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Transcriptional Response of the Cellobiose Dehydrogenase Gene to Cello- and Xylooligosaccharides in the Basidiomycete *Phanerochaete chrysosporium*

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Cellobiose dehydrogenase (CDH) gene transcripts were quantified by reverse transcription-PCR (RT-PCR) in cultures of *Phanerochaete chrysosporium* supplemented with various cello- and xylooligosaccharides in order to elucidate the mechanism of enhanced CDH production in xylan/cellulose culture. Cellotriose and cellotetraose induced *cdh* expression, while xylobiose and xylotriose induced expression of cellobiohydrolase genes, especially *cel7C*.

The basidiomycete *Phanerochaete chrysosporium* produces various extracellular carbohydrate-degrading enzymes (14, 24) and oxidative enzymes (13) that degrade wood, which contains cellulose, hemicellulose, and lignin as major components. Cellulose is a β-1,4-linked homopolymer of glucose, and the cellulolytic system of this fungus involves multiple endoglucanases (EGs) and cellobiohydrolases (CBHs) (20, 21), which hydrolyze cellulose into cellobiose and cellooligosaccharides. In addition to hydrolytic enzymes, redox enzymes such as cellobiose dehydrogenase (CDH) are involved in cellulose degradation (1, 3, 4, 23). CDH oxidizes cellobiose and cellooligosaccharides into the corresponding 1,5-δ-lactones (5, 15). The role of CDH in cellulose degradation has been widely discussed (7, 8, 10, 25), and its relationship with polysaccharides monooxygenase was recently pointed out (12, 16, 22). A study by Dumonceaux and coworkers on a CDH-deficient mutant of the wood-rotting basidiomycete *Trametes versicolor* indicated that CDH is also involved in degradation of other plant cell wall components (2). We have recently reported that arabinoxylan, the major hemicellulose of monocots, enhances fungal growth and CDH production when it is supplemented into cellulose-degrading cultures, although it could not serve as a carbon source for *P. chrysosporium* by itself (6). In the present study, a similar response of the fungus was observed when glucuronoxylan (from beech wood) was added to cellulolytic culture, suggesting that the main-chain structure of xylan affects fungal growth, extracellular protein production and CDH activity. To elucidate the mechanism of these effects, we used reverse transcription-PCR (RT-PCR) to quantify CDH gene transcripts in cultures supplemented with xylose or xylooligosaccharides as well as glucose or cellooligosaccharides.

Phanerochaete chrysosporium strain K-3 (9) was cultivated in Kremer and Wood medium (11) for 4 days with cellulose (CF11; Whatman, Fairfield, NJ), with or without xylan (from oat spelt, Serva Electrophoresis, Heidelberg, Germany; from beech, Sigma-Aldrich, St. Louis, MO). Briefly, xylan from beech wood has side chains of 4-*O*-methyl-β-D-glucuronic acid whereas that from oat spelt has additional side chains of α-L-arabinose. The packed volume of fungal mycelium and protein concentration of the culture filtrate were estimated as described previously (6). Cellobiose dehydrogenase (CDH) activity of the culture filtrates was assayed using cellobiose and cytochrome *c* as described previously (17). For transcriptional analysis in cultures with purified xylooligosac-

Received 18 January 2012 **Accepted** 28 February 2012
Published ahead of print 9 March 2012
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doi:10.1128/AEM.00150-12

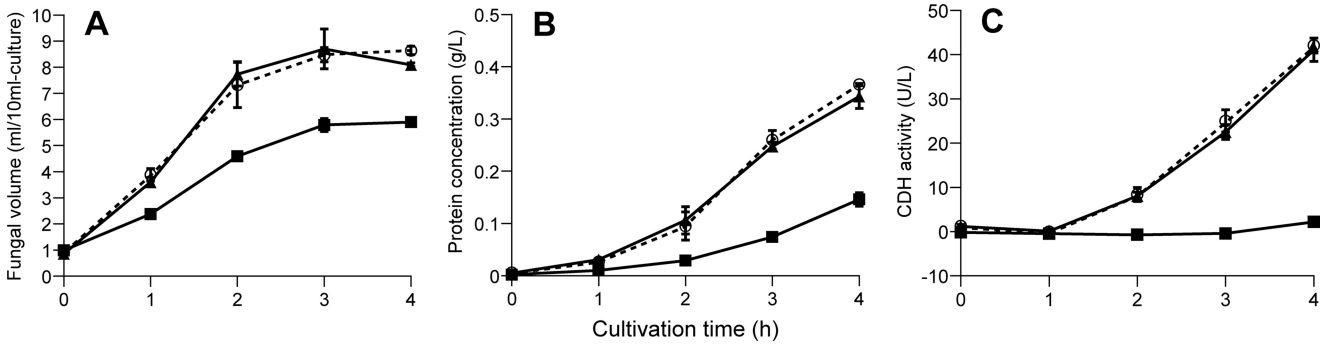

FIG 1 Time courses of fungal volume (A), concentration of extracellular protein (B), and CDH activity (C) of *P. chrysosporium* grown in culture medium containing cellulose (filled square), cellulose and oat spelt xylan (open circle), and cellulose and beech xylan (filled triangle). Error bars show the standard deviations for the results from three experiments.

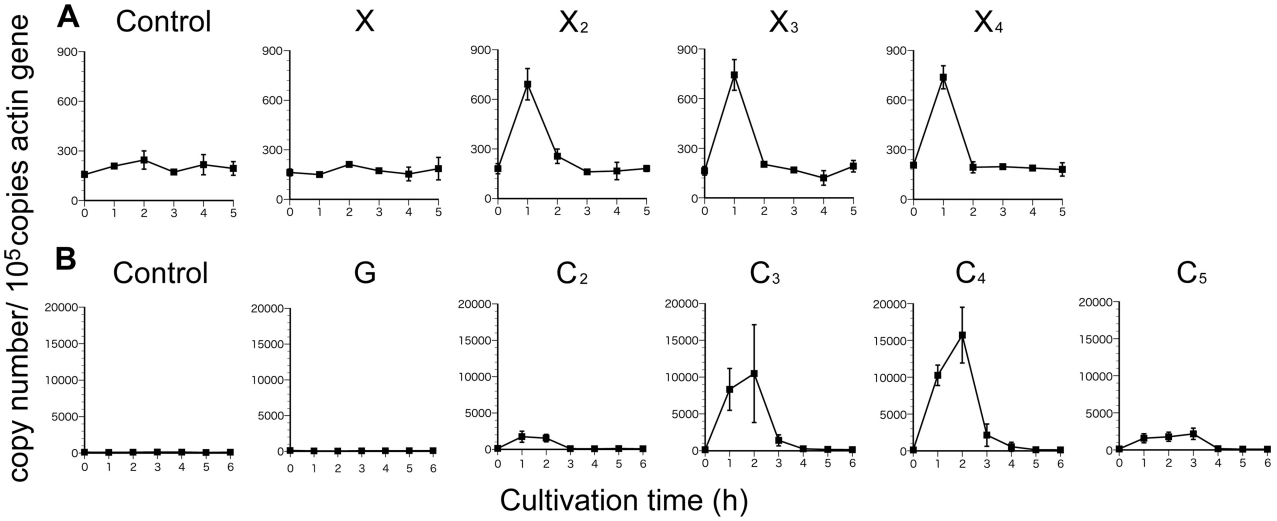

FIG 2 Time course of *cdh* transcripts of *P. chrysosporium* grown in culture medium supplemented with xylooligosaccharides and cellooligosaccharides. (A) X, xylose; X_2, xylobiose; X_3, xylotriose; X_4, xylotetraose. (B) G, glucose; C_2, cellobiose; C_3, cellotriose; C_4, cellotetraose; C_5, cellopentaose. Control means culture without saccharides. Error bars show standard deviations for results from three experiments. Note that the scale on the vertical axis is not the same in panels A and B.

charides, *P. chrysosporium* was precultivated for 3 days and the mycelium was transferred into resting medium as previously described (18). After resting cultivation for 6 h, 100 μM glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, xylose, xylobiose, xylotriose, or xylotetraose was added to the medium. Five milliliters of culture was collected every hour until 5 or 6 h, and the mycelium was immediately frozen in liquid nitrogen for the preparation of total RNA and corresponding cDNA. The gene transcripts of *cdh*, *cel6A*, *cel7C*, and *cel7D* were quantified using quantitative PCR (qPCR) since the corresponding proteins are the

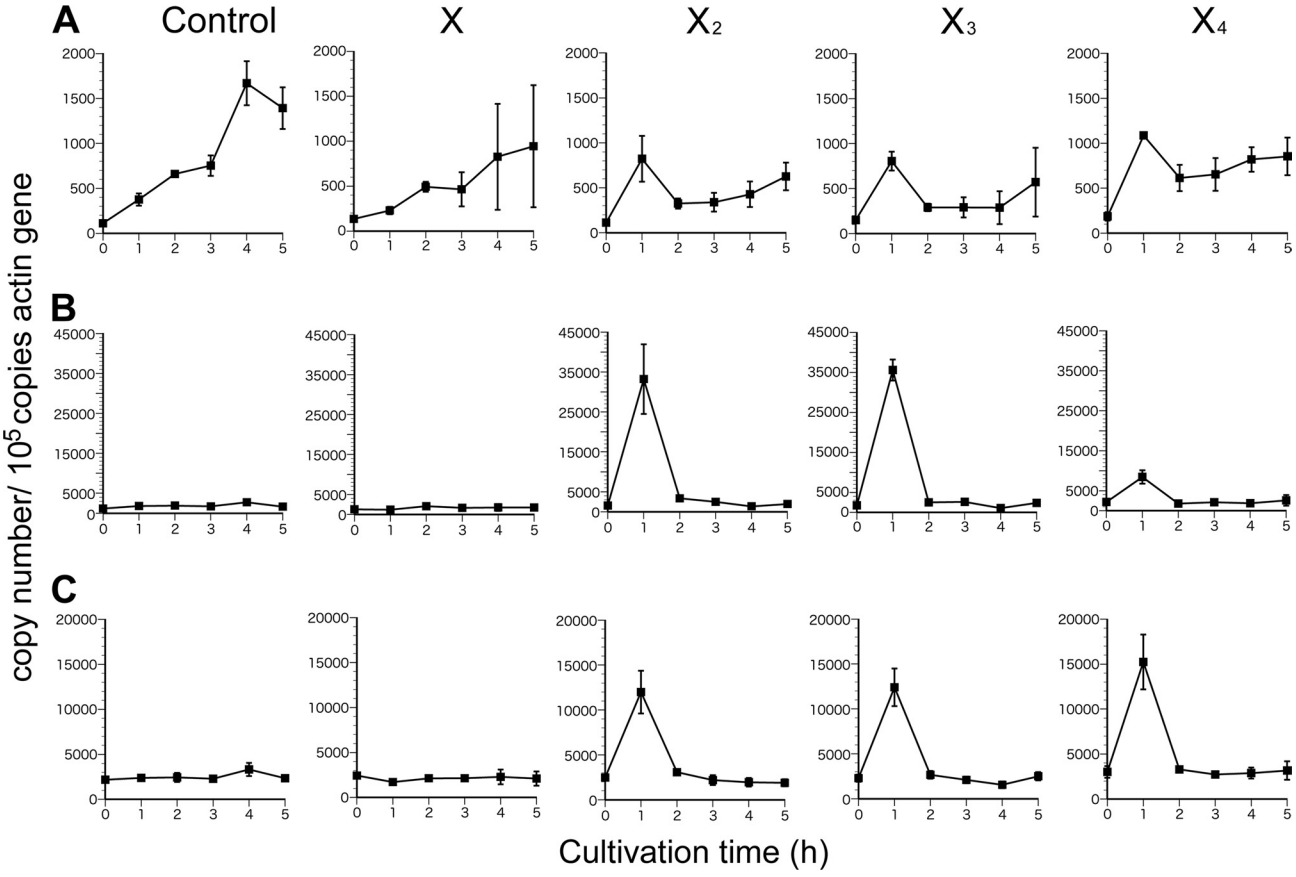

FIG 3 Time courses of *cel6A* (A), *cel7C* (B), and *cel7D* (C) transcripts of *P. chrysosporium* grown in culture medium supplemented with xylose (X), xylobiose (X_2), xylotriose (X_3), and xylotetraose (X_4). Control means culture without saccharides. Error bars show standard deviations for results from three experiments. Note that the scale on the vertical axis is not the same in panels A, B, and C.

dominant cellulases in medium containing cellulose. The primer sets were listed in the previous papers (18, 19). The actin gene was used as an internal standard.

P. chrysosporium was cultivated for 4 days in synthetic media containing cellulose only (C), cellulose and oat spelt xylan (COX), and cellulose and beech xylan (CBX) as carbon sources. As shown in Fig. 1A, the fungal volumes in COX and CBX cultures were larger than that in C culture during cultivation. As shown in Fig. 1B, production of extracellular proteins increased markedly in COX and CBX cultures but only weakly in C culture. The concentrations of extracellular protein in COX and CBX cultures were 0.37 g/liter and 0.34 g/liter, respectively, while that in culture C was 0.14 g/liter. As shown in Fig. 1C, the CDH activity in culture C was 2.2 U/liter on day 4, whereas the corresponding values in COX and CBX cultures were 42 and 41 U/liter, respectively. These results indicate that the effect of xylan is due to the β-1,4-xylan main chain structure of xylans but not the side chains.

P. chrysosporium was cultivated for 5 or 6 h in media containing xylose (X), xylooligosaccharide (DP = 2 to 4, xylobiose [X_2], xylotriose [X_3], or xylotetraose [X_4]), glucose (G), or cellooligosaccharide (degree of polymerization [DP] = 2 to 5, cellobiose [C_2], cellotriose [C_3], cellotetraose [C_4], or cellopentaose [C_5]), and the level of *cdh* gene transcripts was quantified by real-time RT-PCR. As shown in Fig. 2A and B, there was no change of *cdh* expression in the cultures supplemented with X and G compared with the corresponding controls. However, *cdh* was quickly upregulated (within 1 h) in the presence of X_2, X_3, and X_4, reaching maximum values of 7.0×10^2, 7.4×10^2, and 7.4×10^2, respectively. However, in cultures supplemented with C_3 and C_4, the numbers of *cdh* transcripts reached 1.0×10^4 and 1.6×10^4, respectively, at 2 h after supplementation. Thus, *cdh* gene expression is induced more strongly by cellooligosaccharides than by xylooligosaccharides. We previously found that production of glycoside hydrolase (GH) family 10 xylanase was enhanced in xylan-containing culture (6), so we speculated that xylooligosaccharides produced by the xylanase promote CDH production in the culture. However, the results of the present study indicate that upregulation of *cdh* transcription by short and unsubstituted xylooligosaccharides is not sufficient to account directly for the enhanced production of secreted protein. We next examined the effect of xylooligosaccharides on the expression of cellobiohydrolase (*cel*) genes. *cel6A*, *cel7C*, and *cel7D* transcripts were all increased by xylooligosaccharides; in particular, *cel7C* transcripts were increased up to 20-fold by xylobiose and xylotriose (Fig. 3).

In conclusion, the induction of cellulolytic enzymes by xylan in cellulose-degrading culture is not simply due to xylooligosaccharides but is more complex, involving induction by cellotriose and cellotetraose. One possible interpretation is that xylooligosaccharides promote the production of cellobiohydrolases, which produce cellooligosaccharides that in turn enhance the expression of other cellulolytic genes, including *cdh*. We are currently addressing the complex gene regulation system in *P. chrysosporium* by analysis of large-scale transcriptomic data; the results will be reported shortly.

ACKNOWLEDGMENTS

This research was supported by Grants-in-Aid for Scientific Research to M.S. (no. 20380100 and 23248025) from the Japanese Ministry of Education, Culture, Sports, and Technology and a Research Fellowship to C.H. (no. 233288) from the Japan Society for the Promotion of Science (JSPS).

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