JOURNAL OF BACTERIOLOGY, Jan. 1982, p. 47-53
0021-9193/82/010047-07$02.00/0

Vol. 149, No. 1

Induction of Cellulase in *Schizophyllum commune*: Thiocellobiose as a New Inducer

D. RHO,[1] M. DESROCHERS,[1]* L. JURASEK,[1] H. DRIGUEZ,[2] AND J. DEFAYE[2]

Pulp and Paper Research Institute of Canada, Pointe Claire, Quebec, Canada H9R 3J9,[1] and Centre National de la Recherche Scientifique, Centre de Recherches sur les Macromolécules Végétales, 53X-38041, Grenoble, France[2]

Received 27 April 1981/Accepted 17 August 1981

Several mono-, di-, tetra-, and polysaccharides were screened for their ability to induce cellulase production by the tetrapolar hymenomycete *Schizophyllum commune*. Out of 21 carbohydrates screened, 4 (thiocellobiose, carboxymethylcellulose, cellobiose, and xylan) induced all three enzymes tested (carboxymethylcellulase, β-glucosidase, and xylanase). The inducing effect increased with rising concentrations of the inducers up to a certain value, beyond which there was either a leveling off or a decrease of the enzymatic activities. The most powerful inducer, thiocellobiose, showed the highest activity at 0.5 mM. Cellobiose, carboxymethylcellulose, and xylan showed their highest activities at 1 mM and 1%, respectively. Surprisingly, sophorose did not enhance enzyme production. The enzymatic activities were monitored over a period of 24 h. Thiocellobiose elicited a response immediately after incubation, but with all other inducers there was a latency period before their effect could be measured. High-performance liquid chromatography showed no hydrolysis of thiocellobiose when incubated in the presence of *S. commune* extracellular enzymes.

Biological and biochemical treatments of wood or pulp by microorganisms initially cause minor modifications of the properties of these lignocellulosic materials. Such modifications may be useful for accelerating pulping (7) or for upgrading the pulp properties (L. Pilon, M. Desrochers, L. Jurasek, and P. J. Neumann, Proc. TAPPI Annu. Meet. 1981, p. 293). More severe treatments cause complete degradation of lignocelluloses such as conversion of polysaccharides into sugars. The commercial feasibility of enzymatic saccharification of cellulose relies heavily on the cost of enzyme production (4). Therefore, more efficient methods of cellulase production are being sought.

To degrade crystalline cellulose efficiently, fungi such as *Trichoderma reesei* produce at least three enzymes: endo-β-1,4-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (18). The production of these enzymes is induced by addition of cellulose to the culture (20). The induction is probably effected by soluble degradation products of cellulose. Among these, cellobiose has been identified as an inducer of cellulase enzymes (13, 22). Other disaccharides such as sophorose (2-*O*-β-glucopyranosyl-D-glucose) and lactose have also been found to be cellulase inducers (11, 12, 21). Thus, the induction plays an important role in cellulase biosynthesis. A rational control of the induction may significantly contribute to the development of more efficient cellulase production methods. From the pioneering work of Monod (15), it is known that some substrate analogs which can not be enzymatically cleaved are effective inducers for glycosidases. For example alkyl β-D-1-thio-galactosides were shown to induce the production of the β-galactosidase in *Escherichia coli* (3). Accordingly, one could expect that thio-analogs of cellobiose may lead to similar results in the induction of the cellulase system in cellulolytic strains.

We found previously that a wood-destroying basidiomycete, *Schizophyllum commune*, was a productive source of β-glucosidase, xylanase, and endoglucanase (6). As a part of the investigation of the enzyme production by *S. commune* we have now attempted to characterize the induction spectrum of this fungus and examine the possibility of using gratuitous induction for the enhancement of cellulase, β-glucosidase, and xylanase production.

MATERIALS AND METHODS

Microorganism. A *S. commune* Fr. strain 13 (ATCC 38548) was used throughout this work. The isolation and description of this strain has been reported elsewhere (16). The strain was kept on malt agar slants (Difco Laboratories) at 4°C.

Mycelial preparation. The inoculum was started by adding a piece (7 by 7 mm) of an actively growing part of a fungal colony on a malt agar petri dish into a 500-

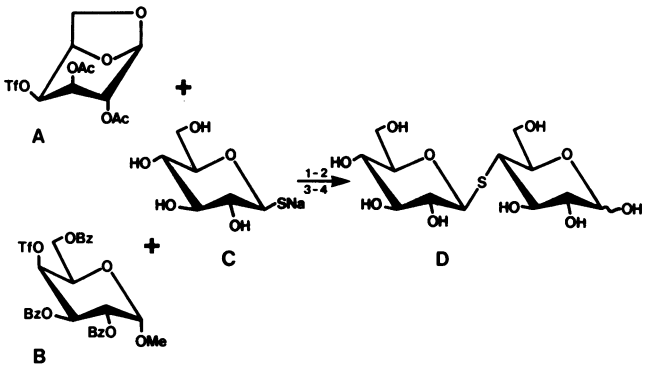

FIG. 1. Synthesis of 4-*S*-β-D-glucopyranosyl-4-thio-D-glucopyranose (thiocellobiose) by condensation of C with A or B. 1, Hexamethylphosphoramide; 2, Ac_2O–C_5H_5N; 3, H_2SO–Ac_2O–AcOH; 4, MeONa–MeOH.

ml polypropylene Erlenmeyer flask containing 200 ml of malt extract broth (Difco) and a 2-cm glass marble. The inoculum flask was shaken at 250 rpm (New Brunswick Scientific Co.; Gyrotory shaker, A 33-500) at 30°C for 3 days. This suspension, in 1.5-ml portions, was used to inoculate 500-ml polypropylene Erlenmeyer flasks containing 150 ml of mycological broth (Difco Laboratories), pH 4.8. The cultures were grown under continuous shaking with a 2-cm glass marble at 250 rpm for 6 days at 30°C. The mycelial content of the flasks was washed four times by centrifugation at 6,500 × *g* for 15 min in 0.1 M potassium phosphate buffer (pH 6.1).

Inducers. 4-*S*-(β-D-Glucopyranosyl)-4-thio-D-glucopyranose (thiocellobiose, Fig. 1D) was obtained in an overall yield of 60 to 70% from the reaction of either 2,3-di-*O*-acetyl-4-*O*-triflyl-1, 6-anhydro-β-D-galactopyranose (Fig. 1A) or methyl 2,3,6-tri-*O*-benzoyl-4-triflyl-α-D-galactopyranose (Fig. 1B) with the sodium salt of 1-thio-β-D-glucopyranose (Fig. 1C) in hexamethylphosphoramide followed by successive acetolysis and de-*O*-acylation of the resulting disaccharide. Up to 1978, the only report on the synthesis of reducing 1-thiodisaccharides was Hutson's preparation of thiogentiobiose (9). Despite the claim of usefulness of such compounds for induction studies, no attempts have been reported toward his goal, probably due to the unavailability of general methods for synthesis of thio-oligosaccharides. Recently a stereoselective method was developed in one of our laboratories (in Grenoble) for the synthesis of 1→4 linked 1-thio-oligosaccharides (Fig. 2). Methyl 4-*S*-β-D-glucopyranosyl-4-thio-α-D-glucopyranoside, already obtained through pathway A (2; M. Blanc-Muesser, J. Defaye, H. Driguez, and E. Ohleyer, 10th Int. Symp. Carbohydr. Chem. Biochem, Sydney, Australia, IL. 3, 1980), can be further deprotected through acetolysis in a yield of 82%. An alternative sequence involving pathway B leads to 4-*S*-β-D-glucopyranosyl-4-thio-D-glucopyranose in yields averaging 60 to 70%. In both pathways, the stereospecificity of the anomeric condensation is complete, and no α-anomer can be detected. Details of the experiments will be reported in another publication.

All other inducing compounds were purchased as follows: Avicel, type PH-101 (FMC Corp.); xylan from larch (Sigma Chemical Co.); carboxymethyl(CM)-cellulose (type 7LT, Hercules Inc.); sophorose monohydrate (Koch-Light Laboratories); turanose, stachyose tetrahydrate, D-mannoheptulose, *N*-acetyl-D-mannosamine, gentiobiose, L-sorbose, and melibiose hydrate (Supelco, Inc.); maltose (Difco Laboratories); L-arabinose, lactose, D-glucose, D-galactose, D-mannose, sucrose, and D-xylose (Fisher Scientific Co.); cellobiose (Eastman Co.).

Induction experiments. The washed mycelial preparation was dispersed in 0.1 M phosphate buffer (pH 6.1) to a final concentration of 2 to 4 mg (dry weight) per ml. A 10-ml sample of the appropriate inducer prepared in the same buffer was added to 10 ml of the mycelial suspension in 50-ml Erlenmeyer flasks containing a 1-cm glass bead. The flasks were incubated at 30°C under continuous agitation (200 rpm) for various lengths of time. Blanks were prepared in the same way, except that no inducer was added.

Enzyme assays. After various incubation times, the

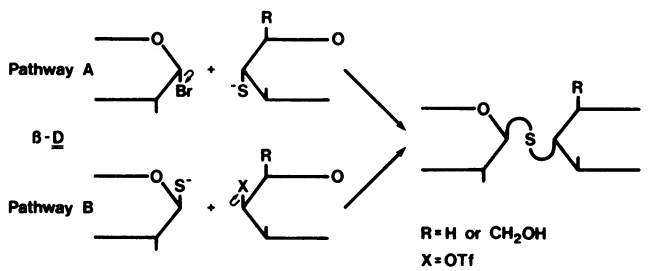

FIG. 2. Alternative pathways for the synthesis of β-1-4-linked D-glucose and D-xylose thio-oligosaccharides.

content of each flask was centrifuged for 15 min at 700 × *g*. The enzymatic activities of the supernatants were determined as follows.

(i) CM-cellulase, endoglucanase. One milliliter of supernatant (full strength or diluted with distilled water) was incubated with 1 ml of 1% CM-cellulose (type 7LT; Hercules, Inc.) in 0.2 M sodium acetate buffer (pH 5.0) for 30 min at 30°C. Reducing sugar concentration was determined by the dinitrosalicylic acid method (14). The optical density was calibrated with glucose solutions of known concentration. Appropriate blanks were used. Enzyme activity was expressed as micromoles of glucose produced per minute.

(ii) β-Glucosidase. *p*-Nitrophenyl-β-glucosidase (β-glucosidase) was assayed by the technique of Kanda et al. (10). Units are defined as micromoles of *p*-nitrophenol produced per minute.

(iii) Xylanase. Xylanase assays were performed as described previously (17). Units are expressed as micromoles of xylose produced per minute.

HPLC. To determine whether thiocellobiose was degraded by *S. commune* extracellular enzymes, high-performance liquid chromatography (HPLC) was used to search for degradation products. A 10 mM thiocellobiose solution prepared in water was added to a *S. commune* dilute enzyme solution (1:40) in a 1:1 ratio at 28°C and analyzed by HPLC by using refractive index detection. The enzyme solution was a filtrate of an optimized culture of *S. commune* (5). For comparison, a similar experiment was carried out with 10 mM cellobiose instead of thiocellobiose. In both experiments, an HPLC apparatus (Waters Associates, Milford, Mass.; model ALC/GPC 204C) was used with a 740 information processor, a 710A sampler processor, and a 6000A solvent delivery system. For the thiocellobiose analysis, a Waters μ Bondapak C_{18} column (300 by 3.9 mm) was used at a solvent (water) flow rate of 0.8 ml/min at room temperature. With injection volumes of 10 μl, the respective retention times of glucose and thiocellobiose were 4.88 and 9.24 min. For the cellobiose analysis an HPX-85 heavy metal column (300 by 7.8 mm) (Bio-Rad Laboratories) with water as eluent (0.3 ml/min) was used at 85°C. With injection volumes of 10 μl, the respective retention times of glucose and cellobiose were 26.3 and 31.9 min.

RESULTS

Screening for inducers. The induction experiments (Table 1) were performed with 21 compounds (mono-, di-, tetra-, and polysaccharides). Each inducer was examined in a range of concentrations (0.25 to 10% for the polysaccharides and 0.1 to 2.5 mM for sugars). The enzyme activities obtained at the optimal inducer concentrations are presented in Table 1. Each value is an average of at least three measurements. Activities which were not significantly higher than blanks at the 99.3% confidence level are presented as zero values.

Fourteen compounds showed induction capabilities for one or more of the enzyme activities, but only thiocellobiose, cellobiose, CM-cellulose, and xylan induced all three enzymes (β-glucosidase, CM-cellulase, and xylanase). Avicel and sophorose caused marginal induction of CM-cellulase and xylanase. Lactose induced β-glucosidase and xylanase, whereas stachyose enhanced xylanase only.

As shown in Table 1, CM-cellulase was induced by thiocellobiose, cellobiose, CM-cellulose, Avicel, xylan, and, to a lesser extent, other compounds. The β-glucosidase production was induced with thiocellobiose, cellobiose, CM-cellulose, and xylan. Xylanase activity seemed to be strongly induced by a wide variety of substances. The most effective were thiocellobiose, xylan, cellobiose, CM-cellulose, stachyose, and Avicel. Apart from a slight increase in β-glucosidase activity, possibly linked to increased metabolism, glucose did not induce any enzymatic activity. Lactose and sophorose were not effective inducers of cellulase in *S. commune*.

The low activities consistently recorded in the blanks show that there is little basal synthesis of the three enzymes tested. These blank values

TABLE 1. Enzyme yields after an incubation of *S. commune* mycelium with various carbohydrates at 30°C for 24 h

Substance[a]	Concn	Enzyme yields (IU/ml)		
		CM-cellulase	β-Glucosidase	Xylanase
Thiocellobiose	0.5 mM	0.075	0.037	0.981
Xylan	1%	0.024	0.011	0.880
CM-cellulose	1%	0.061	0.017	0.822
Cellobiose	1 mM	0.024	0.020	0.692
Lactose	2.5 mM	0	0.008	0.438
Sophorose	2.5 mM	0.008	0	0.153
Stachyose	2.5 mM	0	0	0.851
Avicel	0.5%	0	0	0.835
Gentiobiose	0.5 mM	0	0	0.198
Buffer blanks		0.003	0.004	0.040

[a] In addition to substances listed in the table, melibiose, sorbose, *N*-acetyl-D-mannosamine, L-arabinose, and glucose showed very slight induction of some of the enzyme activities. Galactose, maltose, D-mannoheptulose, D-mannose, sucrose, turanose, and xylose showed no induction.

were not subtracted from enzyme yields in the induction experiments.

Effect of inducer concentration on enzyme production. The effect of the concentration of four selected inducers (thiocellobiose, CM-cellulose, cellobiose, and Avicel) is shown in Fig. 3 and 4. The inducing effect increased with rising concentrations of the inducers up to a certain value, beyond which there was either a leveling off or a decrease of the enzyme activities. The highest activities of all three enzymes were recorded after induction with 0.5 mM thiocellobiose. Further increase of the thiocellobiose concentration did not cause a significant rise of the enzyme yields. With cellobiose and CM-cellulose as inducers, the highest enzyme activities were recorded at 0.75 mM and 1%, respectively. With Avicel a strong xylanase induction was recorded as 1% concentration of the inducer, but at higher concentrations the induction decreased dramatically.

Development of induced enzyme production. The enzyme yields resulting from the induction with four selected compounds were monitored over a period of 24 h. With 0.5 mM thiocellobiose, all three enzyme activities appeared within 8 h and continued to increase throughout the 24 h of incubation (Fig. 5). With cellobiose, there were rapid initial increases in xylanase and CM-cellulase activities followed by a leveling off at around 8 h (Fig. 5). With CM-cellulose as the inducer, the induction of the enzymes was noticeable only after a lag period of 4 h (Fig. 6). With Avicel, there was a rapid but short-lived induction, and after 15 h xylanase and CM-cellulase activities increased again (Fig. 6).

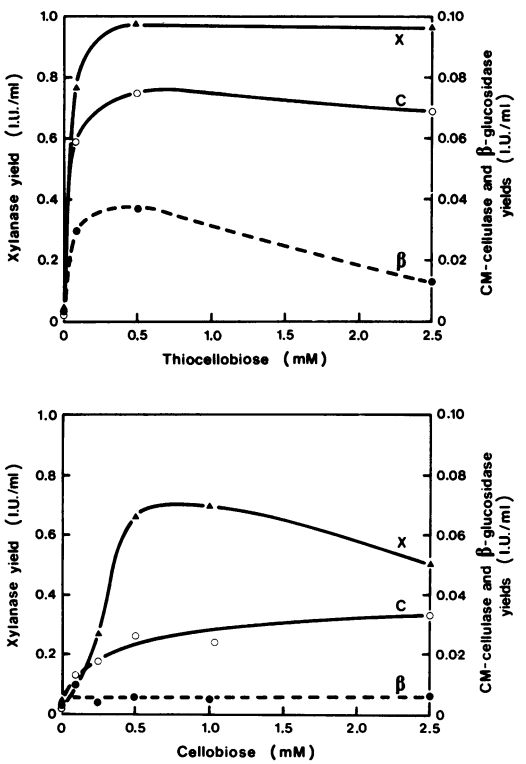

FIG. 3. Enzymatic activities measured after 24 h of incubation at 30°C with different concentrations of thiocellobiose and cellobiose. X, Xylanase; C, CM-cellulase; β, β-glucosidase.

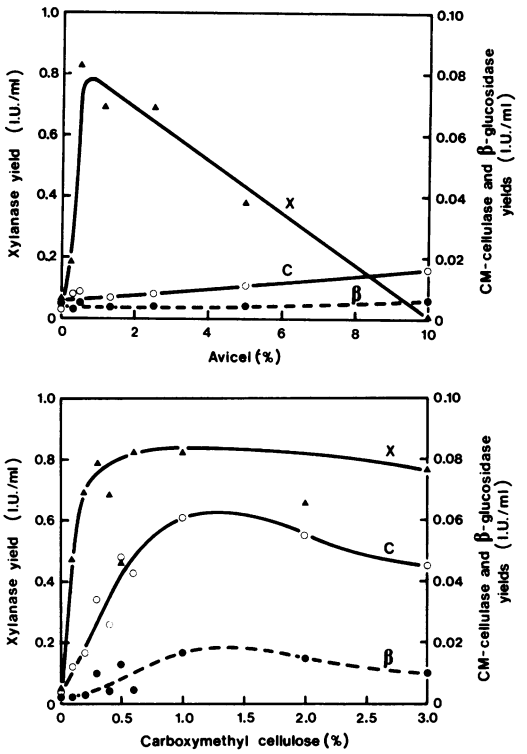

FIG. 4. Enzymatic activities measured after 24 h of incubation at 30°C with different concentrations of Avicel and CM-cellulose. X, Xylanase; C, CM-cellulase; β, β-glucosidase.

Enzymatic hydrolysis of thiocellobiose and cellobiose. Thiocellobiose was not hydrolyzed to glucose when incubated with a complex of *S. commune* extracellular enzymes (Fig. 7A). After incubation for 9 h, there was no significant depletion of thiocellobiose measured by HPLC. However, when cellobiose was mixed with *S. commune* enzymes under the same experimental conditions, glucose resulting from its hydrolysis was detected immediately (Fig. 7B). After 6 h, almost all of the cellobiose was converted into glucose.

DISCUSSION

The mechanism of induction of cellulases by insoluble cellulose is likely to be as follows: very small amounts of enzymes produced by basal

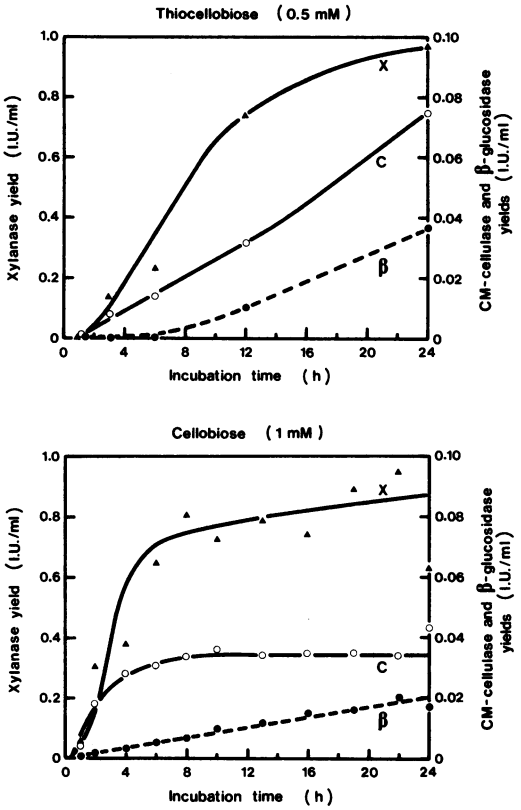

FIG. 5. Enzyme activities recorded over a 24-h incubation period (30°C) at fixed thiocellobiose and cellobiose concentrations. X, Xylanase; C, CM-cellulase; β, β-glucosidase.

enzyme synthesis convert a small part of the substrate into soluble molecules (e.g., cellobiose), which then act as extracellular inducers. This seems to be true at least in the case of *S. commune* since low levels of cellulolytic enzymes were consistently recorded in the blanks in the absence of inducers.

In *S. commune*, the production of cellulolytic enzymes was enhanced by several compounds (Table 1). All of the other sugars mentioned above have been tested, but they showed negligible inducing activities. The most potent inducers were thiocellobiose, cellobiose, CM-cellulose, xylan, and Avicel. All of these compounds are closely related to naturally occurring wood polysaccharides which are used by the fungus as carbon sources. It is apparent (Fig. 5 and 6) that among these inducers the low-molecular-weight compounds elicit the most immediate and rapid response in production of xylanase and CM-cellulase. These enzymes are almost immediately induced by thiocellobiose and cellobiose (Fig. 5). In the case of CM-cellulose and Avicel, there was a lag period of 4 and 16 h, respectively (Fig. 6). This lag is probably due to time required for partial conversion of these polysaccharides into low-molecular-weight inducers. The enzyme production induced by Avicel (Fig. 6) showed an initial short-lived increase, apparently due to the presence of small amounts of easily accessible carbohydrate which may have served as a source of inducing sugars. The delayed major increase observed after 16 h in xylanase and CM-cellulase could be a reflection of the resistance to hydrolysis of the highly crystalline Avicel.

The inducing effect increased with rising concentration of the inducer up to a certain value, beyond which there was either a leveling off or a decrease of the enzymatic activity (Fig. 3 and 4). The highest enzyme activities were recorded with thiocellobiose. CM-cellulose, cellobiose, and Avicel as inducers gave lower enzyme activities. The high enzyme yields found with thiocellobiose are probably a result of the full-capacity production of enzymes by the fungal cells in our experimental conditions.

The results obtained with HPLC (Fig. 7) indicate that thiocellobiose is not being hydrolyzed

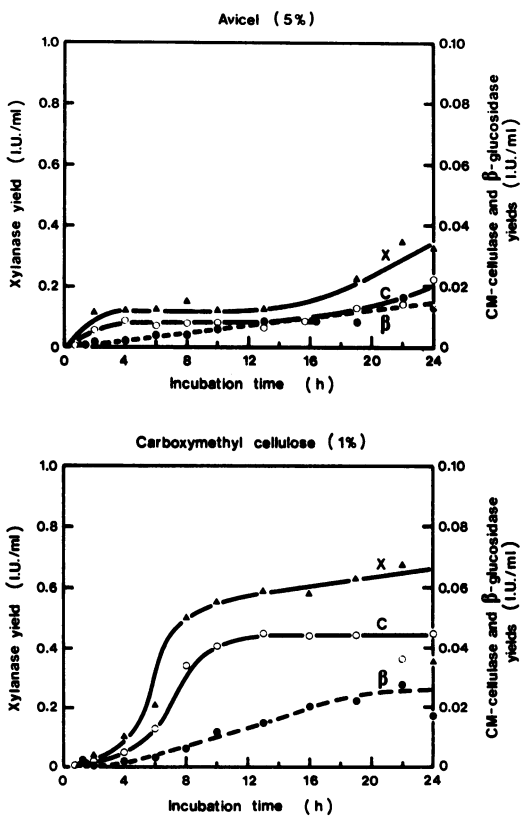

FIG. 6. Enzyme activities recorded over a 24-h incubation period (30°C) at fixed Avicel and CM-cellulose concentrations. X, Xylanase; C, CM-cellulase; β, β-glucosidase.

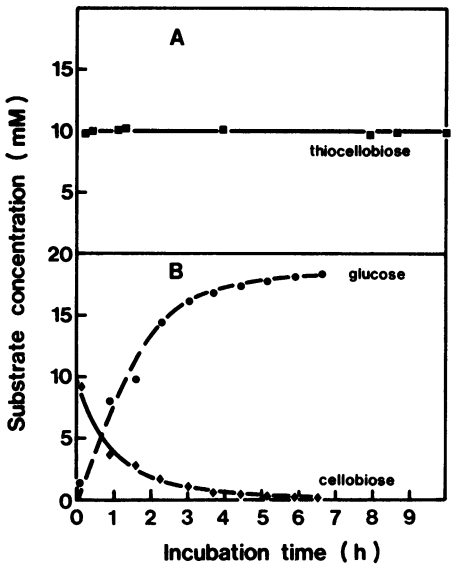

FIG. 7. A, HPLC analysis of a 10 mM solution of thiocellobiose after incubation at 28°C for 9 h in the presence of *S. commune* extracellular enzymes. B, HPLC analysis of a 10 mM solution of cellobiose after incubation at 28°C for 6 h in the presence of *S. commune* extracellular enzymes.

by *S. commune* extracellular enzymes. An efficient induction requires the uptake, intracellular concentration, and activation of the inducer. If this inducer is not a substrate for the cell, it may be referred to as a gratuitous inducer. Thiosugars have been shown to act as gratuitous inducers in the case of β-galactosidase (15). Although the possibility of intracellular breakdown of thiocellobiose has not been excluded, it is likely that this thio-sugar acts as a gratuitous inducer of cellulase, β-glucosidase, and xylanase. When degradable inducers such as cellobiose are used, the inducing effect will level off because of depletion of the inducer. Increasing the inducer concentration will result in catabolite repression due to the accumulation of glucose. This has been shown when cellobiose was used as an inducer in *Sporotrichum pulverulentum* (8). Both the inducer depletion and catabolite repression may be avoided by the use of thiocellobiose.

In *Trichoderma viride*, sophorose has been shown to be the best inducer (11). However, sophorose has not been effective in inducing cellulolytic enzyme production by *S. commune*. Thus, *S. commune* differs from *T. viride* and behaves more like *Sporotrichum pulverulentum* (8) and another basidiomycete isolated by Shewale and Sadana (19). A possible reason for this lack of induction by sophorose is that it might not be carried actively into the *S. commune* cells.

We have shown that in *S. commune*, at least four compounds can induce more than one enzyme activity. *S. commune* is a higher fungus, and the regulation of enzyme synthesis in such a complex organism may not follow the modes of regulation of lactose and tryptophan operons in bacteria. Uptake and activation of the inducers by the cells may partly explain certain variations measured in enzyme activities when different sugars were used. Although thiocellobiose, cellobiose, xylan, and CM-cellulose induced all three enzymes tested, the distinct patterns of induction of some of the enzymes suggest different regulatory systems for each of the enzymes. Some inducers seem to be quite specific. Xylanase, which has been shown to be inducible (1), responds to a large number of inducers, but stachyose seemed to induce this enzyme specifically.

As thiocellobiose proved to be an interesting inducer, other sulfur analogs of sugars are being synthesized; their inducing potencies will be investigated.

ACKNOWLEDGMENTS

We thank B. I. Fleming and M. G. Paice for valuable comments on the manuscript.

Part of this work was supported by a grant from the National Research Council of Canada. H.D. and D.R. were recipients of grants from the COMES of France and DGES of Québec, respectively.

LITERATURE CITED

1. **Biely, P., Z. Kratky, M. Vrsanska, and D. Urmanicova.** 1980. Induction and inducers of endo-1, 4-β-xylanase in the yeast *Cryptococcus albidus*. Eur. J. Biochem. **108**:323–329.
2. **Blanc-Muesser, M., J. Defaye, and H. Driguez.** 1978. Synthèses stéréosélectives de 1-thioglycosides. Carbohydr. Res. **67**:305–328.
3. **Boos, W., Schaedel, P., and K. Wallenfels.** 1967. Untersuchungen zur Induktion der Lac-Enzyme 1. Induktionswirkung und Permeation. Eur. J. Biochem. **1**:382–394.
4. **Dekker, R. F. H., and W. A. Lindner.** 1979. Bioutilization of lignocellulosic waste materials: a review. S. Afr. J. Sci. **75**:65–71.
5. **Desrochers, M., L. Jurasek, and M. G. Paice.** 1981. High production of β-glucosidase in *Schizophyllum commune*: isolation of the enzyme and effect of the culture filtrate on cellulose hydrolysis. Appl. Environ. Microbiol. **41**:222–228.
6. **Desrochers, M., L. Jurasek, and M. G. Paice.** 1981. Production of cellulase, β-glucosidase and xylanase by *Schizophyllum commune* grown on a cellulose-peptone medium. Dev. Ind. Microbiol. **22**:675–684.
7. **Eriksson, K.-E., A. Grünewald, and L. Vallander.** 1980. Studies of growth conditions in wood for three white-rot fungi and their cellulaseless mutants. Biotechnol. Bioeng. **22**:363–376.
8. **Eriksson, K.-E., and S. G. Hamp.** 1978. Regulation of endo-1, 4-β-glucanase production in *Sporotrichum pulverulentum*. Eur. J. Biochem. **90**:183–190.
9. **Hutson, D. H.** 1967. 6-*S*-β-D-glucopyranosyl-6-thio-D-glycopyranose. A thioglycosidic analogue of gentiobiose. J. Chem. Soc. Ser. C, p. 442–444.
10. **Kanda, T., K. Wakabayashi, and K. Nisizawa.** 1976. Purification and properties of an endocellulase of avicelase type from *Irpex lacteus* (*Polyporus tulipiferae*). J. Biochem. **79**:977–988.

11. **Loewenberg, J. R., and C. M. Chapman.** 1977. Sophorose metabolism and cellulase induction in *Trichoderma*. Arch. Microbiol. 113:61–64.
12. **Mandels, M.** 1975. Microbial sources of cellulase. *In* Cellulose as a chemical and energy resource. Biotechnol. Bioeng. Symp. 5:81–105.
13. **Mandels, M., and E. T. Reese.** 1960. Induction of cellulase in fungi by cellobiose. J. Bacteriol. 79:816–826.
14. **Miller, G. L.** 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 31:426–428.
15. **Monod, J.** 1956. Remarks on the mechanism of enzyme induction, p. 313–334. *In* A. Woff and A. Ullmann (ed.), Selected papers in molecular biology by Jaque Monod. Academic Press, Inc., New York.
16. **Paice, M. G., and L. Jurasek.** 1977. Wood-saccharifying enzymes from *Schizophyllum commune*, p. 113–117. *In* TAPPI Forest Biology Wood Chemistry Conference proceedings, Madison, Wis. Technical Association of the Pulp and Paper Industry, Publications, Atlanta.
17. **Paice, M. G., L. Jurasek, M. R. Carpenter, and L. B. Smillie.** 1978. Production, characterization, and partial amino acid sequence of xylanase A from *Schizophyllum commune*. Appl. Environ. Microbiol. 36:802–808.
18. **Ryu, D. D. Y., and M. Mandels.** 1980. Cellulases: biosynthesis and applications. Enzyme Microb. Technol. 2:91–101.
19. **Shewale, J. G., and J. C. Sadana.** 1978. Cellulase and β-glucosidase production by a basidiomycete species. Can. J. Microbiol. 24:1204–1216.
20. **Sternberg, D., and S. Dorval.** 1979. Cellulase production and ammonia metabolism in *Trichoderma reesei* on high levels of cellulose. Biotechnol. Bioeng. 21:181–191.
21. **Sternberg, D., and G. R. Mandels.** 1979. Induction of cellulolytic enzymes in *Trichoderma reesei* by sophorose. J. Bacteriol. 139:761–769.
22. **Vaheri, M. P., M. E. O. Vaheri, and V. S. Kauppinen.** 1979. Formation and release of cellulolytic enzymes during growth of *Trichoderma reesei* on cellobiose and glycerol. Eur. J. Appl. Microbiol. 8:73–80.