

Involvement of a Conidial Endoglucanase and a Plasma-membrane-bound β -Glucosidase in the Induction of Endoglucanase Synthesis by Cellulose in *Trichoderma reesei*

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The induction of endo-1,4- β -glucanase synthesis by *Trichoderma reesei* QM 9414 was investigated in conidia, mycelia and protoplasts. Cellulose induced endoglucanase synthesis only in conidia, but not in glucose-grown mycelia or protoplasts. Cellooligosaccharides and sophorose induced endoglucanase synthesis in mycelia, conidia and protoplasts. Only conidia exhibited detectable basal endoglucanase levels, whereas β -glucosidase activity was found in conidia, mycelia and protoplasts. The β -glucosidase was inhibited *in vitro* by nojirimycin and glucono- δ -lactone. Addition of either of these inhibitors to the induction medium blocked *de novo* synthesis of endo-1,4- β -glucanase with cellulose (conidia) or cellooligosaccharides (protoplasts and mycelia) as inducer, whereas induction by sophorose remained unaffected. The results are consistent with the assumption that basal constitutive levels of endoglucanase and β -glucosidase are involved in the induction of cellulase synthesis by cellulose in *T. reesei*.

INTRODUCTION

Despite enormous efforts there are still many gaps in our understanding of cellulase biosynthesis. One of the unsolved problems concerns the mechanisms controlling cellulase biosynthesis. In wild-type strains of *Trichoderma reesei* – currently the most efficient cellulase producer (Montenecourt, 1983) – endoglucanases (endo-1,4- β -D-glucanase; EC 3.2.1.4) and cellobiohydrolase (1,4- β -D-glucan cellobiohydrolase; EC 3.2.1.91) are subject to catabolite repression by readily metabolizable substrates, and can be induced by growth on cellulose (Merivuori *et al.*, 1984). Since cellulose is insoluble, it has been suggested that the true inducer might be a low molecular mass catabolite, for example the disaccharide sophorose (2-O- β -glucopyranosyl-D-glucose), which has been isolated from culture filtrates of *T. reesei* (Mandels *et al.*, 1962), and which has been shown to be by far the strongest inducer of cellulases *in vivo* (Mandels *et al.*, 1962; Sternberg & Mandels, 1979). The formation of sophorose *in vitro* by a broken cell suspension of *T. reesei* has been demonstrated (Vaheri *et al.*, 1979). It has been thus suggested that a constitutive β -glucosidase may be responsible *in vivo* for formation of sophorose from cellulose hydrolysis products, and that sophorose may in fact be the true inducer. More recently, we have shown (Umile & Kubicek, 1986) that a constitutive β -glucosidase is predominantly located in the plasma membrane of *T. reesei*, which strongly supports its probable role in cellulase induction.

The origin of small amounts of cellulose breakdown products would require the presence of basal cellulase levels that are formed constitutively, but which have so far not been demonstrated. Alternatively, there may be recognition sites for cellulose on the mycelial surface which trigger cellulase biosynthesis (Binder & Ghose, 1978; Berg & Petterson, 1977). However, small amounts of cellulase may be bound to the cell wall surface, as described by Kubicek (1981).

In the present paper the involvement of a conidial-wall-bound endoglucanase and a plasma-membrane-bound β -glucosidase in the induction of cellulase secretion by *T. reesei* QM 9414 is described.

METHODS

Organism and growth conditions. *Trichoderma reesei* QM 9414 was used throughout these studies. It was maintained on malt-agar slants. Inocula were prepared by harvesting 14 d-old conidia in sterile tap water containing 0.1% (w/v) Tween 80. The conidia were added to the medium to a final concentration of 10^8 conidia l^{-1} . The fungus was grown in flasks on a rotary shaker (250 r.p.m.) at 28 °C in the medium described by Mandels & Andreotti (1978) except that the carbon source was 0.5% (w/v) and the pH of the medium was kept at 5.0 with phosphate/citrate buffer as described by Labudova & Farkas (1983). Samples (200 ml) of this medium were added to 1 l wide-mouthed Erlenmeyer flasks.

Isolation of protoplasts. Mycelium (20 h) from cultures grown on glucose was used for the preparation of protoplasts, which was done according to the procedure of Kolar *et al.* (1985), but with the following modifications. After harvesting, the mycelium was washed with 100 ml cold distilled water and 100 ml 50 mM-potassium phosphate buffer, pH 6.5, containing 0.9 M-KCl, 0.1% (v/v) ethanol and 100 µg chloramphenicol ml^{-1} . The mycelium was incubated with Novozym 234 for 15 h at 28 °C under the conditions reported by Kolar *et al.* (1985). After removal of the mycelial debris, protoplasts were collected by centrifugation in a swing-out rotor (100 g, 10 min, 4 °C) and resuspended in an appropriate volume of 25 mM-potassium phosphate buffer, pH 6.0, containing 0.9 M-sorbitol, to give a final density of 10^7 protoplasts ml^{-1} .

Induction of endoglucanase secretion in mycelium of *T. reesei*. Mycelium (20 h), grown on glucose as a carbon source, was harvested by filtration on G1 sintered-glass funnels (25 mm diameter) without applying pressure. After two washings, each with 50 ml sterile distilled water, mycelial suspensions were prepared in 0.25 × strength *Trichoderma* medium (Sternberg & Mandels, 1982) lacking urea and KH_2PO_4 to give a ratio of 1.5 mg mycelial dry weight to 1 ml medium. The pH of this incubation medium was controlled using phosphate/citrate buffer (Labudova & Farkas, 1983). Incubations were done in sterile 100 ml Erlenmeyer flasks, containing 25 ml of the respective mycelial suspension, which were agitated at 200 r.p.m. at 28 °C for appropriate times. Inducing compounds to be investigated were added as 26-fold concentrated solutions or suspensions after 1 h equilibration of the mycelial suspensions in the medium given above.

Induction of endoglucanase secretion in protoplasts of *T. reesei*. This was done essentially as described by Kolar *et al.* (1985).

Induction of endoglucanase secretion in conidia of *T. reesei*. This was done by inoculating 14 d-old conidia into the medium used for growth of the mycelia (see above) to give 10^8 conidia ml^{-1} , and using the inducer as a carbon source (at a concentration of 0.5 mg ml^{-1}).

Assay of enzyme activities. Endoglucanase and β -glucosidase were assayed as described by Kubicek (1981), with carboxymethylcellulose (Serva) or *p*-nitrophenyl- β -D-glucoside as the substrate. One unit (1 U) of enzyme activity is that which releases 1 µmol glucose equivalent min^{-1} . Specific activities are expressed as units of activity (mg protein) $^{-1}$. Protein was estimated by the Coomassie Blue binding method (Bradford, 1976). For inhibition studies, plasma-membrane-bound β -glucosidase was assayed on isolated membranes (Umile & Kubicek, 1986).

Immunoprecipitation of radio-labelled endoglucanase. Mycelia or protoplasts to be investigated were suspended as described above and distributed in 2 ml portions into 20 ml conical Erlenmeyer flasks. Endoglucanase secretion was induced as described above; 1 h after addition of the inducer and/or other compounds, the cells were pulsed with 100 µCi (370 kBq) [^{35}S]methionine ml^{-1} , and incubation was continued for a further 8 h. Thereafter, extracellular supernatant solutions (obtained as described above) were transferred to centrifuge tubes and mixed with 20 µl endoglucanase IgG antibody fraction per each 100 µl supernatant. After 24 h at 4 °C, 50 µl of a 10% (w/v) suspension of heat-killed *Staphylococcus aureus* cells (prepared as described by Cladaras & Kaplan, 1984) were added and the mixture kept at 4 °C for 30 min. The immune complexes were sedimented for 1 min in an Eppendorf centrifuge, resuspended and washed with 0.5 ml phosphate-buffered saline (10 mM-sodium phosphate, 150 mM-NaCl, pH 6.5) containing 0.5% (w/v) Triton X-100. The washing procedure was repeated three times. The final pellet was suspended in 20 µl 0.1 M-Tris/HCl (pH 8.0), containing 1% (w/v) SDS and 1% (v/v) β -mercaptoethanol, and heated in a boiling water bath for 10 min. After centrifugation in an Eppendorf centrifuge for 5 min, the supernatants were withdrawn, kept at 4 °C and analysed by SDS-PAGE within the next 24 h.

Electrophoresis and autoradiography. Culture supernatants and immunoprecipitated proteins were analysed by SDS-PAGE on 7.5% (w/v) polyacrylamide slab gels according to the technique of Laemmli (1970). Samples from the untreated culture supernatants were mixed with 2 vols 0.1 M-Tris/HCl buffer, pH 8.0, containing 1% (w/v) SDS and 1% (v/v) β -mercaptoethanol, and heated in a boiling water bath for 10 min before being applied to the gel. After electrophoresis, the gels were fixed in 7% (v/v) acetic acid containing 10% (v/v) methanol for 45 min, and then soaked in 1 M-salicylic acid for 1 h. The gels were then dried and exposed to Kodak X-Omat film at -70 °C.

Preparation of β -glucosidase-free cellulase digests of cellulose. These were obtained by inducing cellulase secretion by sophorose in mycelial suspensions of *T. reesei* for 20 h, as described above, after which time all the β -glucosidase was still associated with the cell wall of the fungus, whereas most endoglucanase and cellobiohydrolase activities were in the culture supernatant (Sternberg & Mandels, 1979). The cellulases from 1 l of supernatant were

harvested by three-phase partitioning (Odegaard *et al.*, 1984) and subsequent lyophilization. For digesting cellulose, the cellulases (1 mg protein ml⁻¹) were incubated for 48 h with Avicel cellulose (1 mg ml⁻¹) in 10 mM-sodium citrate buffer, pH 5.0, containing 100 µg chloramphenicol ml⁻¹. The residual Avicel was removed by filtration, and the filtrate was then centrifuged (5000 g, 4 °C, 20 min), lyophilized, suspended in a small volume of distilled water and passed over a column of Biogel P-2 (25 × 900 mm) (equilibrated in distilled water) in order to separate enzymes and cellulose digest products (cellooligodextrins). The latter were identified by the phenol-sulphuric acid method (Dubois *et al.*, 1956), lyophilized and stored at -20 °C until use.

RESULTS

Induction of endoglucanase synthesis in T. reesei

Preliminary investigations were done in order to establish a system for endoglucanase synthesis as a basis for the present investigations. Coincident with several other authors (Mandels *et al.*, 1962; Nisizawa *et al.*, 1970; Loewenberg & Chapman, 1977; Sternberg & Mandels, 1979) it was found that best induction (i.e. highest rate of synthesis and highest final activity) was achieved with sophorose. Cellobiose was inactive in the resting cell system used in these studies as, unexpectedly, was cellulose. Prolonged cultivation of the resting cell system on cellulose led to autolysis followed by sporulation and the secretion of some endoglucanase activity.

Some control experiments on endoglucanase induction by sophorose were done. Cycloheximide (20 µg ml⁻¹) blocked secretion indicating that the extracellular appearance of endoglucanase was due to *de novo* synthesis of the enzyme. Immunoprecipitation of ³⁵S-labelled endoglucanase from the culture fluid always yielded results consistent with measurements of the activity from the culture fluid. This was taken as an indication that no substantial inactivation occurred with the secreted endoglucanase. In all further experiments reported, endoglucanase synthesis was thus quantified by measurement of enzyme activities, except for selected experiments as indicated.

Endoglucanase synthesis was induced by sophorose in mycelia, protoplasts and conidia of *T. reesei*; the same result was obtained with cellooligodextrins (obtained by endoglucanase and cellobiohydrolase digestion of cellulose), but only conidia were active in endoglucanase synthesis with cellulose (Table 1). This suggested the absence of an enzyme or another factor involved in endoglucanase induction from mycelia or protoplasts. A measurement of basal levels of endoglucanase and β-glucosidase activity in conidia, mycelia and protoplasts (Table 2)

Table 1. *Induction of endoglucanase synthesis in T. reesei conidia, mycelia and protoplasts*

The concentration of the inducers was 5 mg ml⁻¹; the induction time was 20 h. Values are means of at least three separate experiments, with SD values given in parentheses.

Inducer	Endoglucanase specific activity [U (mg protein) ⁻¹]		
	Conidia	Mycelia	Protoplasts
Cellulose	0.36 (±0.05)	<0.02	<0.02
Cellooligodextrins	0.45 (±0.08)	0.59 (±0.10)	0.12 (±0.02)
Sophorose	0.32 (±0.05)	0.80 (±0.09)	0.16 (±0.02)

Table 2. *Endoglucanase and β-glucosidase activities in conidia, mycelia and protoplasts of T. reesei*

The sources of enzyme were as follows: conidia, 14 d-old; mycelium, 20 h glucose culture; protoplast membranes, prepared as described by Umile & Kubicek (1986). Values are means of at least five independent measurements, with SD values given in parentheses.

	Endoglucanase activity	β-Glucosidase activity
Conidia (U per 10 ⁸ conidia)	0.028 (±0.004)	0.051 (±0.010)
Mycelia [U (g dry wt) ⁻¹]	<0.001	0.082 (±0.013)
Protoplasts [U (g dry wt) ⁻¹]	<0.001	0.063 (±0.010)

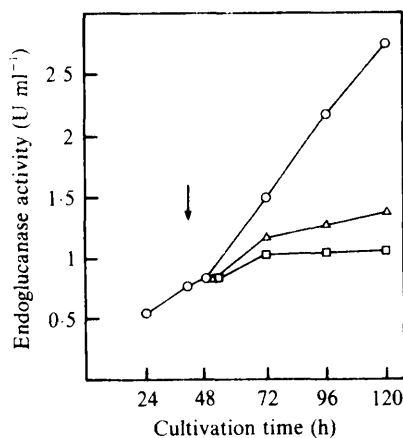


Fig. 1

Fig. 1. Influence of nojirimycin ($20 \mu\text{g ml}^{-1}$ final concentration), and glucono- δ -lactone (0.1 mg ml^{-1}) on endoglucanase induction by growth of a conidial inoculum on crystalline cellulose. The time of addition of the inhibitors is indicated by the arrow. ○, No addition; □, nojirimycin; △, glucono- δ -lactone.

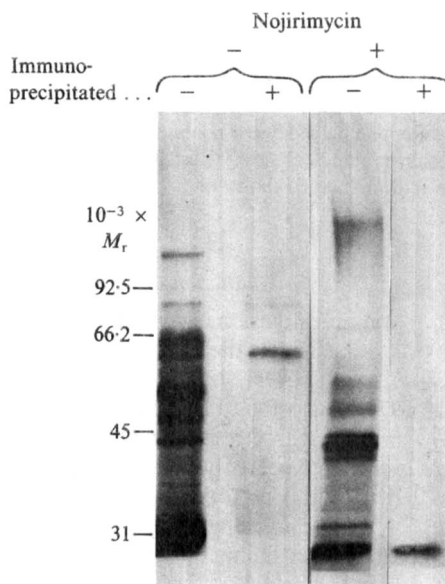


Fig. 2

Fig. 2. Fluorograph of ^{35}S -labelled endoglucanase secreted by mycelia of *T. reesei* induced by cellooligosaccharides showing the effect of nojirimycin ($20 \mu\text{g ml}^{-1}$) addition. Mycelia were grown in the resting-cell inducing medium, pulsed with $100 \mu\text{Ci}$ (370 kBq) [^{35}S]methionine, and processed as described in Methods. Culture supernatant ($10 \mu\text{l}$) or immunoprecipitate were applied to each lane. The M_r values of marker proteins (Pharmacia low molecular weight kit) are indicated.

showed that all contained β -glucosidase, but only conidia contained measurable endoglucanase activity. Conidial endoglucanase activity was periplasmic or surface-bound, since it could be inactivated by 30 min treatment with 0.1 M-HCl at 4°C . Such treatment destroyed the ability of conidia to secrete endoglucanase upon addition of cellulose, but did not affect their ability to germinate and grow on a medium containing glucose as a carbon source. From these results I concluded that the initial attack on the cellulose molecule could be due to basal constitutive levels of endoglucanase, which form the cellooligodextrins necessary for further formation of the inducer.

Involvement of β -glucosidase in inducer formation

In order to check whether the constitutive, plasma-membrane-bound β -glucosidase of *T. reesei* is in fact the enzyme responsible for inducer formation, as has been proposed by some authors (Gritzali & Brown, 1979; Vaheri *et al.*, 1979), I made use of nojirimycin and glucono- δ -lactone which are reputed to be specific inhibitors of β -glucosidase (Dale *et al.*, 1985). These two compounds inhibited the plasma-membrane-bound β -glucosidase from *T. reesei*: for nojirimycin the K_i was $3 \mu\text{M}$ and $2.7 \mu\text{M}$ with *p*-nitrophenyl β -D-glucoside and cellobiose as substrates; for glucono- δ -lactone the respective K_i values were $27 \mu\text{M}$ and $23 \mu\text{M}$. Their use was thus justified. Fig. 1 illustrates the effect of these inhibitors on endoglucanase formation during growth of a conidial inoculum on cellulose: it is evident that both inhibitors strongly decreased the synthesis of endoglucanase. Several control experiments were done in order to confirm that the effect was actually caused by inhibition of plasma-membrane-bound β -glucosidase: (a) ^{35}S -labelling and immunoprecipitation of endoglucanase showed that no enzyme was secreted upon complete inhibition by nojirimycin; this excludes the possibility that nojirimycin inhibits secretion of an active enzyme, e.g. by inhibiting glucosidases involved in its processing (Fig. 2); (b) incubating

Table 3. Effect of *nojirimycin* and *glucono-δ-lactone* on induction of endoglucanase in *T. reesei* mycelium and protoplasts by cellooligodextrins and sophorose

Values are means of at least three separate experiments, with SD values given in parentheses.

Inhibitor	Inducer . . .	Endoglucanase specific activity [U (mg protein) ⁻¹]			
		Mycelium		Protoplasts	
		Cellooligodextrins (5 mg ml ⁻¹)	Sophorose (2 mg ml ⁻¹)	Cellooligodextrins (5 mg ml ⁻¹)	Sophorose (2 mg ml ⁻¹)
None (control)		0.59 (±0.10)	0.80 (±0.09)	0.12 (±0.02)	0.16 (±0.02)
Nojirimycin (0.02 mg ml ⁻¹)		0.08 (±0.02)	0.75 (±0.12)	<0.02	0.16 (±0.03)
Glucono-δ-lactone (0.1 mg ml ⁻¹)		0.13 (±0.04)	0.78 (±0.10)	<0.02	0.18 (±0.02)

conidia with *nojirimycin* or *glucono-δ-lactone* for 1 h at 4 °C and subsequent washing with mineral salts medium (Mandels & Andreotti, 1978) did not affect their ability to secrete endoglucanase upon incubation with cellulose; this confirms that the effect of these inhibitors is reversible on the surface of the cells; (c) *nojirimycin* and *glucono-δ-lactone* did not inhibit endoglucanase secretion when sophorose was the inducer (see below). It therefore appears that the plasma-membrane-bound β-glucosidase is in fact involved in inducer formation from cellulose breakdown products.

The effect of the two inhibitors on induction of endoglucanase synthesis by sophorose and cellooligodextrins in mycelia and protoplasts was also investigated (Table 3): it is evident both in mycelium and protoplasts that the inhibitors were only active with cellooligodextrins but not with sophorose. Since constitutive β-glucosidase is the only enzyme from the whole cellulolytic enzyme complex contained in protoplasts (Kolar *et al.*, 1985; Umile & Kubicek, 1986) it is concluded that β-glucosidase is involved in endoglucanase induction.

DISCUSSION

Although the mechanism by which cellulose induces the synthesis and secretion of enzymes concerned with its catabolism in *T. reesei* is far from clear, two different hypotheses have so far been offered as explanations. One postulates the existence of low constitutive levels of cellulase which carry out the initial attack on cellulose, thereby forming oligosaccharides and cellobiose which then act or become converted to the inducer (Mandels & Reese, 1960; Mandels *et al.*, 1962). The second hypothesis, in contrast, claims that 'binding sites' on the mycelial surface recognize cellulose (Binder & Ghose, 1978), thereby explaining the necessity of contact between *T. reesei* and cellulose for cellulase synthesis. The results from the present paper are so far coincident with the first model, since in all experiments dealing with cellulose as an inducer, induction was observed only with conidia, which – in contrast to mycelia (glucose-grown) and protoplasts – exhibited cell-bound endoglucanase activity. Chaudhary & Tauro (1982) have also recently observed endoglucanase activity in conidia of *T. reesei* QM 9414, but since these authors measured the activity in ultrasonic homogenates the location of their activity is uncertain. Since the activity in the present investigation was destroyed by acid treatment of the conidia, it is very likely that the enzyme is located at the conidial surface.

Although at a first glance contradictory, the present results do not necessarily contrast with those of Binder & Ghose (1978): these authors stated that their mycelia sporulated during the experiments, so they might have contained the basal levels of endoglucanase possibly responsible for the initiation of cellulose degradation. Their conclusion that adsorption of mycelia was merely a physical process was mainly a consequence of their inability to detect cellulase induction by adding extra cellobiohydrolase and endoglucanase to a system where mycelia and cellulose were significantly separated from each other. It is clear that possible cellulose breakdown products might have become considerably diluted during this procedure, and that this situation is not comparable to release of oligosaccharides by an endoglucanase

located very close to the cell membrane. Since endoglucanase strongly adsorbs to cellulose (Klyosov *et al.*, 1986), the results of Binder & Ghose (1978) might well be indicative of an endoglucanase carrying out the initial attack.

The lack of induction of cellulases by cellulose with resting mycelia has also been observed by other authors (Zhu *et al.*, 1982), whereas Mandels *et al.* (1962) stated that induction by cellulose occurred but required time. Under the conditions used in the present work, such prolonged cultivation always resulted in heavy sporulation, which may be an explanation of the apparent difference between the present work and that of Mandels *et al.* (1962).

Although the experiments discussed so far support a possible role of the conidial endoglucanase in the induction process they tell us nothing about the enzyme involved in the formation of the actual inducer. Several laboratories have so far speculated on a 'mycelium-associated' β -glucosidase being involved in inducer formation (Gritzali & Brown, 1979; Vaheri *et al.*, 1979). Additionally, Inglin *et al.* (1980) showed that an intracellular β -glucosidase was involved in the control of intracellular inducer (i.e. sophorose) concentration. The results from the present inhibitor studies are completely in accordance with the first assumptions insofar as they strongly support the involvement of the recently described plasma-membrane-bound β -glucosidase (Umile & Kubicek, 1986) in the induction process. Since nojirimycin and glucono- δ -lactone also inhibit β -glucanases in general it must be noted that the protoplast membranes did not contain measurable activities of any β -glucanase but only β -glucosidase (C. Umile & C. P. Kubicek, unpublished results); thus the effect of these inhibitors on cellulase induction must have been due to inhibition of β -glucosidase.

In the light of these findings it is proposed that the induction of cellulase synthesis by crystalline cellulose in *T. reesei* comprises at least the following two events: (i) initial attack and adsorption of the fungal conidia on cellulose by means of their surface bound basal endoglucanase activities; (ii) conversion of the cellulose breakdown products to the actual intracellular inducer by means of a plasma-membrane-bound β -glucosidase. Since cellulase and β -glucosidase are under separate control in *T. reesei* it should be possible to obtain mutants lacking either of these enzymes, which might be a useful tool in further establishing this mechanism.

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REFERENCES

- BERG, B. & PETTERSON, G. (1977). Location and formation of cellulases in *Trichoderma viride*. *Journal of Applied Bacteriology* **42**, 65–75.
- BINDER, A. & GHOSE, T. K. (1978). Adsorption of cellulose by *Trichoderma viride*. *Biotechnology and Bioengineering* **20**, 1187–1199.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.
- CHAUDHARY, K. & TAURO, P. (1982). Sequential release of cellulose enzymes during germination of *Trichoderma reesei* spores. *Journal of Biosciences* **4**, 281–286.
- CLARADRAS, M. H. & KAPLAN, A. (1984). Maturation of α -mannosidase in *Dictyostelium discoideum*. Acquisition of endoglycosidase H resistance and sulfate. *Journal of Biological Chemistry* **259**, 14165–14169.
- DALE, M. P., ENSLEY, H. E., KERN, K., SASTRY, K. A. R. & BYERS, L. D. (1985). Reversible inhibitors of β -glucosidase. *Biochemistry* **24**, 3530–3539.
- DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for detection of sugars and related substances. *Analytical Chemistry* **28**, 350–356.
- GRITZALI, M. & BROWN, R. D., JR (1979). The cellulase system of *Trichoderma*: relationship between purified extracellular enzymes in induced and cellulose grown cells. *Advances in Chemistry Series* **181**, 237–260.
- INGLIN, M., FEINBERG, B. A. & LOEWENBERG, J. R. (1980). Partial purification and characterization of a new intracellular β -glucosidase of *Trichoderma reesei*. *Biochemical Journal* **185**, 515–519.
- KLYOSOV, A. A., MITKEVICH, O. V. & SINITSYN, A. P. (1986). Role of the activity and adsorption of cellulases in the efficiency of the enzymatic hydrolysis of amorphous and crystalline cellulose. *Biochemistry* **25**, 540–542.
- KOLAR, H., MISCHAK, H., KAMMEL, W. P. & KUBICEK, C. P. (1985). Carboxymethylcellulase and β -glucosidase secretion by protoplasts of *Trichoderma reesei*. *Journal of General Microbiology* **131**, 1339–1347.

- KUBICEK, C. P. (1981). Release of carboxymethyl-cellulase and β -glucosidase from cell walls of *Trichoderma reesei*. *European Journal of Applied Microbiology and Biotechnology* **13**, 226–231.
- LABUDOVA, I. & FARKAS, V. (1983). Multiple enzyme forms in the cellulase system of *Trichoderma reesei* during its growth on cellulose. *Biochimica et biophysica acta* **744**, 135–140.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- LOEWENBERG, J. R. & CHAPMAN, C. M. (1977). Sophorose metabolism and cellulase induction in *Trichoderma*. *Archives of Microbiology* **113**, 61–64.
- MANDELS, M. & ANDREOTTI, R. E. (1978). Problems and challenges in the cellulose to cellulase fermentation. *Process Biochemistry* **13**, 6–13.
- MANDELS, M. & REESE, E. T. (1960). Induction of cellulase in fungi by cellobiose. *Journal of Bacteriology* **79**, 816–826.
- MANDELS, M., PARRISH, F. W. & REESE, E. T. (1962). Sophorose as an inducer of cellulase in *Trichoderma viride*. *Journal of Bacteriology* **83**, 400–408.
- MERIVUORI, H., SIEGLER, K. M., SANDS, J. A. & MONTENECOURT, B. S. (1984). Regulation of cellulase biosynthesis and secretion in fungi. *Biochemical Society Transactions* **13**, 411–414.
- MONTENECOURT, B. S. (1983). *Trichoderma reesei* cellulases. *Trends in Biotechnology* **1**, 156–161.
- NISIZAWA, T., SUZUKI, M., NAKAYAMA, M. & NISIZAWA, K. (1970). Inductive formation of cellulase by sophorose in *Trichoderma viride*. *Journal of Biochemistry* **70**, 375–393.
- ODEGAARD, B. H., ANDERSON, P. C. & LOVRIEN, R. E. (1984). Resolution of the multi-enzyme cellulase complex of *Trichoderma reesei* QM 9414. *Journal of Applied Biochemistry* **6**, 156–183.
- STERNBERG, D. & MANDELS, G. R. (1979). Induction of cellulolytic enzymes in *Trichoderma reesei* by sophorose. *Journal of Bacteriology* **139**, 761–769.
- STERNBERG, D. & MANDELS, G. R. (1982). β -Glucosidase induction and repression in the cellulolytic fungus *Trichoderma reesei*. *Experimental Mycology* **6**, 115–124.
- UMILE, C. & KUBICEK, C. P. (1986). A constitutive, plasma-membrane bound β -glucosidase in *Trichoderma reesei*. *FEMS Microbiology Letters* **34**, 291–295.
- VAHERI, M. P., LEISOLA, M. & KAUPINEN, V. (1979). Transglycosylation products of the cellulase system of *Trichoderma reesei*. *Biotechnology Letters* **1**, 41–46.
- ZHU, Y. S., WU, Y. Q., CHEN, W., TAN, C., GAO, J. H., FEI, J. X. & SHIH, C. N. (1982). Induction and regulation of cellulase synthesis in *Trichoderma pseudokoningii* mutants EA₃-867 and N₂-78. *Enzyme and Microbial Technology* **4**, 3–12.