thiamine hydrochloride 0.1mg/L. The pH of the culture medium was adjusted to 6.0 [10].

2. Preparation of spore Suspension: To stock culture of *penicillium expansum* 10.0ml of sterilized water was added and the surface was gently rubbed with sterilized wire loop. The spore suspension was further diluted to 100ml with sterilized water [11].

3. Hydrolysis of Agriculture wastes: 10.0g of sugarcane bagasse and leaves were hydrolyzed with 800ml of 0.6N NAOH and KOH for two hours on flame, maintaining the level of slurry constant. The digested slurry was autoclaved for 30 minutes at 1.5kg/cm2. The slurry was filtered through Whatman No. 1 filter paper after cooling at room temperature. The filtrate of solubilized sugarcane waste was incorporated into mineral medium as a carbon source. The loss in weight of sugarcane waste was determined after drying at 105°C to constant weight [12].

4. Determination of total carbohydrate: The concentration of carbohydrate in the culture broth was measured by phenolsulphuric acid method [13] with glucose as standard.

5. Determination of reducing sugars: The concentration of reducing sugars in the culture broth was determined by dinitrosalicylic acid (DNS) method [14] with glucose as a standard.

6. Determination of Protein: The protein content of culture broth was determined by Lowry et., al.,

method [15] with bovine serum albumin as a standard.

7. Assay of β-glucosidase activity: β-glucosidase activity was determined as reported by Mandels et., al., [16]. 1.0ml of enzyme sample (culture broth) was mixed with 1.0ml of 1% cellobiose and 2.0ml of sodium acetate buffer pH 4.6. The reaction was carried out at 35°C for one hour. Reducing sugars released were estimated by the dinitrosalisylic acid method [14] with glucose as a standard.

One unit of β-glucosidase activity is the amount of the enzyme that liberates μmol/minute of reducing sugar as glucose from cellobiose under the assay condition.

RESULT AND DISCUSSION

Table 1 represents the results of β-glucosidase production of *Penicillium expansum* grown on mineral medium supplemented with the hydrolysate of 0.6N NaOH treated sugar cane bagasse. The rate of β-glucosidase production and mycelial biomass increases with the increase of time period and reached maximum 4750mol/min and 1186mg/100ml respectively at 240 hours while the concentration of sugar and protein decreases through out study period. The final pH of culture medium was also noted in increasing order during β-glucosidase production.

TABLE-1: Effect of 0.6N NaOH pretreated bagasse as carbon source on the growth of P*enicillium expansum* and production of β-glucosidase culture was incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28± 2°C.

Time period hours	Final pH	Weight Of mycelia mg/100ml	TSuga mg/ml broth	R.Sugar mg/ml broth	T.Protein mg/ml broth	β-gluco-sidase activity units/ml broth
24	6.15	360.00	14.22	7.78	1.055	246.6
48	6.44	440.00	13.92	7.64	1.052	568.3
72	6.81	520.00	13.79	7.46	1.041	778.3
96	7.08	580.00	13.66	7.44	1.007	1393.3
120	7.18	660.00	13.56	7.41	0.966	1570.0
144	7.23	820.00	13.50	7.26	0.933	2100.0
168	7.30	900.00	13.46	7.17	0.910	2316.6
192	7.35	1002.00	12.90	7.11	0.865	3316.6
216	7.44	1136.00	12.24	7.01	0.836	4100.0
240	7.71	1186.00	11.25	6.96	0.781	4750.0

Glucosidase production by *Penicillium expansum* grown on 0.6N NaOH pretreated sugar cane leaves reaches maximum (58 units/units/ml) at 192 hours and then declined as shown in Table-2. The concentration of total sugar, reducing sugar and total protein in the culture medium decreases while final pH and mycelial biomass increases with the increase in time period.

TABLE-2: Effect of 0.6N NaOH pretreated sugar cane leaves as a carbon source on the growth of *penicillium expansum* and production of β-glucosidase culture was incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28± 2°C.

Time period hours	Final pH	Weight Of mycelia mg/100ml	TSuga mg/ml broth	R.Sugar mg/ml broth	T.Protein mg/ml broth	β-gluco-sidase activity units/ml broth
24	4.40	4.400	18.73	15.87	0.898	8.36
48	4.48	5.200	17.58	15.54	0.883	13.30
72	4.51	6.200	17.44	15.44	0.874	18.50
96	4.65	7.600	17.04	15.01	0.851	26.10
120	4.71	8.800	16.40	14.22	0.839	29.30
144	4.80	10.200	15.97	13.92	0.826	38.10
168	4.95	11.600	15.05	13.62	0.807	52.20
192	5.04	13.600	14.91	12.57	0.793	58.46
216	4.91	15.600	13.70	11.28	0.781	41.02
240	4.88	16.800	12.92	10.62	0.771	32.02

The result of β-glucosidase production by *Penicillium expansum* grown on 0.6N KOH treated sugar cane bagasse shown in Table-3, clearly indicates that maximum (2023.3 units/ml) yield is achieved at 192 hours and then decreases. The concentration of total sugar reducing sugar and total protein in the culture medium decreases during fermentation period. However, final pH of the medium and mycelial biomass increases throughout study period.

TABLE-3: Effect of 0.6N KOH pretreated bagasse as a carbon source on the growth of P*enicillium expansum* and production of β-glucosidase culture was incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28± 2°C.

Time period hours	Final pH	Weight Of mycelia mg/100ml	TSuga mg/ml broth	R.Sugar mg/ml broth	T.Protein mg/ml broth	β-gluco-sidase activity units/ml broth
24	5.92	360.00	12.57	6.29	0.968	785.00
48	5.98	560.00	12.24	6.27	0.959	1038.30
72	6.08	820.00	12.14	6.24	0.945	1393.30
96	6.16	960.00	11.94	6.20	0.918	1545.00
120	6.25	1100.00	11.81	6.17	0.902	2165.00
144	6.42	1240.00	11.51	5.98	0.900	2561.00
168	6.41	1300.00	11.35	5.79	0.843	2121.60
192	6.42	1420.00	10.92	5.53	0.806	2023.30
216	6.50	1620.00	10.26	5.25	0.775	1891.60
240	6.52	1840.00	9.43	5.18	0.728	1816.60

The yield of β-glucosidase production increases with the increase in time period by *Penicillium expansum* grown on 0.6N KOH treated sugar cane leave medium as shown in Table-4. the final pH of the culture medium and mycelial biomass increases with time but the concentration of total sugar, reducing sugar and total protein decreases with the growth of penicillium expansum.

TABLE-4: Effect of 0.6N KOH pretreated sugar cane leaves as a carbon source on the growth of *penicillium expansum* and production of cellulases. Culture was incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28± 2°C.

Time period hours	Final pH	Weight Of mycelia mg/100ml	TSuga mg/ml broth	R.Sugar mg/ml broth	T.Protein mg/ml broth	β-gluco-sidase activity units/ml broth
24	5.74	48.00	10.82	8.67	1.26	55.00
48	5.81	56.00	10.62	8.54	1.226	403.30
72	5.88	70.00	9.95	8.40	1.205	938.30
96	5.95	84.00	9.77	8.26	1.195	1716.60
120	6.12	102.00	9.60	8.17	1.185	1975.00
144	6.20	116.00	9.36	8.02	1.150	3083.30
168	6.63	136.00	8.92	7.87	1.138	3318.30
192	6.71	156.00	8.71	7.66	1.089	3566.60
216	6.83	162.00	8.24	7.31	1.072	3875.00
240	6.91	182.00	8.17	7.02	1.054	4583.30

It is concluded that the highest amount of β-glucosidase (4750units/ml) was produced at 240 hours when *Penicillium expansum* was grown on 0.6N NaOH pretreated sugar cane bagasse. There is no definite relationship between time of growth and β-glucosidase production but nature of carbon source, pH of culture medium and medium composition also play an essential role in enzyme synthesis by microorganisms [17-19]. It is known that synthesis of hydrolytic enzyme by fungi is subject to catalytic repression by high pure sugar concentration affecting inducible and constitutive enzymes [20,21]. On the other hand, the consistence and size of particles in media component interfered during fermentation [22].

REFERENCES

1. T. Funaguma and Hara, Bio. Chem., **52**: 749-755, (1988).
2. T. M. Wood and S.I. McCare, Biochem, J., **171**: 61 (1978)
3. Beg, Q. K., M. Kepoor, L. Mahajan and G.S. Hoondal, Appl. Microbiol. Biotechnol. **56**: 326-38 (2001)
4. Wood T. M., Biochem. Soc. Trans. **13**: 407-410 (1985).
5. Durand, H., M. Clanet and G. Tiraby, Enzyme Microbiol. **10**: 341-346 (1988)
6. D. Sternberg, Appl. Environ. Microbio., **31**: 648-654 (1976)
7. Calsen M., α-amylase production by Aspergillus oryzae. Ph.D. thesis, Department of Biotechnology. The technical University of Denmark (1994).
8. Jorgensen, H., T. Eriksson, J. Borjesson, F. Jerneld and L. Olsson, Enzyme and Microbial Technology **32**:851-861 (2003).
9. R. G. Burrel, C.W. Clayton, M.F. Gallegly and V.D. Lilly, Phytopathology, **56**:422 (1966).
10. P. Lilensiek, M.S. Fisher and E.P Baker J. Biochem **226**:981-991 (1994)
11. Y.K. Park., H. H. Sato and G.M. Pastore., Korean J, Biochem, **18**:13 (1986)
12. M. Hanif Noomrio and M. Umar Dahot, All Pak. Sci. Conf. 16-21 May (1992)
13. R. Montgomery, Biochem. Biophys. Acta **48**: 591-593 (1961).
14. G.L. Miller, Anal. Chem. **31**:426 (1959).
15. O. H. Lowry, N.J. Rosebrough, A.L, Farr and R.J. Randall, J. Bio. Chem. **193**: 265-275, (1951).
16. D. Stenberg, P. Vijakumar and E.T. Reese, Can. J. Microbial. **23**: 139 (1977).
17. Takasaki, Y., Agric. Biol. Chem., **40**: 1523 (1976)
18. Macris, B.J., M. Paspaliari and D. Kekos., Biotechnol. Lett., **4**: 785 (1982)
19. Shoemarker, S.P., J.C.Raymond and R. Burner., Basic Life Sci., **18**: 89 (1981)
20. Aguillar, G. and C. Hutron, Enz. Micobiol. Technol. **9**: 690-696 (1987)
21. Guevara, M.A., M.T. Gonzalez-Jen and P. Estevez, Can. J. Microbiol., **43**: 245-253 (1997)
22. Mitchell, D.A., N. Krieger, D.M. Stuart and A. Pandey, Process. Biochem. **35**: 1211-1225 (2000).