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Cellulase production by *Penicillium pinophilum*, *Aspergillus quadricinctus* and *Gliomastix murorum*

J K SETHI and G S RAWLA
Department of Botany, Panjab University, Chandigarh 160 014, India

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Abstract. *Penicillium pinophilum*, *Aspergillus quadricinctus* and *Gliomastix murorum* secreted a complete extra-cellular cellulase complex comprising of endo-β-glucanase, exo-β-glucanase and β-glucosidase. The enzyme production was optimum with absorbent cotton and a combination of ammonium sulphate, urea and peptone in *Penicillium pinophilum*, with carboxymethylcellulose-7 MT and ammonium chloride in *Aspergillus quadricinctus*, and with absorbent cotton and peptone in *Gliomastix murorum*. The biosynthesis of cellulase was repressed by sugars in the presence of cellulose and only negligible amounts of cellulolytic enzymes were produced when sugars formed the sole carbon sources.

Keywords. Cellulase production; *Penicillium pinophilum*; *Aspergillus quadricinctus*; *Gliomastix murorum*.

1. Introduction

Cellulose being the earth's most abundant renewable organic compound, represents an inexpensive source of biomass which can be converted into sugar, protein, fuels and other chemicals (Spano *et al* 1976; Wang *et al* 1978). The utilization of this resource for such purposes is greatly simplified if cellulose is first hydrolysed to its monomer glucose. This conversion could be accomplished either by acid or enzymatic hydrolysis but the latter has attracted considerable interest in recent years. It requires the participation of at least 3 components of cellulase viz endo-1,4-β-glucanase, exo-1,4-β-glucanase and β-glucosidase. The production of these enzymes in appreciable amounts for commercial exploitation has been reported from only a few fungal species such as *Trichoderma reesei*, *T. viride*, *T. lignorum*, *T. koningii*, *Fusarium solani*, *Penicillium funiculosum* and *Sclerotium rolfsii* (Ryu and Mandels 1980; Sadana *et al* 1979). However, many other cellulolytic fungi capable of hydrolysing cellulose more efficiently than those reported above might be existing. Therefore, the search for a more potent source of cellulase still continues. The present paper reports studies on cellulase production by *Penicillium pinophilum*, *Aspergillus quadricinctus* and *Gliomastix murorum* under different cultural conditions.

2. Materials and methods

The fungi used in the present investigation were collected from rotting paper. Several monosporic cultures of each of these fungi raised on PDA exhibited no morphological variation. Therefore, only one representative isolate of each fungus was maintained at 4°C and revived after every two months.

The following materials were obtained from the suppliers indicated: cellulose powders CP-X, CP-60, CP-100 (Cellulose Products of India Ltd., Ahmedabad), CP-

123 (Schleicher and Schül, Dassel, West Germany), CP MN 300 (Macherey Nagel and Co., D-516, West Germany), carboxymethylcellulose-7 MT, DS = 0·7 and medium viscosity (Hercules Incorporated Wilmington, Delaware, USA), absorbent cotton (Bengal Chemical and Pharmaceutical Works Ltd., Calcutta), 3,5-dinitrosalicylic acid and *p*-nitrophenyl-*β*-*D*-glucoside (Sigma Chemical Co., St. Louis, Missouri, USA). All other chemicals used were from commercial sources and were of analytical grade.

The natural cellulosic raw materials such as wheat straw, maize cobs, rice husk and sugarcane bagasse were dried in the sun (24 h) and in the oven at 60°C (48 h). They were finally ground in an electric grinder and passed through a sieve (120 mesh). A part of each of these powders was used as such (untreated) and a part was treated with sodium hydroxide 1% aq. in the ratio of 1 : 18 at 15 lbs pressure for 1 h. The contents were filtered, washed alkali-free, dried in the oven at 60°C and again pulverized into a fine powder.

The basal medium of Reese and Mandels (1963b) was used for cellulase production. It contained g/l KH_2PO_4 2·0, $(NH_4)_2SO_4$ 1·4, NH_2CONH_2 0·3, $MgSO_4.7H_2O$ 0·3, $CaCl_2.2H_2O$ 0·3, proteose peptone 1·0 and mg/l $FeSO_4.7H_2O$ 5·0, $MnSO_4.H_2O$ 1·6, $ZnCl_2$ 1·7 and $CoCl_2.2H_2O$ 2·0. Cellulose substrates (10 g/l) were added to the basal medium and the pH of the medium was adjusted to an optimum of 5·0 before autoclaving. Erlenmeyer conical flasks (capacity 250 ml) each containing 50 ml of the above medium were autoclaved at 15 lbs pressure for 20 min and the medium after cooling was inoculated with 2 ml of standardized spore suspension (2000 spores/ml). After incubation under stationary conditions at 28°C for 14 days, the cultures were filtered, the filtrate centrifuged at 10,000 g for 10 min and the supernatant assayed for various enzyme activities. Endo- and exo-*β*-glucanase activities were determined as per methods described by Mandels (1974) and *β*-glucosidase activity was assayed following the procedure of Berghem and Pettersson (1973). Three replicates were kept for assaying each enzyme activity.

2.1 *Endo-β-glucanase (CM case) activity*

0·5 ml of appropriately diluted culture filtrate was added to 0·5 ml 1% carboxymethylcellulose (CMC) (in 0·05 M citrate buffer pH 4·8) and incubated at 50°C for 30 min. The amount of reducing sugars liberated by enzymatic action was determined by the dinitrosalicylic acid (DNS) method of Miller (1959). Unit endo-*β*-glucanase activity was defined as *μ*mol glucose produced/ml/h at 50°C.

2.2 *Exo-β-glucanase activity (cotton degrading activity)*

100 mg of absorbent cotton was incubated with 2·0 ml culture filtrate and 0·1 ml 1 M citrate buffer (pH 4·8) at 50°C for 24 h and the amount of reducing sugars estimated by the DNS method. Unit exo-*β*-glucanase activity was expressed as mg of glucose released/ml/24 h at 50°C.

2·3 *β-glucosidase activity*

To 1·0 ml 1 mM *p*-nitrophenyl-*β*-*D*-glucoside (in 0·05 M acetate buffer pH 5·0) and

0·1 ml suitably diluted culture filtrate, after incubation at 40°C for 30 min was added, 2 ml 1 M Na_2CO_3 and the absorbance was noted at 410 nm. Unit β-glucosidase activity was taken as μ mol *p*-nitrophenol liberated/ml/h at 40°C.

3. Results and discussion

3.1 *Cellulase production in relation to different cellulose substrates*

The effect of 16 different cellulose substrates on cellulase production by the 3 fungi was investigated (table 1). The production of all the 3 components of cellulase was maximum with absorbent cotton in *G. murorum*. While endo- and exo-β-glucanase activities in *P. pinophilum* and *A. quadricinctus* were the highest with absorbent cotton and CMC-7 MT respectively, maximum β-glucosidase production occurred with untreated sugarcane bagasse in *P. pinophilum* and with untreated wheat straw and maize cobs and alkali-treated sugarcane bagasse in *A. quadricinctus*. But considering the optimum overall yield of the cellulase complex, absorbent cotton for *P. pinophilum* and *G. murorum* and CMC-7 MT for *A. quadricinctus* were selected as the best cellulose substrates for subsequent investigations. Considerably low cellulase yields were recorded with CP-X, CP-60, CP-100, CP-123, CP MN-300, filter paper, wheat straw, maize cobs, rice husk and sugarcane bagasse in *P. pinophilum* and *G. murorum*. In *A. quadricinctus* cellulase production was moderate with CP-60, CP-123, wheat straw, maize cobs and sugarcane bagasse. Therefore it resembles *A. terreus* GN_1 (Garg and Neelkantan 1982) and *A. terreus* 17P (Ismailova and Loginova 1975) which have been reported to give good cellulase yield with sugarcane bagasse and wheat straw respectively. These differences in the cellulase inducing efficiencies of various cellulose substrates probably result from the variations in surface area, degree of hydration and other factors which influence the availability of substrate to organism or adsorption of the enzyme from culture filtrate (Mandels and Reese 1957).

3.2 *Cellulase production with the selected concentrations of cellulose substrates*

Using the best selected cellulase inducers i.e. absorbent cotton for *P. pinophilum* and *G. murorum* and CMC-7 MT for *A. quadricinctus* the effect of different cellulose concentrations on cellulase production by these fungi was examined (table 2). As maximum production of all the 3 components of cellulase occurred with 20 g/l absorbent cotton in *G. murorum* which was the maximum concentration used in this experiment, the concentration range of cellulose was extended upto 40 g/l for *G. murorum* (table 3). An increase in the yield of all the 3 cellulase components was recorded with increase in cellulose concentration upto 7·5, 10 and 20 g/l for *P. pinophilum*, *A. quadricinctus* and *G. murorum* respectively. This was followed by a decrease in enzyme production in *P. pinophilum*. In *A. quadricinctus* and *G. murorum*, however, the cellulase yield remained constant from 10–15 and 20–30 g/l respectively, but showed a gradual decrease beyond these cellulose concentrations. This decrease in cellulase production with increasing cellulose concentration beyond the optimum may be attributed either to a physical adsorption of the enzyme on cellulose (Reese and Mandels 1963a), or to a repression of cellulase synthesis due to accumulation of the

Table 1. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase by the 3 fungi with different cellulose sources.

Cellulose source (10 g/l)	*P. pinophilum*			*A. quadricinctus*			*G. murorum*		
	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control (without cellulose)	0	0	0	0	0	0	0	0	0
CMC-7MT	16	1·15	5·3	21·0	0·65	8·6	10·0	0·22	2·6
CP-X	5	0·01	2·8	0·3	0·02	0·8	2·0	0·01	1·4
CP-60	8	0·02	1·6	14·0	0·23	4·3	0·1	0·01	0·1
CP-100	0	0·02	4·5	0·3	0·02	1·4	0·1	0·01	1·4
CP-123	4	0·02	4·0	13·0	0·03	6·7	0·1	0·01	0·1
CPMN-300	4	0·02	5·3	8·0	0·09	2·1	0·1	0·01	1·6
Filter paper	11	0·10	1·6	6·0	0·11	8·7	1·0	0·02	0·1
Absorbent cotton	32	1·40	5·3	12·0	0·17	8·6	16·0	0·23	2·6
Wheat straw (−)	2	0·14	4·6	11·0	0·09	10·7	0·1	0·01	1·6
Maize Cobs (−)	9	0·56	9·1	16·0	0·44	10·7	0·1	0·01	1·6
Rice husk (−)	4	0·44	6·5	4·0	0·03	4·5	0·1	0·01	1·4
Sugarcane bagasse (−)	11	0·44	10·7	14·0	0·15	9·1	0·1	0·02	1·6
Wheat straw (+)	2	0·16	3·2	16·0	0·15	9·1	2·0	0·02	1·4
Maize cobs (+)	8	0·44	2·6	15·0	0·65	9·1	3·0	0·02	1·4
Rice husk (+)	5	0·14	4·8	4·0	0·15	4·3	1·0	0·02	1·6
Sugarcane bagasse (+)	12	0·27	2·4	15·0	0·37	10·7	3·0	0·02	1·6

(−), Untreated; (+), alkali-treated.

Table 2. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase in relation to different cellulose concentrations using absorbent cotton for *P. pinophilum* and *G. murorum* and CMC-7MT for *A. quadricinctus* as the cellulose substrates.

Cellulose concentration (g/l)	*P. pinophilum*			*A. quadricinctus*			*G. murorum*		
	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control (without cellulose)	0	0	0	0	0	0	0	0	0
1·0	5	0·30	3·4	1	0·13	6·7	2	0	1·2
2·5	13	1·25	3·8	6	0·33	7·5	3	0	1·6
5·0	16	1·32	5·8	9	0·40	7·5	4	0	2·1
7·5	43	3·00	6·2	17	0·50	8·7	8	0·13	2·2
10·0	33	1·52	5·3	20	0·65	9·1	15	0·22	2·4
15·0	32	1·50	3·4	20	0·62	9·1	16	0·22	2·6
20·0	28	1·10	3·0	17	0·50	8·6	20	0·31	3·6

Table 3. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase by *G. murorum* with different concentrations of cellulose.

Cellulose concentration (g/l)	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control without (cellulose)	0	0	0
20	20	0·31	3·6
25	19	0·30	3·6
30	19	0·30	3·2
35	16	0·30	3·2
40	15	0·30	2·8

products of hydrolysis in the medium (Horton and Keen 1966) or to the acid conditions that may develop at high cellulose levels (Sternberg 1976).

3.3 *Cellulase production in relation to different nitrogen sources*

The effect of 10 different nitrogen sources, used singly in the basal medium, was investigated in comparison to the combined source of nitrogen (ammonium sulphate, urea and peptone) as present in the Reese and Mandels medium (table 4). Nitrogen free basal medium was prepared and supplemented with different nitrogen sources singly in amounts equivalent to the amount of N present in the original Reese and Mandels medium. The cellulose substrate, absorbent cotton (7·5 g/l) for *P. pinophilum*, CMC-7 MT (10 g/l) for *A. quadricinctus* and absorbent cotton (20 g/l) for *G. murorum* served as the carbon source.

The results indicate that in *P. pinophilum* none of the single sources of nitrogen tested gave better cellulase yield than with the combined nitrogen source as has also been reported by many workers in other fungi (Gupta *et al* 1972; Trivedi and Rao 1979). In *A. quadricinctus*, ammonium chloride significantly promoted the yield of endo-β-glucanase. However, the production of exo-β-glucanase was equal and the yield of β-glucosidase lesser with this nitrogen source than with the combination of ammonium sulphate, urea and peptone. Narania and Reddy (1978) have reported similar effect of ammonium chloride in *Fusarium oxysporum*. An interesting point is that the production of all the 3 components of cellulase in *G. murorum* was enhanced with peptone. Glycine and urea also supported moderate enzyme yields in this fungus. Lizak (1975) also reports the stimulatory effect of peptone on cellulase production in *Stysanus stemonites*, *Periconia stemonites*, *Alternaria tenuis*, *Cladosporium herbarum* and *Stemphyllium botryosum*. In showing good cellulase production with organic nitrogen sources this fungus resembles *G. convoluta* (Siu and Sinden 1951).

3.4 *Cellulase production with selected nitrogen concentrations*

The nitrogen sources giving the optimum yield of cellulase with these fungi (ammonium sulphate, urea and peptone combination for *P. pinophilum*, ammonium chloride for *A. quadricinctus* and peptone for *G. murorum*) were taken and the effect of varying nitrogen concentration on cellulase production was studied (table 5). With an increase in the concentration of N from 200–600 mg N/l in *P. pinophilum* and to 400 mg N/l in *G. murorum*, there was a concomitant increase in the production of cellulase. No further increase in the enzyme yield occurred with further increase in the N concentration; instead a decrease in the yield was observed beyond 400 mg N/l in *G. murorum*. In *A. quadricinctus* cellulase production did not increase beyond 200 mg N/l concentration. Hence, N concentrations of 600 mg N/l for *P. pinophilum*, 200 mg N/l for *A. quadricinctus* and 400 mg N/l for *G. murorum* were used in further experiments on cellulase production.

3.5 *Cellulase production in relation to some selected sugars*

The effect of 6 selected sugars on cellulase production by the 3 fungi was studied both in the absence of cellulose (so that sugar was the sole carbon source) and in the

Table 4. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase by 3 fungi with different nitrogen sources.

Nitrogen source (600 mg N/l)	*P. pinophilum*			*A. quadricinctus*			*G. murorum*		
	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control (without N)	0	0	0	0	0	0	0	0	0
Ammonium sulphate, urea and peptone combination	44	3·00	6·3	20	0·62	8·7	19·0	0·30	3·3
Potassium nitrate	25	1·92	6·5	17	0·42	4·1	16·0	0·23	2·6
Calcium nitrate	33	2·07	6·5	8	0·28	4·0	16·0	0·22	2·2
Magnesium nitrate	33	1·50	6·6	6	0·28	4·1	15·0	0·17	3·1
Ammonium chloride	31	0·75	2·1	29	0·65	3·8	2·5	0	1·9
Ammonium phosphate	33	0·92	3·0	18	0·30	4·0	2·5	0·01	1·6
Ammonium sulphate	33	0·30	2·2	20	0·30	3·6	3·0	0·01	1·6
Ammonium nitrate	20	0·52	2·4	19	0·40	4·3	3·0	0	1·9
Glycine	2	0·28	6·7	3	0·48	3·6	11·0	0·20	1·6
Urea	38	3·00	4·0	3	0·50	9·1	14·0	0·14	1·9
Peptone	24	1·50	10·1	15	0·52	15	22·0	0·40	4·7

Table 5. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase in relation to different nitrogen concentrations using ammonium sulphate, urea and peptone combination for *P. pinophilum*, ammonium chloride for *A. quadricinctus* and peptone for *G. murorum* as the nitrogen sources.

Nitrogen concentration (mg N/l)	*P. pinophilum*			*A. quadricinctus*			*G. murorum*		
	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control (without N)	0	0	0	0	0	0	0	0	0
200	32	2·00	5·3	43	1·10	7·3	3	0·02	2·1
400	40	2·50	6·2	33	0·60	4·1	27	0·38	5·0
600	45	3·00	6·5	28	0·60	4·0	23	0·38	4·8
800	44	3·00	6·3	28	0·60	4·0	16	0·29	3·8
1000	43	2·95	6·3	28	0·60	4·0	15	0·29	3·2

Table 6. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase by the 3 fungi with different sugars as sole carbon sources.

Sugar (10 g/l)	*P. pinophilum*			*A. quadricinctus*			*G. murorum*		
	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control (without sugar)	0	0	0	0	0	0	0	0	0
Glucose	0·5	0·10	5·0	0·6	0·17	3·2	0	0	3·6
Fructose	0·5	0·10	3·3	2·0	0·17	3·5	0	0	3·2
Cellobiose	1·4	0·10	3·1	0·6	0·17	3·6	0	0	3·5
Lactose	1·4	0·20	2·9	7·0	0·57	4·5	1	0·01	4·8
Maltose	0·5	0·10	2·0	0·6	0·15	3·7	0	0	5·6
Sucrose	0·5	0·18	3·5	0·6	0·15	3·6	0·8	0·01	5·0

Table 7. Production (units/ml culture filtrate) of endo-β-glucanase, exo-β-glucanase and β-glucosidase by 3 fungi with sugars (2·5 and 5·0 g/l) in the presence of cellulose.

		P. pinophilum			*A. quadricinctus*			*G. murorum*		
Sugar added	Concentration (g/l)	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
Control*	—	43	3·10	6·7	41	1·02	6·7	28·0	0·37	4·6
Glucose	2·5	34	0·97	9·1	41	0·77	6·2	18·0	0·35	4·6
	5·0	25	0·60	6·8	28	0·60	5·5	0·8	0·02	4·6
Fructose	2·5	29	0·95	9·2	33	0·70	5·5	24·0	0·35	3·6
	5·0	21	0·60	7·7	29	0·57	5·5	0·3	0	3·5
Cellobiose	2·5	36	0·60	7·7	41	1·10	6·2	24·0	0·22	5·3
	5·0	34	0·60	6·7	36	0·80	6·2	4·0	0·11	4·6
Lactose	2·5	23	1·50	6·2	28	0·62	6·2	17·0	0·37	4·6
	5·0	13	0·50	5·3	26	0·57	5·5	12·0	0·10	4·0
Maltose	2·5	26	1·15	8·7	29	0·57	5·5	20·0	0·20	4·6
	5·0	21	0·75	6·7	29	0·55	5·5	1·0	0·10	4·5
Sucrose	2·5	25	1·50	9·4	33	0·70	6·2	21·0	0·12	4·0
	5·0	21	0·77	7·6	25	0·52	5·5	0·8	0·06	3·6

*Basal medium with cellulose and without the tested sugars.

Table 8. Comparison of cellulase production by the 3 fungi with *T. reesei* QM 9414, in still culture.

	Cellulase yield (units/ml culture filtrate)		
Organism	Endo-β-glucanase	Exo-β-glucanase	β-glucosidase
T. reesei QM 9414	41	3·00	3·0
P. pinophilum	43	3·10	6·7
A. quadricinctus	41	1·02	6·7
G. murorum	28	0·37	4·6

*Grown in Reese and Mandels medium with absorbent cotton (7·5 g/l) as the cellulose source at 28°C, pH 5·0 for 14 days.

presence of optimum levels of the best selected cellulose source for each fungus. The data in table 6 reveal that cellulase yield in all the 3 fungi was negligible with glucose, fructose, cellobiose, lactose, maltose and sucrose as sole sources of carbon as compared to that obtained with cellulose (table 7). Negligible cellulase production with sugars as sole carbon sources has been similarly reported by several workers in different fungi (Bastawde *et al* 1977; Gupta *et al* 1972; Shewale and Sadana 1978) and attributed to the inducible nature of cellulase. However, significant yields of cellulase with sugars as sole carbon sources have been reported in the case of *Trichoderma* sp. (Feniksova *et al* 1978) and *Polyporus schweinitzii* (Bailey *et al* 1969).

The supplementation of cellulose medium with sugars singly at the rate of 2·5 and 5·0 g/l (table 7) resulted in decreased cellulase synthesis in all the 3 fungi indicating that the cellulolytic enzymes elaborated by these fungi are catabolite-repressed. The repression was more pronounced at 5·0 g/l than at 2·5 g/l concentration of the sugar. Other workers have similarly reported inhibition of cellulase synthesis by sugars in many fungi although the sensitivity of fungi to repression with sugar concentration varies (Gupta *et al* 1972; Horton and Keen 1966; Kassim 1982; Rapp *et al* 1982).

The 3 fungi fall in the following order based on their efficacy to synthesize a complete, well balanced and active cellulase complex: *P. pinophilum* > *A. quadricinctus* > *G. murorum*. A comparison of the optimum cellulase yields recorded for the 3 fungi with the cellulase yield obtained with *T. reesei* QM 9414 in still culture (table 8) shows that *P. pinophilum* secretes equally good amounts of endo- and exo-β-glucanase as *T. reesei* and more of β-glucosidase. *A. quadricinctus* produces equal amount of endo-β-glucanase, less of exo-β-glucanase and more of β-glucosidase and *G. murorum* synthesizes less of endo-β-glucanase and exo-β-glucanase and more of β-glucosidase than *T. reesei*. Therefore, only the cellulolytic activity of *P. pinophilum* is comparable to that of *T. reesei* QM 9414. Also, β-glucosidase production is higher in all the 3 fungi than that recorded for *T. reesei* QM 9414. β-glucosidase catalyses the last reaction in the hydrolysis of cellulose and, therefore, is of potential importance if glucose is the desired end product in cellulose saccharification (Sternberg 1976). Most *Trichoderma* cellulase preparations are generally deficient in this component (Sternberg 1976) and, therefore, supplementation of such complexes with β-glucosidase from other sources has been attempted. Sternberg *et al* (1977) have reported a significant increase in the rate of saccharification and production of glucose as the predominant product by supplementation of *Trichoderma* cellulase with β-glucosidase from *Aspergillus* spp. It is in this context that the higher production of β-glucosidase along with the endo- and exo-β-glucanases is of interest and merits further investigation.

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