Induction of cellulose- and xylan-degrading enzyme systems in *Aspergillus terreus* by homo- and heterodisaccharides composed of glucose and xylose

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(Received 10 August 1990; revised 15 November 1990; accepted 21 November 1990)

Synthetic heterodisaccharides composed of glucose and xylose were tested as inducers of cellulose- and xylan-degrading enzymes in *Aspergillus terreus*, and the inducing abilities were compared with those of sophorose and xylobiose or their positional isomers. Measurement of secreted and cell-associated enzyme activities revealed that the heterodisaccharides induced the synthesis of the cellulolytic and xylanolytic enzymes, 2-*O*- β -D-glucopyranosyl D-xylose (Glc β 1-2Xyl) being the most powerful inducer. Sophorose and 2-*O*- β -D-xylopyranosyl D-xylose (Xyl β 1-2Xyl), or their positional isomers, selectively induced the synthesis of cellulases and β -xylanases, respectively. An analysis of the extracellular enzymes (which were separated by isoelectric focusing followed by detection using chromogenic and fluorogenic substrates) showed that Glc β 1-2Xyl initiated the synthesis of specific endo-1,4- β -glucanases and specific endo-1,4- β -xylanases identical to those produced separately in response to sophorose or Xyl β 1-2Xyl. Glc β 1-2Xyl also induced specific endo-1,4- β -glucanases that hydrolysed 4-methylumbelliferyl β -lactoside at the agluconic bond. The results strengthen the concept of separate regulatory control of the synthesis of cellulases and β -xylanases. The results also suggest that mixed disaccharides, composed of glucose and xylose moieties, which may occur in nature, could play an important role in regulating the synthesis of wood-degrading enzymes.

Introduction

A generally accepted view on the regulation of synthesis of enzymes degrading polymeric substrates is that low constitutive levels of polysaccharide hydrolases interact with the polymer to produce small soluble 'signal' fragments, which enter the cell and induce the synthesis of the corresponding enzyme, thus permitting utilization of the polysaccharide (Reese, 1977; Sternberg & Mandels, 1979). The most potent low-molecular-mass inducers of cellulase include sophorose, cellobiose and lactose (Mandels *et al.*, 1962; Nisizawa *et al.*, 1971), while those of β -xylanase comprise xylobiose and higher homologous xylooligosaccharides (Biely & Petráková, 1984a; Hrmová *et al.*, 1984) or non-metabolizable alkyl- and aryl- β -D-xylosides (Nakanishi *et al.*, 1976; Biely *et al.*, 1980). Another explanation for the induced synthesis of polysaccharide-degrading enzymes emphasizes a need for physical contact between the cell and the

polysaccharide (Berg & Pettersson, 1977; Binder & Ghose, 1978).

The principal components of wood include cellulose and hemicelluloses (e.g. xylans, mannans, xyloglucans, glucomannans, galactoglucomannans) cross-linked with lignin which are spread throughout the plant cell wall. Since the chemical structure of these natural wood composites is highly complex, the character of the true natural inducers of cellulases and β -xylanases in plants is obscure. These natural inducers may be represented not only by homodisaccharides such as cellobiose and xylobiose, but also by various heterodisaccharides, originating from (e.g.) xyloglucans. One such heterodisaccharide, 4-*O*- β -D-xylopyranosyl L-arabinose, has been already tested in our laboratory for the production of xylan-degrading enzymes by the yeasts *Trichosporon* (Hrmová *et al.*, 1984) and *Cryptococcus* (Biely & Petráková, 1984a).

Using the homodisaccharides cellobiose (or its positional isomers) and xylobiose, we showed that the synthesis of cellulases and β -xylanases in *Trichoderma reesei* QM 9414 (Hrmová *et al.*, 1986) and *Aspergillus terreus* (Hrmová *et al.*, 1989) is under separate regulatory control. It was therefore of interest to investigate

Abbreviations: Umb-Lac, 4-methylumbelliferyl β -lactoside; 4-NPh-Lac, 4-nitrophenyl β -lactoside; HEC, hydroxyethylcellulose; OBR-HEC, Ostazin Brilliant Red-hydroxyethylcellulose; RBB-xylan, Remazol Brilliant Blue-xylan.

whether mixed disaccharides, such as glucosylxyloses and xylosylglucoses, would be able to induce both types of polysaccharide hydrolases in *A. terreus*. To our knowledge, such a complete group of these compounds has not been tested so far. Our aim was also to determine the substrate specificities and numbers of multiple enzyme forms of cellulases and β -xylanases induced by different disaccharides.

Methods

Chemicals. 2-*O*- β -D-Glucopyranosyl D-xylose (Glc β 1-2Xyl), 3-*O*- β -D-glucopyranosyl D-xylose (Glc β 1-3Xyl), 4-*O*- β -D-glucopyranosyl D-xylose (Glc β 1-4Xyl) and 4-*O*- β -D-xylopyranosyl D-glucose (Xyl β 1-4Glc) were prepared by the Helferich modification of the Koenigs-Knorr synthesis (Helferich & Ost, 1962). The source of 2-*O*- β -D-glucopyranosyl D-glucose (Glc β 1-2Glc, sophorose), 3-*O*- β -D-glucopyranosyl D-glucose (Glc β 1-3Glc), 4-*O*- β -D-glucopyranosyl D-glucose (Glc β 1-4Glc, cellobiose), 6-*O*- β -D-glucopyranosyl D-glucose (Glc β 1-6Glc), 2-*O*- β -D-xylopyranosyl D-xylose (Xyl β 1-2Xyl), 3-*O*- β -D-xylopyranosyl D-xylose (Xyl β 1-3Xyl), 4-*O*- β -D-xylopyranosyl D-xylose (Xyl β 1-4Xyl, xylobiose), 4-NPh-Lac, Umb-Lac and all other inducers and chemicals of p. a. purity is reported elsewhere (Hrmová *et al.*, 1984).

Micro-organism and induction experiments. *Aspergillus terreus* strain F-413 was obtained from the Czechoslovak Collection of Micro-organisms (Brno). Induction experiments were done as previously described (Hrmová *et al.*, 1989) using 2 or 4 mM concentrations of saccharides tested as inducers in screening experiments and in time course studies, and 0.6, 2, 4 and 10 mM in concentration-dependence experiments. Each experiment was repeated at least three times; the standard deviation was not more than $\pm 20\%$ of the mean.

Enzyme assays. The activity of cellulase was determined using filter paper (Mandels & Weber, 1969) and that of endo-1,4- β -glucanase (EC 3.2.1.4) using HEC (Hrmová *et al.*, 1984) following the liberation of reducing sugars. The activity of cellulolytic enzymes releasing aglycones from β -lactosides, referred to here as β -lactosidases, was assayed colorimetrically using 4-NPh-Lac (Hrmová *et al.*, 1989) or fluorometrically (excitation at 340 nm and emission at 440 nm) with Umb-Lac as described by van Tilbeurgh & Claeysens (1985). Endo-1,4- β -xylanase (β -xylanase; 1,4- β -D-xylan xylanohydrolase, EC 3.2.1.8), β -glucosidase (β -D-glucoside glucohydrolase, EC 3.2.1.21), β -xylosidase (β -D-xylan xylohydrolase, EC 3.2.1.37) and β -galactosidase (β -D-galactoside galactohydrolase, EC 3.2.1.23) were assayed on beechwood xylan, 4-nitrophenyl β -D-glucoside, 4-nitrophenyl β -D-xyloside and 4-nitrophenyl β -D-galactoside, respectively, as described previously (Hrmová *et al.*, 1989). Total cell-associated β -glucosidase and β -xylosidase activities were determined using toluene-permeabilized cells as the enzyme source (Hrmová *et al.*, 1984). All activities were determined in duplicate; reproducibility was $\pm 5\%$.

One unit of enzyme activity is defined as 1 μ mole of glucose or xylose equivalent, or as 1 μ mol of 4-nitrophenol or 4-methylumbelliferone, released per min from the appropriate substrate under the given conditions. 1 U corresponds to 16.67 nanokatal.

Analysis of enzyme composition. Dialysed and lyophilized extracellular enzyme preparations, dissolved in distilled water (30–40 times more concentrated than those obtained after induction), were separated by analytical isoelectric focusing according to Radola (1980). Enzyme activities were detected by a zymogram technique using OBR-HEC, RBB-xylan and Umb-Lac as substrates (Biely *et al.*, 1985; van Tilbeurgh *et al.*, 1982; Biely & Markovič, 1988). The pI values of

enzyme components were determined by using standard pI marker proteins from Serva.

Test for hydrolysis and transformation of disaccharides. Hydrolysis of Glc β 1-2Xyl, Xyl β 1-2Xyl, Xyl β 1-3Xyl and Xyl β 1-4Xyl was examined at 1 mM concentration, and transformation of Glc β 1-2Xyl at 40 mM concentration using Glc β 1-2Xyl-induced and non-induced toluene-permeabilized cells of *A. terreus* as the enzyme source. At intervals during 19 h incubation at 30 °C, aliquots were removed and centrifuged, and reducing saccharides were measured or analysed by thin-layer chromatography in supernatants (Hrmová *et al.*, 1984).

Other methods. Concentration of disaccharides in the induction media was determined by the phenol-sulphuric acid method (Dubois *et al.*, 1956). Reducing sugars were estimated by the Somogyi-Nelson procedure (Paleg, 1959) and protein content by the Lowry method using bovine serum albumin as the standard.

Results

Screening for induction by glucobioses, xylobioses, glucosylxyloses and one xylosylglucose

Fig. 1 contains structural formulae and abbreviations of compounds screened as inducers of cellulose- and xylan-degrading enzyme systems in *A. terreus* with their corresponding extracellular enzyme activities. After 24 h incubation of the fungus with 4 mM-saccharides, the endo-1,4- β -glucanase, β -lactosidase and endo-1,4- β -xylanase activities showed that 1,2- β -linked homodisaccharides had selective inducing abilities. All the glucobioses, the best being Glc β 1-2Glc, induced the synthesis of endo-1,4- β -glucanase and β -lactosidase, but none induced the synthesis of endo-1,4- β -xylanase activity. On the other hand, the xylobioses Xyl β 1-2Xyl or Xyl β 1-3Xyl served as excellent inducers of endo-1,4- β -xylanase, but produced only low levels of endo-1,4- β -glucanase. Heterodisaccharides composed of glucose and xylose units behaved differently. As a rule, they served as hybrid inducers, promoting the synthesis of both cellulose- and xylan-degrading enzymes. Glc β 1-2Xyl was the most efficient inducer, also initiating a high level of β -lactosidase activity. It is noteworthy that a certain activity, liberating a fluorogenic aglycone from Umb-Lac, was also recorded in the control enzyme samples (glucose or xylose medium or medium without inducer). This may indicate a constitutive production of the enzymes releasing 4-methylumbelliferone from the substrate. However, for unknown reasons, these activities were not found after the separation by isoelectric focusing followed by detection using Umb-Lac. β -Galactosidase activity was negligible in all cases, indicating that the hydrolysis of Umb-Lac was not due to a two-step reaction catalysed by β -galactosidase and β -glucosidase.

Fig. 1. Activities of extracellular endo-1,4-β-glucanase (open columns), β-lactosidase, assayed on Umb-Lac (dotted columns), and endo-1,4-β-xylanase (filled columns) of *A. terreus* after 24 h incubation with 4 mM-homodisaccharides (glucobioses and xylobioses), heterodisaccharides (glucosylxyloses and xylosylglucose) and monosaccharides tested as the inducers. Structural formulae and abbreviations of the compounds are given. Values are given for a single experiment, but repetition yielded consistent results.

Enzyme induction as a function of time

The best inducers of extracellular cellulases and β-xylanases were 1,2-β-linked disaccharides, i.e. Glcβ1-2Glc among glucobioses, Xylβ1-2Xyl among xylobioses and Glcβ1-2Xyl among glucosylxyloses. Therefore, further studies were performed using these compounds.

The results of time course studies of induction of cellulase and β-xylanase in *A. terreus* by 4 mM-Glcβ1-2Glc, Xylβ1-2Xyl and Glcβ1-2Xyl are shown in Fig. 2. These concentrations of the inducers were found to be optimal at about 2 mg cell dry weight ml⁻¹ (data not shown). It is clear from Fig. 2 that endo-1,4-β-glucanase activity appeared in the medium after 3 h in the presence of Glcβ1-2Glc or Glcβ1-2Xyl, while extracellular β-lactosidase activity, induced by Glcβ1-2Xyl, increased without a lag. The activities of all extracellular enzymes induced by Glcβ1-2Xyl were maximal after 9 h incubation, when only a low amount of Glcβ1-2Xyl was detected in the medium (Fig. 3). The greatest β-lactosidase and cellulase activities on filter paper were detected in the presence of Glcβ1-2Xyl (Fig. 2). On the other hand, the best inducer of endo-1,4-β-glucanase was Glcβ1-2Glc; however, very little β-lactosidase activity was found in this case. These results may explain the overall lower cellulase activity on filter paper detected in the presence of Glcβ1-2Glc. The time lag for the induction of endo-1,4-β-xylanase by Xylβ1-2Xyl was

about 3 h and thereafter the activity increased almost linearly. In the absence of the disaccharides, the specific activities of the polysaccharide hydrolases were less than 5% of the maximum levels.

In a further experiment, we tried to find out whether Glcβ1-2Glc and Xylβ1-2Xyl, present simultaneously in the medium, would be complementary to the inducing effect of Glcβ1-2Xyl, i.e. whether they would induce the same spectrum of cellulolytic and xylanolytic enzymes. Therefore, Glcβ1-2Glc and Xylβ1-2Xyl were added together, each in 2 mM concentration, to the medium. Fig. 2 shows that endo-1,4-β-glucanase, β-lactosidase, cellulase on filter paper and endo-1,4-β-xylanase activities appeared as before. However, the time course of production and yields of the enzymes were substantially different to those observed in the presence of Glcβ1-2Xyl. Clearly, no synergism was found between the effects of Glcβ1-2Glc and Xylβ1-2Xyl with respect to the production of the cellulolytic and xylanolytic enzymes induced by Glcβ1-2Xyl. When both Glcβ1-2Glc and Xylβ1-2Xyl were tested at 4 mM, the activities of all enzymes were lower than at 2 mM (data not shown), probably owing to a repression of enzyme synthesis.

Fig. 3 shows the time course of appearance of the extracellular and the total cell-associated β-glucosidase and β-xylosidase during 24 h incubation of *A. terreus* with 1,2-β-linked disaccharides. The maximum levels of total cell-associated β-glycosidases were reached in all

Fig. 2. Time course of induction of extracellular endo-1,4-β-glucanase (○), β-lactosidase, assayed on 4-NPh-Lac (●), cellulase, using filter paper (□), and endo-1,4-β-xylanase (△) activities of *A. terreus* by (a) 4 mM-Glcβ1-2Xyl, (b) Glcβ1-2Glc and Xylβ1-2Xyl (both 2 mM), (c) 4 mM-Glcβ1-2Glc and (d) 4 mM-Xylβ1-2Xyl.

cases after about 6 h incubation, i.e. when the residual levels of the inducers amounted to about 10% of their original concentration. The extracellular β-glucosidase and β-xylosidase induced by the compounds tested increased steadily throughout the experiment and attained the highest values after 24 h incubation.

Analysis of cellulases and β-xylanases induced by Glcβ1-2Glc, Xylβ1-2Xyl and Glcβ1-2Xyl

Proteins secreted by *A. terreus* in response to the three selected 1,2-β-linked disaccharides were resolved by analytical isoelectric focusing. As shown in Figs 4(a) and 4(b), detection of the enzyme activities induced by Glcβ1-2Glc and Xylβ1-2Xyl with OBR-HEC and RBB-xylan revealed the presence of specific endoglycanases degrading cellulose and xylan. The same forms of specific endo-1,4-β-glucanases and specific endo-1,4-β-xylanases were induced by Glcβ1-2Xyl. However, one other group of specific endo-1,4-β-glucanases was also found (see below). The group of specific endo-1,4-β-glucanases hydrolysing exclusively HEC (thick arrows in Fig. 4a), detected also in the cellulose culture superna-

tant (Hrmová *et al.*, 1989), was represented by an alkaline (pI 7.7) and several acidic components (pI 6.6, and between 6.1 and 4.3). It is worth mentioning that the specific endo-1,4-β-glucanase with pI 6.1 was found to be produced in substantially different amounts in response to Glcβ1-2Glc or Glcβ1-2Xyl (white arrow in Fig. 4a). This may well be true also for some other forms of specific endoglycanases. The second group of specific endoglycanases (thick arrows in Fig. 4b), induced by Xylβ1-2Xyl and Glcβ1-2Xyl, included alkaline endo-1,4-β-xylanases (pI values between 9.6 and 7.8) and a neutral one (pI 6.5). The group of endo-1,4-β-glucanases (pI values 7.5 and 6.9), identified on the basis of OBR-HEC and Umb-Lac hydrolysis (thin arrows in Figs 4a and 4c), was induced exclusively by Glcβ1-2Xyl. These enzymes, also present in the cellulose-spent growth medium, but in higher numbers of multiple enzyme forms (Hrmová *et al.*, 1989), were called specific endo-1,4-β-glucanases since they did not hydrolyse xylan, but they exhibited an affinity for Umb-Lac. It can therefore be said that the low hydrolytic activity on filter paper detected in the presence of Glcβ1-2Glc (Fig. 2) was not due to the presence of different endo-1,4-β-glucanases from those

Fig. 3. Time course of induction of extracellular (○) and total cell-associated (△) β -glucosidase and extracellular (●) and total cell-associated (▲) β -xylosidase activities of *A. terreus* by 4 mM-disaccharides. Dotted lines show the level (100% = 4 mM) of disaccharides in the culture medium.

induced by Glc β 1-2Xyl, but rather due to the lack of specific endo-1,4- β -glucanases hydrolysing Umb-Lac at the agluconic bond.

Fate of Glc β 1-2Xyl in the induced cells

It was of interest to investigate whether the inducing effect of Glc β 1-2Xyl was due to its slower hydrolysis rate inside the cells or its transformation into some other active compound, as was shown with positional isomers of xylobiose in *Cryptococcus albidus* (Biely & Petrkov, 1984b). The former hypothesis was tested using cells induced by Glc β 1-2Xyl, which were permeabilized with toluene. Fig. 5 shows the kinetics of hydrolysis of Glc β 1-2Xyl and of three xylobioses. It is clear that Glc β 1-2Xyl was hydrolysed almost completely after 19 h by the Glc β 1-2Xyl-induced or even by the non-induced cells. The most resistant xylobioses were Xyl β 1-2Xyl and Xyl β 1-3Xyl, which may explain their high capabilities for the induction of β -xylanase. The possibility that 40 mM Glc β 1-2Xyl is transformed into some other transglycosylation product was tested with Glc β 1-2Xyl-induced cells (having specific activities of 35, 97 and

119 mU (mg protein)⁻¹ for the total cell-associated β -xylosidase, β -glucosidase and β -xylanase, respectively). Thin-layer chromatography analysis of the products showed that the cell-associated enzymes only catalysed hydrolysis of Glc β 1-2Xyl to glucose and xylose. It can therefore be concluded that, in the cells of *A. terreus*, Glc β 1-2Xyl is hydrolysed but not transformed into any other transglycosylation product and that Glc β 1-2Xyl may serve as a direct inducer of cellulases and β -xylanases.

Discussion

In the present work, our knowledge of the regulation of the synthesis of cellulose- and xylan-degrading enzyme systems in fungi was extended using a series of synthetic glucobioses, xylobioses, glucosylxyloses and one xylosylglucose. The synthesis of naturally occurring Glc β 1-2Xyl had already been published (Jones & Reid, 1960); however, the inducing capabilities of Glc β 1-2Xyl and other related heterodisaccharides for cellulase and β -xylanase production were examined here for the first time.

Fig. 4. Endo-1,4- β -glucanases, endo-1,4- β -xylanases and β -lactosidases of *A. terreus* induced by 4 mM-Glc β 1-2Glc (lane 1), Glc β 1-2Xyl (lane 2) and Xyl β 1-2Xyl (lane 3). The dialysed and lyophilized enzymes were prepared from cultures grown for 24 h, and were resolved by isoelectric focusing (pH span 3–10). The enzyme activities were detected by a zymogram technique using OBR-REC (a, first replica from the separation gel), RBB-xylan (b, first replica from the OBR-HEC detection gel) and Umb-Lac (c, second replica from the separation gel). The large open arrow denotes the position of the sample application. The thick arrows pointing to panels (a) and (b) mark specific endo-1,4- β -glucanases and specific endo-1,4- β -xylanases, respectively. The thin arrows pointing to panels (a) and (c) mark specific endo-1,4- β -glucanases attacking both OBR-HEC and Umb-Lac. The white arrow in panel (a) points to specific endo-1,4- β -glucanase induced by Glc β 1-2Xyl which is synthesized in a larger amount than that induced by Glc β 1-2Glc. pI values for the main enzyme components are given near the arrows.

Fig. 5. Time course of Glc β 1-2Xyl (○), Xyl β 1-2Xyl (△), Xyl β 1-3Xyl (□) and Xyl β 1-4Xyl (●) hydrolysis catalysed by Glc β 1-2Xyl-induced and permeabilized cells of *A. terreus*.

It was found with *A. terreus* that the mixed disaccharides, containing glucose and xylose moieties, were able to initiate the synthesis of both cellulase and β -xylanase. The substitution of xylose by glucose in the heterodisac-

charide molecule led to β -xylanase and a group of specific endo-1,4- β -glucanases attacking HEC and Umb-Lac not being synthesized. On the other hand, β -xylanases (but not cellulases) were produced when xylobioses were applied. These findings support the concept of separate regulatory control of the synthesis of cellulases and β -xylanases, as was already suggested for *T. reesei* QM 9414 (Hrmová *et al.*, 1986) and *A. terreus* (Hrmová *et al.*, 1989).

The other important finding, that 1,2- β -linked disaccharides, i.e. Glc β 1-2Glc among glucobioses, Xyl β 1-2Xyl among xylobioses and Glc β 1-2Xyl among glucosylxyloses, were the best inducers of cellulases and β -xylanases in *A. terreus*, is difficult to understand at present. Nevertheless, the results of this work indicate that, in *A. terreus*, transglycosylation reactions might give rise to positional isomers which could be more effective inducers than the oligosaccharides arising from polysaccharide hydrolysis. This has already been shown by Nisizawa *et al.* (1971) and Vaheri *et al.* (1979) for *Trichoderma*.

Resolution of the cellulolytic and xylanolytic enzymes induced by Glc β 1-2Xyl revealed the presence of specific endo-1,4- β -glucanases and specific endo-1,4- β -xylanases which were identical to those produced separately in

response to Glc β 1-2Glc or Xyl β 1-2Xyl. Glc β 1-2Xyl was found to mimic the effects of both Glc β 1-2Glc and Xyl β 1-2Xyl with respect to onset of the synthesis of specific endo-1,4- β -glycanases. However, Glc β 1-2Xyl alone initiated the synthesis of another group of endo-1,4- β -glucanases which also exhibited β -lactosidase activity. This observation could indicate that Glc β 1-2Xyl interacts not only with the regulatory molecules for the synthesis of specific endo-1,4- β -glucanases as sophorose and xylobiose do, but also with other regulatory molecules which trigger the synthesis of endo-1,4- β -glucanases of a different type. In other words, in *A. terreus*, the synthesis of endo-1,4- β -glucanases with differing substrate affinities may not be under coordinate regulation by just one 'signal' molecule (Zhu *et al.*, 1982), implying that a variety of 'signal' molecules may be required to initiate the synthesis of the complete spectrum of cellulases (Sternberg & Mandels, 1979; Messner *et al.*, 1988) produced during growth of *A. terreus* in cellulose-containing medium (Hrmová *et al.*, 1989). Consistent with the results of the previous work (Hrmová *et al.*, 1989), specific exo-1,4- β -glucanase, of the cellobiohydrolase I type found in *Trichoderma* (Hrmová *et al.*, 1986), was not detected with Umb-Lac.

It is of interest that the group of heterodisaccharides examined cannot be regarded as universal inducers of cellulases and β -xylanases in fungi, since in *T. reesei* QM 9414, these compounds were ineffective (unpublished results). Differences among genera and species in this respect have already been observed. For example, sophorose, an excellent inducer of cellulase in the genus *Trichoderma*, was found to be less efficient in *Schizophyllum commune* (Rho *et al.*, 1982) and in another basidiomycete isolated by Shewale & Sadana (1978). Similarly, cellobiose failed to induce cellulase in some *Trichoderma* strains (Nisizawa *et al.*, 1971; Sternberg & Mandels, 1979; Royer & Nakas, 1990).

References

- BERG, B. & PETTERSSON, G. (1977). Location and formation of cellulases in *Trichoderma viride*. *Journal of Applied Bacteriology* **42**, 65–75.
- BIELY, P. & MARKOVIČ, O. (1988). Resolution of glycanases of *Trichoderma reesei* with respect to cellulose and xylan degradation. *Biotechnology and Applied Biochemistry* **10**, 99–106.
- BIELY, P. & PETRÁKOVÁ, E. (1984a). Novel inducers of the xylan-degrading enzyme system of *Cryptococcus albidus*. *Journal of Bacteriology* **160**, 408–412.
- BIELY, P. & PETRÁKOVÁ, E. (1984b). Glycosidic bond rearrangements in isomeric xylobioses by yeast xylan-degrading enzymes. *FEBS Letters* **178**, 323–326.
- BIELY, P., KRÁTKÝ, Z., VRŠANSKÁ, M. & URMANIČOVÁ, D. (1980). Induction and inducers of endo-1,4- β -xylanase in the yeast *Cryptococcus albidus*. *European Journal of Biochemistry* **108**, 323–329.
- BIELY, P., MISLOVIČOVÁ, D. & TOMAN, R. (1985). Soluble chromogenic substrates for the assay of endo-1,4- β -xylanases and endo-1,4- β -glucanases. *Analytical Biochemistry* **144**, 142–146.
- BINDER, A. & GHOSE, T. A. (1978). Adsorption of cellulase by *Trichoderma viride*. *Biotechnology and Bioengineering* **20**, 1187–1199.
- DUBOIS, M. K., GILLES, A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.
- HELFERICH, B. & OST, W. (1962). Synthese einer β -D-Xylopyranoside. *Chemische Berichte* **95**, 2612–2615.
- HRMOVÁ, M., BIELY, P., VRŠANSKÁ, M. & PETRÁKOVÁ, E. (1984). Induction of cellulose- and xylan-degrading enzyme complex in the yeast *Trichosporon cutaneum*. *Archives of Microbiology* **138**, 371–376.
- HRMOVÁ, M., BIELY, P. & VRŠANSKÁ, M. (1986). Specificity of cellulase and β -xylanase induction in *Trichoderma reesei* QM 9414. *Archives of Microbiology* **144**, 307–311.
- HRMOVÁ, M., BIELY, P. & VRŠANSKÁ, M. (1989). Cellulose- and xylan-degrading enzymes of *Aspergillus terreus* and *Aspergillus niger*. *Enzyme and Microbial Technology* **11**, 610–616.
- JONES, J. K. N. & REID, P. E. (1960). The synthesis of 2-O- β -D-glucopyranosyl-D-xylose. *Canadian Journal of Chemistry* **38**, 944–949.
- MANDELS, M. & WEBER, J. (1969). The production of cellulases. In *Cellulases and their Applications*, Advances in Chemistry Series 95, pp. 391–413. Edited by F. Gould. Washington: American Chemical Society.
- MANDELS, M., PARRISH, F. W. & REESE, E. T. (1962). Sophorose as an inducer of cellulase in *Trichoderma viride*. *Journal of Bacteriology* **83**, 400–408.
- MESSNER, R., GRUBER, G. & KUBICEK, C. P. (1988). Differential regulation of synthesis of multiple forms of specific endoglucanases by *Trichoderma reesei* QM 9414. *Journal of Bacteriology* **170**, 3689–3693.
- NAKANISHI, K., YASUI, T. & KOBAYASHI, T. (1976). Inducers for xylanase production by *Streptomyces* sp. *Journal of Fermentation Technology* **54**, 801–807.
- NISIZAWA, T., SUZUKI, H., NAKAYAMA, M. & NISIZAWA, K. (1971). Inductive formation of cellulase by sophorose in *Trichoderma viride*. *Journal of Biochemistry* **70**, 375–385.
- PALEG, L. G. (1959). Citric acid interference in the estimation of reducing sugars with alkaline copper reagent. *Analytical Chemistry* **31**, 1902–1904.
- RADOLA, B. J. (1980). Ultrathin-layer isoelectric focusing in 50–100 μ m polyacrylamide gels on silanized glass plates or polyester films. *Electrophoresis* **1**, 43–56.
- REESE, E. T. (1977). Degradation of polymeric carbohydrates by microbial enzymes. In *Recent Advances in Phytochemistry*, pp. 311–367. Edited by F. A. Loewus & V. C. Runeckles. New York: Plenum Publishing Corporation.
- RHO, D., DESROCHERS, M., JURASEK, L., DRIGUEZ, H. & DEFAYE, J. (1982). Induction of cellulase in *Schizophyllum commune*: thiocellobiose as a new inducer. *Journal of Bacteriology* **149**, 47–53.
- ROYER, J. C. & NAKAS, J. P. (1990). Interrelationship of xylanase induction and cellulase induction of *Trichoderma longibrachiatum*. *Applied and Environmental Microbiology* **56**, 2535–2539.
- SHEWALE, J. G. & SADANA, J. C. (1978). Cellulase and β -glucosidase production by a basidiomycete species. *Canadian Journal of Microbiology* **24**, 1204–1216.
- STERNBERG, D. & MANDELS, G. R. (1979). Induction of cellulolytic enzymes in *Trichoderma reesei* by sophorose. *Journal of Bacteriology* **139**, 761–769.
- VAN TILBEURGH, H. & CLAEYSSENS, M. (1985). Detection and differentiation of cellulase components using low molecular mass fluorogenic substrates. *FEBS Letters* **187**, 283–288.
- VAN TILBEURGH, H., CLAEYSSENS, M. & DE BRUYNE, C. K. (1982). The use of 4-methylumbelliferyl and other chromophoric glycosides in the study of cellulolytic enzymes. *FEBS Letters* **149**, 152–156.
- VAHERI, M., LEISOLA, M. & KAUPINEN, V. (1979). Transglycosylation products of 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.