The cellulase of *Penicillium pinophilum*

Synergism between enzyme components in solubilizing cellulose with special reference to the involvement of two immunologically distinct cellobiohydrolases

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Two immunologically unrelated cellobiohydrolases (I and II), isolated from the extracellular cellulase system elaborated by the fungus *Penicillium pinophilum*, acted in synergism to solubilize the microcrystalline cellulose Avicel; the ratio of the two enzymes for maximum rate of attack was approx. 1:1. A hypothesis to explain the phenomenon of synergism between two endwise-acting cellobiohydrolases is presented. It is suggested that the cellobiohydrolases may be two stereospecific enzymes concerned with the hydrolysis of the two different configurations of non-reducing end groups that would exist in cellulose. Only one type of cellobiohydrolase has been isolated so far from the cellulases of the fungi *Fusarium solani* and *Trichoderma koningii*. Only cellobiohydrolase II of *P. pinophilum* acted synergistically with the cellobiohydrolase of the fungi *T. koningii* or *F. solani* to solubilize Avicel. Cellobiohydrolase II showed no capacity for co-operating with the endo-1,4- β -glucanase of *T. koningii* or *F. solani* to solubilize crystalline cellulose, but cellobiohydrolase I did. These results are discussed in the context of the hypothesis presented.

INTRODUCTION

It is now well established (Wood & McCrae, 1979) that degradation of native hydrogen-bond-ordered cellulose by cellulolytic fungi is dependent to a large extent on the synergistic action of three types of enzyme, namely endo-1,4- β -glucanases (1,4- β -D-glucan glucanohydrolase EC 3.2.1.4), cellobiohydrolase (1,4- β -D-glucan cellobiohydrolase, EC 3.2.1.91) and β -glucosidases (β -D-glucoside glucosylhydrolase, EC 3.2.1.21). All of these enzyme types exist in multiple forms, but it is not known how many are synthesized by the fungus in the form in which they appear in the culture solution and how many are artifacts resulting from post-translational modification.

Notwithstanding these problems, synergism manifested between endoglucanase and cellobiohydrolase in solubilizing hydrogen-bond-ordered cellulose has been demonstrated many times (Wood & McCrae, 1972; Streamer *et al.*, 1975; Halliwell, 1975; Ogawa *et al.*, 1982). The phenomenon is therefore well established, but unfortunately it is not well understood (Wood, 1972, 1985; Wood & McCrae, 1978b). Many assume that the mechanism of cellulase action can be discussed in terms of sequential action, where the randomly endoglucanase initiates the attack and the new chain ends generated are then hydrolysed by the endwise-acting cellobiohydrolase; and this does seem likely. This mechanism, however, is acceptable only as far as it goes. It does not explain why both endoglucanase and cellobiohydrolase are individually both capable of degrading 'swollen' amorphous cellulose and not highly ordered cellulose, yet when they are acting in concert they can hydrolyse highly ordered cellulose with comparative ease (Wood, 1975). In other words, it does not explain the precise mechanism of synergistic action on the face of the cellulose crystallite.

There are many anomalous observations recorded in the literature that make it clear that the mechanism of cellulase action cannot be described simply in terms of

sequential action of endoglucanase and cellobiohydrolase (Wood, 1981). One of the most interesting and unexpected is the demonstration by Fagerstam & Pettersson (1980) of the existence of synergism between two immunologically unrelated cellobiohydrolases of the fungus *Trichoderma reesei* in solubilizing crystalline cellulose. More recently, during our studies on the cellulase of the fungus *Penicillium pinophilum*, we have isolated two immunologically distinct cellobiohydrolases (I and II) (Wood & McCrae, 1985). In the present paper the role of these two immunologically unrelated cellobiohydrolases in solubilizing crystalline cellulose is now examined, and a hypothesis to explain the synergistic action between the two cellobiohydrolases is presented.

Part of this work has been presented in preliminary form (Wood, 1985).

EXPERIMENTAL

Materials

Penicillium pinophilum I.M.I. 87160iii was obtained from the Commonwealth Mycological Institute, Kew, Surrey, U.K. Avicel (PH101) was purchased from Honeywell and Stein, London W.1, U.K. All reagents were AnalaR grade or equivalent and were bought either from BDH Chemicals, Poole, Dorset, U.K., or from Sigma Chemical Co., Poole, Dorset, U.K. Cellofas B (CM-cellulose) was a gift from I.C.I. Ltd., Stevenston, Ayrshire, U.K.

Preparation of purified enzyme components

Fractionation of *P. pinophilum* enzyme system by ion-exchange chromatography. Cellobiohydrolases I and II were separated from the other enzyme components of the *P. pinophilum* cellulase system by a two-stage separation involving the ion-exchangers DEAE-Sephadex and DEAE-Sephacrose. In the first stage, which was

similar to that used previously (Wood *et al.*, 1980), two protein components, accounting for 71% of the total protein isolated from the column (fractions 1 and 2), and an endoglucanase, were eluted under the starting conditions (60 mM-acetate buffer, pH 4.8) from a column of DEAE-Sephadex. By applying a gradient involving a simultaneous increase in ionic strength (0.06 to 0.1) and decrease in pH (4.8 to 4.0), two other components (fractions 4 and 5) were separated. For the purposes of the present investigation only fractions 1 and 2 were purified further.

Fraction 2 has already (Wood & McCrae, 1977) been demonstrated to consist of a cellobiohydrolase (herein designated cellobiohydrolase I): this enzyme was obtained in a highly purified state by chromatofocusing as detailed below.

Purification of *P. pinophilum* cellobiohydrolase I by chromatofocusing. Chromatofocusing was carried out with Polybuffer PBE94 exchanger packed into a column and equilibrated with start buffer according to the procedure recommended in the Pharmacia booklet. Start buffer was 25 mM-piperazine/HCl, pH 5.5, and the eluent was Polybuffer 74, diluted 10-fold and adjusted to pH 4.0 with HCl. The column of Polybuffer PBE94 (0.9 cm $\times$ 28.5 cm) carrying a load of 5.8 mg of cellobiohydrolase protein (desalted on Bio-Gel P-2 and redissolved in start buffer), when eluted with the 200 ml of the eluting buffer, provided a pH gradient covering the range 4.0–5.0. Cellobiohydrolase I, free from contaminating carbohydrase activities, was eluted at pH 4.4. The cellobiohydrolase I was precipitated with $(\text{NH}_4)_2\text{SO}_4$ (85% saturation) and isolated by centrifugation. The pellet was dissolved in 10 mM-ammonium acetate buffer, pH 5.0 and the residual SO_4^{2-} was separated from the protein on a column of Bio-Gel P-2. Purified cellobiohydrolase I gave only one protein band (Coomassie Blue stain) on isoelectric focusing in polyacrylamide gels, and on electrophoresis in polyacrylamide gels in non-denaturing and denaturing conditions.

Isolation and purification of *P. pinophilum* cellobiohydrolase II. Fraction 1 from the DEAE-Sephadex column was associated with 81% of the xylanase, 36% of the β -glucosidase, 34% of the endo-1,4- β -glucanase (CM-cellulase) and 36% of the protein eluted from the column, and was further fractionated on a large column of DEAE-Sephadex. The starting conditions (10 mM-sodium phosphate buffer, pH 6.9) were such that many of the enzyme activities previously eluted without adsorption from the DEAE-Sephadex (60 mM-acetate buffer, pH 4.8) column were now bound to the column. Elution with the start buffer resulted in the complete separation of a xylanase and an endoglucanase. Stepwise increases of buffer molarity yielded another major endoglucanase, two peaks of xylanase activity, and a large protein component that was associated with traces of xylanase and endoglucanase activity but that was rich in activity towards Avicel. An increase in molarity and a decrease in pH of the eluent were necessary to effect the removal of the β -glucosidase from the column.

As the major protein component possessed cellulose-hydrolysing activities previously found to be associated with cellobiohydrolase activity (Berghem & Pettersson, 1973) in fungal cellulase systems, it was purified further by chromatofocusing.

Purification of *P. pinophilum* cellobiohydrolase II by chromatofocusing. Fractions containing the major protein component were pooled. The enzyme was precipitated with $(\text{NH}_4)_2\text{SO}_4$ (85% saturation), isolated by centrifugation and redissolved in 5 ml of 10 mM-ammonium acetate buffer, pH 5.0. The solution was desalted on a column of Bio-Gel P-2 equilibrated with 10 mM-ammonium acetate buffer, pH 5.0, and the enzyme was isolated by freeze-drying before being redissolved in 6 ml of 25 mM-histidine/HCl buffer, pH 6.4. This solution was applied to a column of PBE94 chromatofocusing medium equilibrated with 25 mM-histidine/HCl buffer, pH 6.2, and eluted with Polybuffer 74 that had been diluted 9-fold and adjusted to pH 4.5 with conc. HCl. The pH gradient produced covered the pH range 4.8–6.4, and this resulted in the elution of the cellobiohydrolase as a single protein peak at pH 5.45.

Isoelectric focusing in a polyacrylamide gel carried out on a 2.5 μ l sample of each fraction containing the cellobiohydrolase, with carrier ampholyte covering the pH range 3.5–10, showed only one protein band when stained with Coomassie Blue. These fractions were therefore pooled, and the enzyme was precipitated with $(\text{NH}_4)_2\text{SO}_4$ (85% saturation) and isolated by centrifugation. The pellet was redissolved in 10 mM-ammonium acetate buffer, pH 5.0, desalted on a column of Bio-Gel P2 equilibrated with 10 mM-ammonium acetate buffer, pH 5.0, freeze-dried and dissolved in the minimum of 10 mM-ammonium acetate buffer, pH 5.0. Typically, of the protein applied to the column, 80–85% was recovered.

Electrophoresis of the purified and concentrated enzyme under non-denaturing and denaturing conditions in polyacrylamide gels showed only one band on staining for protein (Coomassie Blue), and it was judged to be homogeneous.

Preparation of purified enzyme components of the fungi *Fusarium solani* and *Trichoderma koningii*. Cellobiohydrolase, endoglucanase and β -glucosidase of the fungi *F. solani* and *T. koningii* were separated and purified by the methods described by Wood (1969) and Wood & McCrae (1977).

Enzyme assays

Activity towards Avicel and H_3PO_4 -swollen cellulose. A 2 ml suspension (1% w/v) of Avicel (PH101) or H_3PO_4 -swollen cellulose (Wood, 1971) in 0.1 M-sodium acetate buffer, pH 5.0, was mixed with 0.1 ml of enzyme solution, and incubated at 37 °C for 2 h in the case of H_3PO_4 -swollen cellulose and for 18 h with Avicel. The reaction mixture was centrifuged, and the supernatant was assayed for reducing sugar (Nelson, 1952) or total sugar by the phenol/ H_2SO_4 method (Dubois *et al.*, 1956). In each case the reagents were calibrated with D-glucose as standard.

Activity towards cotton. Activity towards cotton (dewaxed; Corbett, 1963) was measured with either a 2 mg (Wood, 1969) or a 20 mg sample (Wood & McCrae, 1978a). The 2 mg sample was incubated for 7 days at 37 °C before the residual cellulose was determined, but the 20 mg sample was incubated for 2 h only.

Activities towards CM-cellulose (endo-1,4- β -glucanase) and *o*-nitrophenyl β -glucoside (β -glucosidase). These

assays were carried out as previously described (Wood & McCrae, 1977).

Preparation of cellobiohydrolase I antiserum

A 0.1 ml portion (containing 100 μ g of cellobiohydrolase I protein) of water-in-oil emulsion prepared with Freund's complete adjuvant was injected intradermally into a rabbit at intervals of 3 weeks for the first three injections, then at intervals of 6 weeks. Samples (20 ml) of blood were taken at approx. 4-week intervals, and the serum was removed after clotting. Immunoglobulins were precipitated with $(\text{NH}_4)_2\text{SO}_4$ (50% saturation) at 1 °C, isolated by centrifugation, redissolved in water, and dialysed at 4 °C against water and then against 50 mM-sodium acetate buffer, pH 5.0. Insoluble lipoprotein was removed by centrifugation, and the α -globulin fraction containing the antibodies was purified by ion-exchange chromatography in accordance with the method described in LKB Manual of Quantitative Immuno Electrophoresis (1973).

The antibodies were precipitated with $(\text{NH}_4)_2\text{SO}_4$ (25 g/100 ml) at 1 °C, isolated by centrifugation, redissolved in a few millilitres of 0.1 M-NaCl containing 15 mM-Na N_3 and dialysed against the same buffer solution.

Ouchterlony double immunodiffusion

Double immunodiffusion was carried out by the technique of Ouchterlony (1967), with Ionagar (1% w/v) containing a 1:1 dilution of 0.28 M-NaCl/100 mM-Tris adjusted with HCl to pH 7.4 as the diffusion medium. To three of the outer wells were added separately 22, 33 and 46 μ g of purified cellobiohydrolase I; the other three outer wells contained 24, 36 and 48 μ g of purified cellobiohydrolase II. The central well contained a portion (700 μ g of protein; Folin/Lowry method) of the dialysed solution of antibodies to cellobiohydrolase I. Immunodiffusion was allowed to proceed at room temperature in a damp atmosphere until precipitin arcs appeared. When precipitation appeared to be complete, unprecipitated antibodies were removed from the gel by washing twice with 0.9% NaCl solution. The precipitin arcs were stained for protein with 0.1% Coomassie Brilliant Blue R-250 in

ethanol/acetic acid/water (9:2:9, by vol.), and destained after 15 min with ethanol/acetic acid/water (9:2:9, by vol.).

Precipitin arcs were obtained only between the central wells and those containing purified cellobiohydrolase I. Antibodies to purified cellobiohydrolase I did not show any reaction at all with purified cellobiohydrolase II under the conditions tested.

RESULTS AND DISCUSSION

Cellobiohydrolases I and II were isolated and purified by ion-exchange chromatography and chromatofocusing as detailed in the Experimental section. Each component purified in this way gave a single protein band (Coomassie Brilliant Blue) after isoelectric focusing and polyacrylamide-gel electrophoresis in non-denaturing and denaturing (SDS) conditions. Antibodies to purified cellobiohydrolase I did not show any reaction with purified cellobiohydrolase II.

Neither of the purified enzymes (50 μ g of protein) when assayed with cotton fibre (2 mg) under the conditions described by Wood (1969) was capable of effecting any loss in weight of the substrate in 7 days (Table 1). With larger amounts of cotton (20 mg), which would obviously have more available non-reducing end groups for the endwise-acting enzyme to attack, there was a small amount of hydrolysis effected by each enzyme, but this was never more than 0.3%.

Synergism involving cellobiohydrolase and endoglucanase

In the assay involving 2 mg of cotton, no synergism was apparent between cellobiohydrolases I and II in solubilizing this substrate (Wood, 1985), but a marked synergism was observed when 50 μ g of either enzyme was remixed with a reconstituted mixture of the separated endoglucanase activities (Table 1). However, as an assay mixture containing the same amount of total cellobiohydrolase (50 μ g), but made up of 25 μ g of each cellobiohydrolase, produced an even higher degree of solubilization when acting together with endoglucanase, it appeared that the cellobiohydrolases were acting co-operatively. This was investigated further.

Table 1. Cellulase (cotton-solubilizing) activity of enzyme components of *P. pinophilum* acting alone and in combination

The assays were carried out with 2 mg of cotton fibre (see the Experimental section), and the assay mixtures contained 50 μ g of cellobiohydrolase, 1000 units of endoglucanase (CM-cellulase) and 10 units of β -glucosidase. Endoglucanase was a reconstituted mixture of the partially purified endoglucanases fractionated on DEAE-Sephadex and Sepharose (Wood & McCrae, 1985) and recombined in the proportions in which they were eluted from the columns. In assays indicated by an asterisk (*) the 50 μ g of cellobiohydrolase protein was made up of 25 μ g of cellobiohydrolase I and 25 μ g of cellobiohydrolase II.

Enzyme	Solubilization (%)
Cellobiohydrolase I	Nil
Cellobiohydrolase II	Nil
Endoglucanase	8
β -Glucosidase	Nil
Cellobiohydrolases I + II	Nil
Cellobiohydrolase I + endoglucanase	22
Cellobiohydrolase II + endoglucanase	27
Cellobiohydrolase I + endoglucanase + β -glucosidase	24
Cellobiohydrolase II + endoglucanase + β -glucosidase	30
Cellobiohydrolases I* + II* + endoglucanase	51
Cellobiohydrolases I* + II* + endoglucanase + β -glucosidase	51

Fig. 1. Hydrolysis of microcrystalline cellulose by *P. pinophilum* cellobiohydrolases I and II, alone and in combination

See the Experimental section for details of the assay. Abbreviation: CBH, cellobiohydrolase. ----, Theoretical CBH I + CBH II (by sum, Σ ; i.e. hydrolysis expected if CBH I and II were acting independently). The difference between the hydrolysis observed and the theoretical value is the true synergism.

Fig. 2. Hydrolysis of H_3PO_4 -swollen cellulose by *P. pinophilum* cellobiohydrolases I and II, alone and in combination

See the Experimental section for details of the assay. Abbreviation: CBH, cellobiohydrolase. ----, Theoretical CBH I + CBH II (by sum, Σ).

Synergism involving only cellobiohydrolase components

Although no synergism was detectable between cellobiohydrolases I and II with a small sample of cotton (2 mg), with a larger amount of cotton (20 mg), which would contain a larger number of end groups, some

degree of co-operation was apparent between the two enzymes. However, the effects were more obvious with Avicel, which has a much lower degree of polymerization (approx. 200) than cotton (approx. 10000) and therefore has more end groups available for attack by the cellobiohydrolase. As Fig. 1 shows, the degree of hydrolysis of Avicel effected with a mixture of *P. pinophilum* cellobiohydrolases I and II was greater than the sum of the individual actions. Similar synergistic effects were obtained with filter paper, but with H_3PO_4 -swollen cellulose the degree of co-operation was negligible (Fig. 2).

The study was extended further to include the effect of different ratios of the two enzymes on the solubilization of an amorphous cellulose (H_3PO_4 -swollen cellulose) and a crystalline cellulose (Avicel). As Fig. 3 shows, altering the ratios of the two cellobiohydrolases did not result in significantly higher activities towards H_3PO_4 -swollen cellulose than when cellobiohydrolase II was acting alone. However, in the case of the hydrogen-bond-ordered substrate Avicel there was a definite ratio (approx. 1:1)

Fig. 3. Effect of cellobiohydrolase I/cellobiohydrolase II ratio on the solubilization of different celluloses

The assay mixtures contained 20 mg of Avicel (PH101) or 20 mg (dry wt.) of H_3PO_4 -swollen cellulose in a reaction mixture containing 2.0 ml of 0.1 M-acetate buffer, pH 5.0, enzyme and water to give a total volume of 2.1 ml. Incubation was for 2 h at 37 °C. Total sugar was determined by the phenol/ H_2SO_4 method. Each assay contained a total of 100 μg of purified *P. pinophilum* cellobiohydrolase I (CBH I) and cellobiohydrolase II (CBH II).

Fig. 4. Possible mechanism of synergistic action of *P. pinophilum* cellobiohydrolases I and II in solubilizing crystalline cellulose

As cellobiose is the repeating unit in the cellulose chain, theoretically there would be two types (I and II) of non-reducing end groups [(a) and (b)] in the cellulose crystallite. These end groups will be held rigidly in position by the non-covalent bonds existing in highly ordered cellulose, and will require two different stereospecific enzymes for hydrolysis. (c) shows cellobiohydrolase type I action only: cellobiohydrolase type II will act only on the other non-reducing type of end group when this end group has been exposed by cellobiohydrolase I action on the neighbouring chain.

Fig. 5. Hydrolysis of microcrystalline cellulose by *P. pinophilum* cellobiohydrolase I or II and *T. koningii* cellobiohydrolase

Reaction mixtures (see the Experimental section) were incubated for 18 h at 37 °C and the total sugar released was measured by the phenol/H₂SO₄ method. See Fig. 1 for release of sugars by cellobiohydrolases I and II of *P. pinophilum* acting alone. Abbreviations: Tk, *Trichoderma koningii*; Pp, *P. pinophilum*; CBH, cellobiohydrolase. ---, Theoretical hydrolysis expected if *T. koningii* CBH I and *P. pinophilum* CBH I were acting independently (Σ_1). - - - - , Theoretical hydrolysis expected if *T. koningii* CBH I and *P. pinophilum* CBH II were acting independently (Σ_2).

required for maximum efficiency in terms of solubilization of this substrate.

These results, along with those in Table 1, would seem to indicate that the two cellobiohydrolases have a definite role to play in the solubilization of crystalline cellulose. However, the need for, and the action of, two immunologically unrelated cellobiohydrolases in solubilizing crystalline cellulose is difficult to explain. It seems possible, however, that the explanation is connected in some way with the stereochemistry of the cellulose chains and the fact that cellobiose is the repeating unit in the cellulose crystallite.

A reasonable speculation might be that cellobiohydrolases I and II are in fact two different stereospecific enzymes that have been synthesized to attack the two sterically different non-reducing end groups that would be expected to exist in the cellulose crystallite (Fig. 4). Clearly, if cellobiohydrolases I and II are two such enzymes, one could suggest that the synergism observed could be explained if one envisages that the hydrolytic action of one cellobiohydrolase removing cellobiose units successively from one type of non-reducing chain end could expose, on another neighbouring chain, a non-reducing end group with the correct configuration for attack by another stereospecific cellobiohydrolase. Synergism of this type would not exist in the attack on H₃PO₄-swollen cellulose, where most chain ends would be easily accessible to the different cellobiohydrolases; and this is exactly what was observed (Fig. 2).

Only one type of cellobiohydrolase has been isolated so far from the cellulases of the fungi *Fusarium solani* (Wood & McCrae, 1977) and *Trichoderma koningii* (Wood & McCrae, 1972). If the above hypothesis is tenable then clearly only one of the two cellobiohydrolases of *P. pinophilum* would be expected to act in synergism with the cellobiohydrolase of *T. koningii* and *F. solani*. Figs. 5 and 6 show in fact that only cellobiohydrolase II of *P. pinophilum* could act in synergism with the cellobiohydrolases of the other fungal species, and this supports the hypothesis.

Synergism between cellobiohydrolase and endoglucanases of different fungal species

Further interesting information concerning the interaction of enzyme components and the mechanism of cellulase action was obtained from a study in which cellobiohydrolases I and II of *P. pinophilum* were mixed, independently, with the endoglucanases from a number of fungal sources, and the resulting mixtures tested for their abilities to solubilize highly ordered cotton fibre. Significant differences were found in the abilities of the two cellobiohydrolases of *P. pinophilum* to co-operate with the endoglucanases of other fungi. As shown in Table 2, although both cellobiohydrolases I and II could act synergistically with the endoglucanase of *P. pinophilum*, remarkably only cellobiohydrolase I could co-operate with the endoglucanases of the fungi *Trichoderma koningii* and *Fusarium solani* when assayed under the

Fig. 6. Hydrolysis of microcrystalline cellulose by *P. pinophilum* cellobiohydrolase I or II and *F. solani* cellobiohydrolase

Details are similar to these given in the legend to Fig. 5, except that *F. solani* cellobiohydrolase (*Fs* CBH) replaced *T. koningii* cellobiohydrolase.

same conditions. Cellobiohydrolase II showed little or no capacity for co-operating with the endoglucanases of other fungi at a number of different enzyme concentrations.

In previous reports we have speculated that the formation of a 'loose' cellobiohydrolase-endoglucanase complex on the face of the cellulose crystallite may be an essential prerequisite of hydrolysis by mixtures of the randomly acting endoglucanase and the endwise-acting cellobiohydrolase (Wood & McCrae, 1978a; Wood *et al.*, 1980). The results in Table 2 certainly could be interpreted to support that speculation, but steric considerations may also be important. Thus it could be suggested that, if *P. pinophilum* produces two stereospecific cellobiohydrolases capable of attacking both configurations of non-reducing end groups likely to be encountered

in the cellulose crystallite, then it is logical to expect that two stereospecific endoglucanases would also exist, and that these would generate the two sterically different non-reducing end-groups for the cellobiohydrolases to attack. The possibility that two different stereospecific endoglucanases exist in these enzyme systems has not to our knowledge been addressed, but it is certainly worthy of investigation. At least four endoglucanase components have been found in *P. pinophilum* cellulase preparations (Wood & McCrae, 1985).

As *T. koningii* and *F. solani* each appear to produce only one type of cellobiohydrolase (Wood & McCrae, 1972, 1977), then only one type of endoglucanase may exist in these cellulase enzyme systems, and this would explain why only one of the two immunologically unrelated cellobiohydrolases produced by *P. pinophilum* acts synergistically with the endoglucanases of these fungi.

If neither of these explanations is acceptable, the observations recorded in Table 2 are still extremely interesting. A detailed comparison of active sites and subsites of the two cellobiohydrolase enzymes may now provide a solution to the phenomenon of synergism between cellobiohydrolase and endoglucanase that results in solubilization of crystalline cellulose.

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Table 2. Synergistic action between cellobiohydrolases I and II of *P. pinophilum* and the endoglucanases of other fungi in solubilizing cotton fibre

The assay mixtures (Wood & McCrae, 1977) contained, where appropriate, 50 μg of cellobiohydrolase (CBH), 1000 units (Wood, 1968) of endoglucanase (CM-cellulase) and 10 units (Wood, 1968) of β-glucosidase, and where necessary purified β-glucosidase from *F. solani* (Wood, 1968) to make all assays equivalent in this activity.

Source and type of cellobiohydrolase	Source of endoglucanase	Solubilization of cotton in 7 days (%)		
		CBH alone	Endoglucanase + β-glucosidase	CBH + endoglucanase + β-glucosidase
<i>P. pinophilum</i> CBH I	<i>P. pinophilum</i>	Nil	8	24
<i>P. pinophilum</i> CBH II	<i>P. pinophilum</i>	Nil	8	30
<i>P. pinophilum</i> CBH I	<i>F. solani</i>	Nil	3	45
<i>P. pinophilum</i> CBH II	<i>F. solani</i>	Nil	3	3
<i>P. pinophilum</i> CBH I	<i>T. koningii</i>	Nil	9	57
<i>P. pinophilum</i> CBH II	<i>T. koningii</i>	Nil	9	11

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