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Cellotriose and Cellotetraose as Inducers of the Genes Encoding Cellobiohydrolases in the Basidiomycete *Phanerochaete chrysosporium*[▽]

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The wood decay basidiomycete *Phanerochaete chrysosporium* produces a variety of cellobiohydrolases belonging to glycoside hydrolase (GH) families 6 and 7 in the presence of cellulose. However, no inducer of the production of these enzymes has yet been identified. Here, we quantitatively compared the transcript levels of the genes encoding GH family 6 cellobiohydrolase (*cel6A*) and GH family 7 cellobiohydrolase isozymes (*cel7A* to *cel7F*/*G*) in cultures containing glucose, cellulose, and cellooligosaccharides by real-time quantitative PCR, in order to evaluate the transcription-inducing effect of soluble sugars. Upregulation of transcript levels in the presence of cellulose compared to glucose was observed for *cel7B*, *cel7C*, *cel7D*, *cel7F*/*G*, and *cel6A* at all time points during cultivation. In particular, the transcription of *cel7C* and *cel7D* was strongly induced by cellotriose or cellotetraose. The highest level of *cel7C* transcripts was observed in the presence of cellotetraose, whereas the highest level of *cel7D* transcripts was found in the presence of cellotriose, amounting to 2.7×10^6 and 1.7×10^6 copies per 10^5 actin gene transcripts, respectively. These numbers of *cel7C* and *cel7D* transcripts were higher than those in the presence of cellulose. In contrast, cellobiose had a weaker transcription-inducing effect than either cellotriose or cellotetraose for *cel7C* and had little effect in the case of *cel7D*. These results indicate that cellotriose and cellotetraose, but not cellobiose, are possible natural cellobiohydrolase gene transcription inducers derived from cellulose.

Cellulose existing in the form of microfibrils in plant cell wall is the most abundant renewable carbon source on earth (14). Native cellulose microfibrils are highly crystalline, consisting of parallel-stacked linear chains of β-1,4-linked glucose residues (15). The degradation of cellulose in nature is performed mainly by microorganisms, which produce a set of extracellular cellulose-hydrolyzing enzymes (6, 7, 13, 48). Among these enzymes, cellobiohydrolase (CBH; EC 3.2.1.91) is essential for deconstruction of the crystalline part of cellulose (10, 11, 20), suggesting that it plays an important role in the carbon cycle in nature. Fungal CBHs are mainly classified into glycoside hydrolase (GH) families 7 (Cel7; formally known as CBHI) and 6 (Cel6; formally known as CBHII), respectively, based on amino acid sequence similarities (18–20). Many cellulolytic basidiomycetes and ascomycetes produce one or more GH family 6 and 7 CBHs, as listed in the Carbohydrate-Active enZymes (CAZy) website (http://www.cazy.org/).

The filamentous ascomycete *Hypocrea jecorina* (anamorph, *Trichoderma reesei*), which is the best-studied microorganism from the viewpoint of cellulose degradation, has one gene for Cel7A (CBHI) (39, 45) and also one for Cel6A (CBHII) (46). *H. jecorina* has another gene for GH family 7 hydrolase (Cel7B) (32), but Cel7B was functionally characterized as an endoglucanase (EG; EC 3.2.1.4) (16). CBHs are induced in the presence of cellulose (24), even though cellulose is insoluble and may not be directly recognizable by the fungus. Several studies have indicated that a water-soluble, low-molecular-weight inducer is necessary for efficient production of CBHs (reviewed in reference 42). Sophorose, a β-1,2-diglucoside, is a potent inducer produced from cellulose (26, 40, 41) and may be formed by transglycosylation of cellooligosaccharides catalyzed by a β-glucosidase (BGL) (50), although there is no report of any purified BGL that can convert cellooligosaccharides to sophorose. Antibody competition and antisense RNA experiments have led to the proposal that low basal levels of cellulases (mainly Cel7A and Cel6A) are necessary for further induction (5, 12). Analysis of CBH gene expression by using cellulase deletion mutants strongly suggested that basal expression of Cel6A and GH family 5 EG (Cel5A) is indispensable for the formation of the inducer(s) of CBHs from cellulose (37, 38).

Although basidiomycetes efficiently degrade crystalline cellulose by utilizing CBHs, relatively little is known about the induction of the genes encoding CBHs, compared with the case of ascomycetes. The wood-rotting basidiomycete *Phanerochaete chrysosporium* has one gene coding for Cel6A (CBHII) (28, 47) and seven genes encoding proteins belonging to GH family 7 (Cel7A to Cel7F/G); the latter are located at seven different loci and include a duplication of the same sequence (*cel7F* and *cel7G*) (8, 9, 54). It was found that *cel7C* and *cel7D* are coordinately expressed under various conditions (3, 4, 47), and their expression is repressed by glucose (4, 9). The amounts of the *cel7* gene transcripts, as estimated by competitive reverse transcription-PCR (RT-PCR) analysis, were different from each other in cellulose medium supplemented with

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0.1% cellobiose (9, 52). The *cel7D* transcripts were the most abundant, while *cel7A* and *cel7B* transcripts were expressed at constitutively low levels, and *cel7C* transcripts were highly expressed in the presence of cellulose. However, no inducer for the expression of *cel7* genes has yet been identified.

Since cellobiose and cellooligosaccharides are the major products of cellulose hydrolysis, it is possible that cellulolytic organisms employ them to regulate cellulase production. Indeed, specific transporters of cellobiose and cellotriose have been identified in the cellulolytic bacterium *Streptomyces reticuli* (35, 36). Moreover, it was reported that *Clostridium thermocellum* assimilates cellopentaose preferentially during growth on cellulose (57). In addition, the transcription of endoglucanase genes is induced by cellotriose rather than cellobiose in *Ruminococcus flavefaciens* (56). These findings indicate the presence of selective assimilation and response mechanisms for specific cellooligosaccharides in these bacteria. In *H. jecorina*, cellobiose was speculated to be a natural inducer (27, 50), although its inductive effect was weaker than that of sophorose. In the case of cellulolytic basidiomycetes, responsiveness to cellooligosaccharides has been reported only in the case of *Polyporus arcularius*, which showed upregulation of cellobiohydrolase and endoglucanase gene expression in culture medium containing cellopentaose (30, 31).

Here, we performed quantitative transcription analysis of *cel7* genes in *P. chrysosporium* cultured in media containing glucose, cellulose, and cellooligosaccharides and compared the transcription levels of the *cel7* genes in the presence of these sugars. In addition, the transcription of *cel6A* was quantified and compared with that of the *cel7* genes.

MATERIALS AND METHODS

Fungal strain and culture conditions. *P. chrysosporium* strain K-3 (23) and modified Kremer and Wood medium (25) with the composition described previously (44) were used in this study. For transcription analysis in the presence of cellulose and glucose, 1×10^9 liter^{-1} spores of the fungus were inoculated in 400 ml of this medium containing 2% cellulose (CF11; Whatman, Fairfield, NJ) or 100 mM glucose (Wako Pure Chemical Industries, Osaka, Japan) as the sole carbon source. The inoculated media were maintained at 37°C and shaken at 150 rpm for 5 days. A 5-ml aliquot of the culture was harvested every 24 h. For cultivation with cellooligosaccharides, the spores were inoculated in 200 ml of the medium and cultivated under the same conditions used for pregrowth. After 3 days of cultivation, the mycelia were harvested, washed three times with 100 ml of the same medium containing no carbon source, and transferred to 200 ml of fresh medium containing 20 mM glycerol (Wako). After 6 h of cultivation, 100 μM glucose (Wako), cellobiose, cellotriose, cellotetraose, or cellopentaose (Seikagaku Corporation, Tokyo, Japan) was added to the medium and cultivation was continued for another 6 h. A 5-ml aliquot of the culture was harvested every hour for the determination of sugar concentration and mRNA extraction.

Measurement of glucose and cellooligosaccharide concentration in the culture supernatant. The culture supernatant was boiled for 5 min to inactivate enzymes secreted by the fungus. The concentrations of glucose and cellooligosaccharides in the supernatant were measured by high-performance liquid chromatography (HPLC; LC-2000 series; Jasco, Tokyo, Japan), using a corona-charged aerosol detector (ESA Biosciences, Chelmsford, MA) based on our previous report (21). The supernatants were filtered using a MultiScreen HTS 96-well filtration system (Millipore Corporation, Billerica, MA) and then separated on a Shodex Asahipak NH2P-50 4E (Showa Denko K.K., Kanagawa, Japan) with isocratic elution (65% acetonitrile, 35% H_2O [vol/vol]). In addition, isocratic elution with 75% acetonitrile–25% H_2O was used to separate cellobiose from other disaccharides. The amount of each sugar was quantified by using glucose (BioUltra; Sigma-Aldrich, St. Louis, MO) and cellooligosaccharides with degree of polymerization (DP) values of 2 to 6 (Seikagaku Corporation) as standards.

Real-time RT-PCR analysis of cellulolytic gene transcripts. Mycelia collected from the culture aliquots were immediately frozen in liquid nitrogen and stored at −80°C to extract RNA. Frozen fungal mycelia were ground to a fine powder using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan), and then total RNA was extracted with a RNeasy plant mini kit (Qiagen, Valencia, CA) and treated with an RNase-free DNase set (Qiagen), according to the manufacturer's instructions. First-strand cDNA was synthesized using ReverTra Ace (Toyobo, Osaka, Japan), as described previously (44). Real-time RT-PCRs were performed in an Mx3000P real-time QPCR (quantitative PCR) system (Stratagene, La Jolla, CA) as follows. Five microliters of template solution was mixed with 20 μl of solution containing 12.5 μl of Brilliant II Fast SYBR green QPCR master mix (Stratagene), 0.5 μl of 10 μM forward primer, 0.5 μl of 10 μM reverse primer, 0.375 μl of 6-carboxy-X-rhodamine (ROX) reference dye solution, and 6.125 μl of sterile distilled water. The mixtures were initially incubated at 95°C for 2 min, followed by amplification for up to 40 cycles of 95°C for 5 s and 60°C for 20 s. After thermal cycling, reaction mixtures were heated from 60°C to 94°C for 20 min at a constant rate and the fluorescence of SYBR green I was measured continuously during heating for dissociation curve analysis. Fluorescence data were analyzed using MxPro version 4.0 software (Stratagene). The sequences of the oligonucleotide primers used for amplification of the cDNA fragments derived from *cel7A*, *cel7B*, *cel7C*, *cel7D*, *cel7E*, *cel7F*/*G*, and *cel6A* genes (GenBank accession no. X54411 for both *cel7A* and *cel7B*, Z22528, L22656, Z11727, Z11729, and S76141) were designed based on the corresponding sequences in *P. chrysosporium* strain K-3 and listed in our previous reports (43, 44). The transcript number of the actin gene was quantified as an internal standard by using the following primers: actin-F (5′-GCATGTGCAAGGCTG GCTTTG-3′) and actin-R (5′-AGGGCGACCAACGATGGATG-3′).

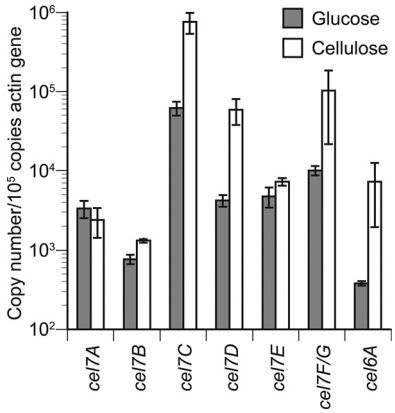

FIG. 1. Copy numbers of *cel7A* to *-F*/*G* and *cel6A* transcripts quantified by real-time PCR in 2-day-old culture with 100 mM glucose or 2% cellulose as a carbon source. The vertical axis indicates the number of transcripts normalized with respect to 10^5 copies of actin gene transcripts in the same sample. Error bars show the standard deviation in triplicate tests.

RESULTS

Transcript levels of *cel7A* to *cel7F*/*G* and *cel6A* were quantified in media containing 2% cellulose and 100 mM glucose by real-time RT-PCR. In 2-day-old cellulose culture, the numbers of gene transcripts of *cel7A* to *-F*/*G* and *cel6A* were 2.4×10^3, 1.3×10^3, 7.6×10^5, 5.9×10^4, 7.2×10^3, 1.0×10^5, and 7.2×10^3 copies per 10^5 copies of actin gene transcript, respectively (Fig. 1). The numbers of transcripts were higher in cellulose culture than in glucose culture for most of these genes, although little difference was observed for *cel7A* and *cel7E*. Throughout the cultivation, *cel7B*, *cel7C*, *cel7D*, *cel7F*/*G*, and *cel6A* transcript levels were higher in cellulose- versus glucose-grown cultures, while there were no significant differences in the transcript levels of *cel7A* and *cel7E* under these conditions. In the case of cellulose culture, the transcript numbers of *cel7C*, *cel7D*, *cel7E*, and *cel7F*/*G* were decreased from day 2 to

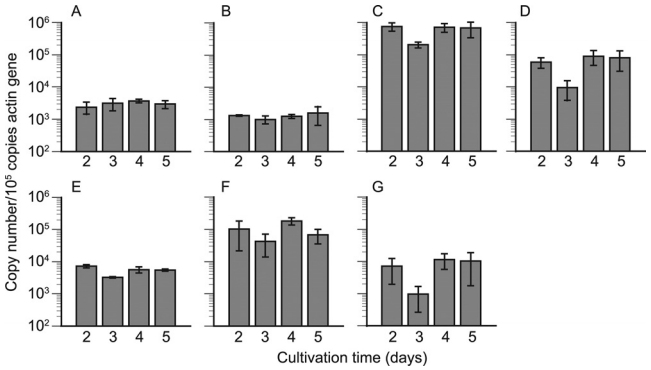

FIG. 2. Copy numbers of *cel7A* (A), *cel7B* (B), *cel7C* (C), *cel7D* (D), *cel7E* (E), *cel7F*/*G* (F), and *cel6A* (G) transcripts quantified by real-time PCR during 2 to 5 days of cultivation with 2% cellulose as a carbon source. Template cDNAs were prepared from the culture every 24 h. The vertical axis indicates the number of transcripts normalized with respect to 10^5 copies of actin gene transcripts in the same sample. Error bars show the standard deviation in triplicate tests.

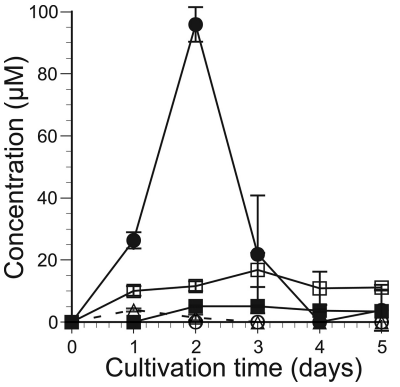

FIG. 3. Concentrations of glucose and cellooligosaccharides in extracellular fluid from culture in 2% cellulose medium. The concentration was quantified by HPLC every 24 h for 5 days. Open squares, glucose; filled circles, cellobiose; open triangles, cellotriose; filled squares, cellotetraose; open circles, cellopentaose. Error bars show the standard deviation in triplicate tests.

day 3 by 73%, 84%, 55%, and 59%, respectively, and then increased from days 3 to 4 (Fig. 2C to F). There were no clear alterations during day 4 and day 5, except in the case of *cel7F*/*G*, for which the transcript level was decreased again by 63% at this time point. In contrast, no marked alteration of transcript numbers was observed for *cel7A* and *cel7B* (Fig. 2A and B). The transcript numbers of *cel6A* were decreased from day 2 to day 3 by 87% (Fig. 2G). As regards the absolute number of transcripts, *cel7C* was most abundant, while *cel7D* and *cel7F*/*G* transcripts were at similar levels in cellulose culture.

Accumulation of soluble cellooligosaccharides in the supernatant of cellulose-containing culture was quantified by HPLC (Fig. 3). Under the conditions used, approximately 100 μM cellobiose accumulated in 2 days and then was completely lost by day 4. The cellotriose concentration reached about 4 μM in day 1 and then decreased. Production of cellotetraose was observed from day 2 and reached approximately 5 μM during further cultivation. In contrast, no accumulation of cellopentaose or cellohexaose was detected in the culture medium.

To evaluate the transcript levels of cellulolytic genes in the presence of soluble cellooligosaccharides, *P. chrysosporium* was cultivated in medium containing 20 mM glycerol as a carbon source supplemented with 100 μM glucose or cellooligosaccharides with a DP value of 2 to 5. The time courses of the concentrations of these sugars were quantified using HPLC during 6 h of cultivation. In the culture with glucose added, the initial concentration of glucose was 120 μM (Fig. 4A), because 20 μM glucose remained after the precultivation. A similar amount of glucose was present in all media tested (Fig. 4). In the cellobiose culture, cellobiose was completely consumed within 3 h (Fig. 4B). In the culture supplemented with oligosaccharides with DP values of 3 to 5, oligosaccharides with lower DP values appeared during cultivation. As shown in Fig. 4C, cellotriose was totally assimilated or hydrolyzed within 4 h, and 23 μM cellobiose was produced within 1 h in the cellotriose culture. In the culture with cellotetraose, hydrolysis or assimilation of cellotetraose occurred within 1 h and 150 μM cellobiose was produced, although no cellotriose was detected (Fig. 4D). All possible hydrolysis products were detected in the cellopentaose culture (Fig. 4E). In the case of the control cultivation without any additive, only glucose was detected, as shown in Fig. 4F.

In the culture containing glucose or cellooligosaccharide, the time courses of transcript levels of cellulolytic genes were quantified by real-time RT-PCR. Throughout the cultivation, no apparent alteration of transcript number was detected for any of the genes tested in the control culture containing only glycerol; thus, it was confirmed that glycerol acts as a neutral carbon source for cellulolytic gene expression under the conditions used. The transcript patterns in response to cellooligosaccharides differed among the genes, as shown in Fig. 5. The transcription of *cel7C* was apparently upregulated by cellotriose and cellotetraose (Fig. 5C), and the maximum amount of *cel7C* transcripts, corresponding to a 970-fold increase, was obtained after 1 h in cellotetraose culture. Addition of cellobiose caused a 190-fold increase of *cel7C* transcripts after 1 h. In cellopentaose culture, the number of *cel7C* transcripts increased to about the same level as in cellobiose culture, although more slowly. In the case of *cel7D* (Fig. 5D), in contrast, maximum transcript levels (340-fold increase) were seen after 2 h in cellotriose culture rather than in cellotetraose culture (120-fold). Conversely, addition of cellobiose and cellopentaose had little effect on *cel7D* transcript levels. As for *cel7F*/*G* (Fig. 5F), transcription was upregulated in cellotriose and cellotetraose cultures, and the largest number of transcripts was detected after 2 h in cellotetraose culture (30-fold increase), while addition of cellobiose or cellopentaose slightly increased *cel7F*/*G* transcripts. The expression of *cel6A* (Fig. 5G) was upregulated by cellotriose and cellotetraose as a 76-fold increase in cellotetraose culture. However, transcripts of *cel7A*, *cel7B*, and *cel7E* showed little variation among culture conditions throughout the cultivation (Fig. 5A, B and E). In addition, repression of transcript levels in glucose culture was observed only for *cel7D* and *cel6A*, for up to 2 h under the culture conditions used.

To compare the amounts of substrate metabolized by the fungus in each culture, the cumulative sugar assimilation dur-

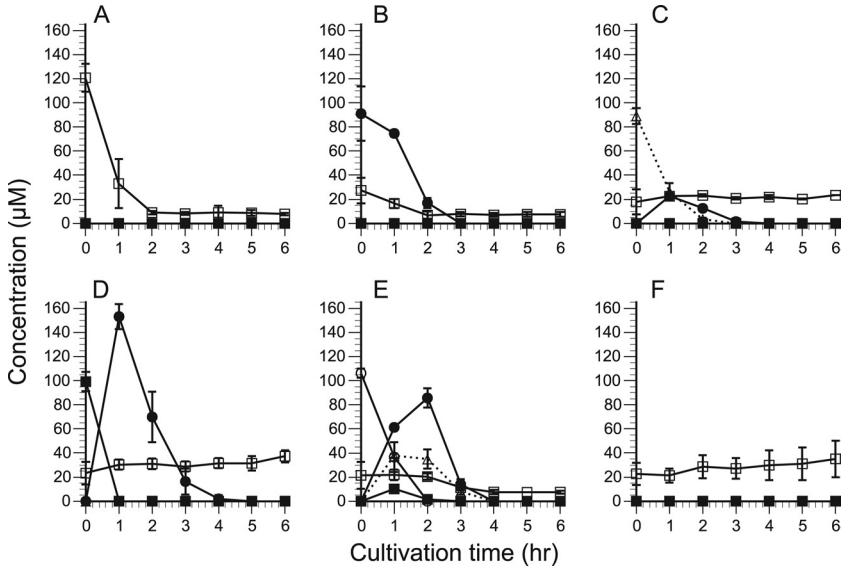

FIG. 4. Time course of concentrations of glucose and cellooligosaccharides in extracellular fluid from 20 mM glycerol culture, supplemented with 100 μM glucose (A), cellobiose (B), cellotriose (C), cellotetraose (D), or cellopentaose (E) or with no addition as a control (F). The concentration was quantified by HPLC for 6 h after the addition of each sugar. Open squares, glucose; filled circles, cellobiose; open triangles, cellotriose; filled squares, cellotetraose; open circles, cellopentaose. Error bars show the standard deviation in triplicate tests.

ing cultivation with cellooligosaccharides was calculated as the product of the decrease from the initial concentration and the DP value of each oligosaccharide and expressed in terms of the concentration of glucose residue (Fig. 6). By 2 h, the concentration of assimilated glucose residue was higher in cellotriose or cellotetraose culture than in cellobiose culture, implying that more carbon was metabolized in cellotriose and cellotetraose culture. After 3 h, most of the supplemented sugar was assimilated, so the concentration of assimilated glucose residue was higher in cultures containing cellooligosaccharides with higher DP values.

DISCUSSION

In cellooligosaccharide-mediated induction of cellulolytic gene expression, two modes were expected: i.e., an extracellular receptor-mediated signaling or uptake and intracellular binding to signaling molecules. There is no information about

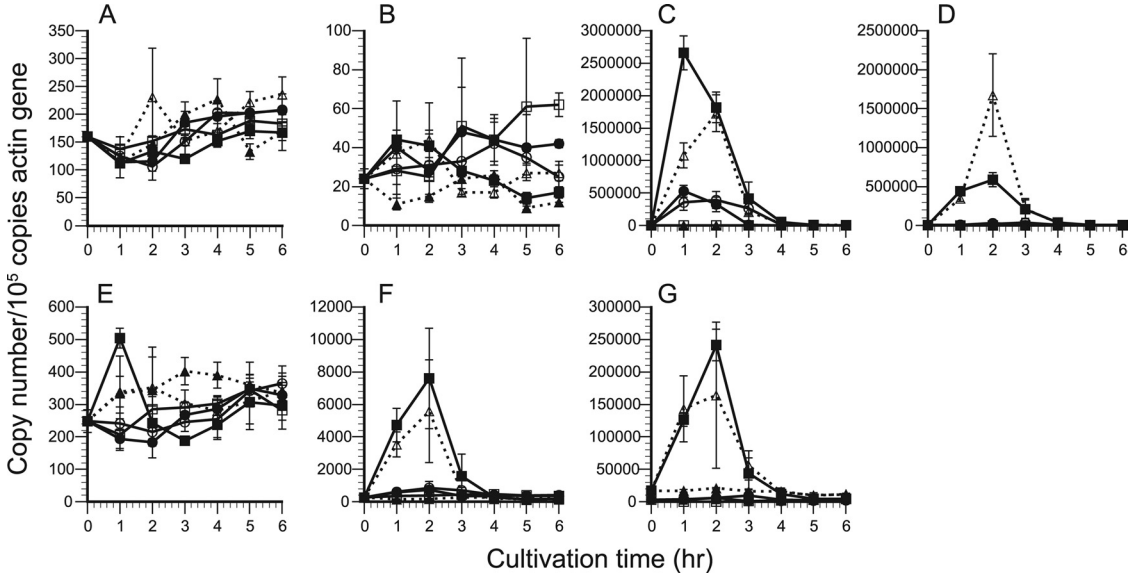

FIG. 5. Time courses of the copy numbers of cellulolytic genes during 6 h of cultivation in culture medium supplemented with glucose (open squares), cellobiose (filled circles), cellotriose (open triangles), cellotetraose (filled squares), or cellopentaose (open circles) or with no addition as a control (filled triangles). The transcript numbers of *cel7A* (A), *cel7B* (B), *cel7C* (C), *cel7D* (D), *cel7E* (E), *cel7F*/*G* (F), and *cel6A* (G) were quantified by real-time PCR and normalized as described in the legend to Fig. 1. Error bars show the standard deviation in triplicate tests.

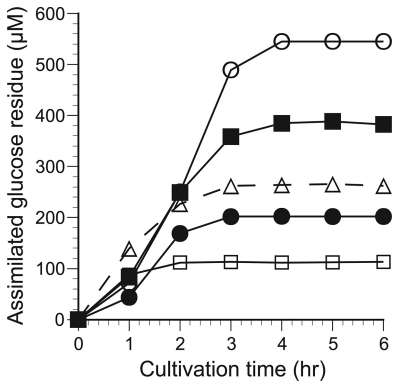

FIG. 6. Time course of sugar assimilation during 6 h of cultivation in culture medium supplemented with glucose (open squares), cellobiose (filled circles), cellotriose (open triangles), cellotetraose (filled squares), or cellopentaose (open circles). The vertical axis indicates total assimilated glucose residue from the culture, calculated as the product of the decrease from the initial concentration and the DP value of each oligosaccharide.

these signal-transducing molecules in *P. chrysosporium* at all, as far as we know, and thus the induction mechanism by cellooligosaccharides remains uncertain. Nevertheless, our results indicate that the addition of cellotriose and cellotetraose clearly increasees transcript levels of several cellulolytic genes in *P. chrysosporium*, although that of cellobiose has little effect. Interestingly, the transcriptional responses of various *cel7* genes to these sugars varied. In the case of *cel7C*, induction in cellobiose culture was clearly observed, but it was 5-fold lower than that in cellotetraose culture (Table 1). Thus, cellobiose, which appeared after 1 h in the cellotetraose culture, probably affected the expression of *cel7C* in that culture, but its effect was considered to be small compared to that of cellotetraose itself. Moreover, only a small amount of cellobiose was formed in cellotriose culture, so that the potent *cel7C*-inducing effect is likely to be due to cellotriose itself. Similar results were observed for *cel7F*/*G*, although in this case, the inducing effect of cellobiose would be even weaker. Addition of cellobiose had little effect in the cases of *cel7D* and *cel6A*, indicating that expression of these genes would not be significantly induced by the formation of cellobiose. In the case of *cel7D*, cellotriose appeared to be a better inducer than cellotetraose, in contrast to the case of *cel7C*. The effect of cellopentaose itself was difficult to estimate, because of the complicating effect of shorter oligosaccharides formed during the cultivation. Nevertheless, cellopentaose appeared not to be a potent inducer, because all five genes were only weakly upregulated in cellopentaose culture.

During the metabolism of cellooligosaccharides by fungi, carbon catabolite repression would be caused by glucose, which is a hydrolysis product of cellooligosaccharides, produced by BGL. In the case of *cel7C*, for example, expression was upregulated by 2 h of cultivation in the presence of cellobiose, cellotriose, or cellotetraose, and at that time point, larger amounts of glucose residue had been assimilated in cellotriose or cellotetraose culture than in cellobiose culture. In *P. chrysosporium*, hydrolysis of cellooligosaccharide may be mainly catalyzed by intracellular BGL (BGL1B) rather than extracellular BGL (BGL3A) (49). The catalytic efficiency (k_{cat}/K_m) of BGL1B is much greater for cellotriose (21×10^1 s^{-1} mM^{-1}) and cellotetraose (16×10^1 s^{-1} mM^{-1}) than for cellobiose (75 s^{-1} mM^{-1}) (T. Tsukada et al., unpublished data). Thus, the intracellular concentration of glucose is considered to be higher in cellotriose and cellotetraose cultures, which may therefore be subject to higher levels of catabolite repression than that seen in cellobiose culture. Consequently, it appears that the lower inductive activity of cellobiose can not be ascribed to catabolism and consequent repression by glucose.

It was previously shown by transcript analysis that the expression levels of *cel7A* and *cel7E* are higher, while that of *cel7D* is lower, in colonized aspen wood (51) than in submerged cellulose culture (9, 52). Moreover, homology models of the three-dimensional structure of Cel7s have shown several structural differences in the tunnel-forming loops; Cel7A and Cel7B exhibit an endoglucanase-like structure, but four other Cel7s are very similar and might not show marked functional differences (29). Thus, it is expected that Cel7A, Cel7B, and Cel7E have characteristically different roles from other Cel7s in cellulose degradation. The results obtained in the present work strongly support this hypothesis, because the transcription of *cel7A*, *cel7B*, and *cel7E* was not affected by cellooligosaccharides, which apparently induced expression of other cellulolytic genes. Furthermore, it is possible that Cel7A and Cel7B do not participate in cellulose degradation, because their gene expression was only slightly altered during cultivation with cellulose, whereas other genes examined in this work all showed similar changes. However, the relationship between the function and structure of Cel7E requires further investigation.

In addition to the transcript numbers in cellulose- and cellooligosaccharide-supplied culture, we compared the maximum

TABLE 1. Maximum values of gene transcripts during cultivation

Gene	Maximum no. of copies of transcript in culture with:				
	Carbon starvation[a]	Cellulose	Cellobiose	Cellotriose or cellotetraose	Glycerol
cel7A	5.22×10^2	3.67×10^3	2.07×10^2	2.36×10^2	2.27×10^2
cel7B	1.30×10^2	1.58×10^3	4.80×10^1	4.40×10^1	2.60×10^1
cel7C	3.78×10^4	7.58×10^5	5.28×10^5	2.66×10^6	3.59×10^3
cel7D	1.39×10^5	9.04×10^4	2.86×10^4	1.67×10^6	5.10×10^3
cel7E	3.28×10^2	7.23×10^3	3.49×10^2	5.04×10^2	4.02×10^2
cel7F/*G*	2.93×10^3	1.83×10^5	7.34×10^2	7.60×10^3	2.81×10^2
cel6A	1.83×10^5	1.15×10^4	6.16×10^3	2.41×10^5	3.18×10^3

[a] Data taken from our previous reports (43, 44).

numbers of gene transcripts under a carbon-starved condition (43, 44), which provokes carbon catabolite derepression of *cel7C*, *cel7D*, and *cel6A* (Table 1). In the case of *cel7C*, the maximum amounts of gene transcripts were higher in cellotriose or cellotetraose culture but lower in carbon-starved culture than in cellulose culture. Therefore, induction by cellotriose and cellotetraose is considered to be a major factor determining *cel7C* transcription, rather than derepression. The transcripts of *cel7D* are at a higher level in both cellotriose and carbon-starved culture than in cellulose culture, indicating that the transcription of *cel7D* is regulated by both induction and derepression. Similarly, the transcript levels of *cel6A* appear strongly regulated by derepression, as well as by cellotriose- and cellotetraose-mediated induction. In the 2-day-old cellulose culture, transcript levels of *cel7C*, *cel7D*, and *cel6A* were considered to be dominantly affected by cellobiose, because the maximum numbers of transcripts in cellobiose culture were close to those in cellulose culture. Indeed, cellobiose was accumulated, but cellotriose and cellotetraose were produced only in small amounts in cellulose culture medium. In contrast, the expression characteristics of *cel7F*/*G* are different from those of *cel7C* and *cel7D*. The transcript number of *cel7F*/*G* in culture with any cellooligosaccharide or in carbon-starved culture did not reach the levels observed in cellulose cultures. Furthermore, a decrease in the number of transcripts from day 4 to day 5 in cellulose culture was observed only for *cel7F*/*G*. These results indicate that, although *cel7F*/*G* seems to be related to cellulose degradation, some other soluble compound, not cellooligosaccharides, is involved in the induction of *cel7F*/*G*. In addition, the transcript levels of *cel7A*, *cel7B*, and *cel7E* were higher in the presence of cellulose than under other culture conditions. HPLC analysis of the supernatant from the cellulose culture showed several unidentified peaks, in addition to the peaks of cellooligosaccharides. Identification and analysis of the effects of the compounds contained in these peaks will be necessary to understand the modes of expression control of the genes.

Recently, transcriptome and secretome analyses of *P. chrysosporium* have revealed a complex pattern of production of wood-degrading enzymes (33, 34, 53, 55), but detailed investigation of the regulatory mechanisms involved remains to be undertaken. The results presented here indicate that cellotriose and cellotetraose are candidates for natural inducers generated by cellulose hydrolysis, whereas cellobiose was not effective. In the extracellular cellulolytic system of *P. chrysosporium*, cellobiose could be oxidized to cellobiono-1,5-lactone if cellobiose dehydrogenase is secreted (1, 2, 17). Cellobiono-1,5-lactone was reported to be an inducer of cellulase production in *H. jecorina* (22). Accordingly, further investigation of the inductive effect of cellobiono-1,5-lactone on transcription of *cel7* and *cel6* genes in *P. chrysosporium* seems worthwhile. It is noteworthy that the *cel7* genes of *P. chrysosporium* were differentially regulated in a complex manner, but not all of the Cel7 enzymes seem to participate in cellulose degradation. Although the machinery of receptors or transporters for cellooligosaccharides remains unknown, the extracellular concentration and/or uptake of cellotriose and cellotetraose could be a signal for cellulolytic enzyme production by *P. chrysosporium*. However, some of our present results are not consistent with those of a previous study using homokaryotic strain RP-78 or its dikaryotic parent, BKM-F-1767. For example, *cel7D* was the most abundant transcript among *cel7* genes in BKM-F-1767 (52), while *cel7C* transcripts were most abundant in heterokaryotic strain K-3. Also, *cel7E* of strain RP-78 is upregulated in cellulose culture compared to glucose culture (53), whereas we found no difference between these carbon sources in strain K-3. Future studies may determine whether these different transcript patterns are due to culture conditions and/or strain variation.

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