APPLIED AND ENVIRONMENTAL MICROBIOLOGY, NOV, 2002, p. 5765–5768 Vol. 68, No. 11
0099-2240/02/$04.00+0 DOI:10.1128/AEM.68.11.5765–5768.2002
Copyright © 2002, American Society for Microbiology. All Rights Reserved.

Transcript Analysis of Genes Encoding a Family 61 Endoglucanase and a Putative Membrane-Anchored Family 9 Glycosyl Hydrolase from *Phanerochaete chrysosporium*

Amber Vanden Wymelenberg,[1] Stuart Denman,[2]† Diane Dietrich,[3] Jennifer Bassett,[1]‡ Xiaochun Yu,[4] Rajai Atalla,[3,4] Paul Predki,[5]§ Ulla Rudsander,[2] Tuula T. Teeri,[2] and Daniel Cullen[1,3]*

Department of Bacteriology [1] and Department of Chemical Engineering, [4] University of Wisconisin—Madison, Madison, Wisconsin 53706; Department of Biotechnology, Royal Institute of Technology, Stockholm Centre for Physics, Astronomy, and Biotechnology, SE-106 91 Stockholm. Sweden[2]; USDA Forest Products Laboratory, Madison, Wisconsin 53705 [3]; and U.S. Department of Energy, Joint Genome Institute, Walnut Creek, California 93598[5]

Received 13 May 2002/Accepted 19 August 2002

***Phanerochaete chrysosporium* cellulase genes were cloned and characterized. The *cel61A* product was structurally similar to fungal endoglucanases of glycoside hydrolase family 61, whereas the *cel9A* product revealed similarities to *Thermobifida fusca* Cel9A (E4), an enzyme with both endo- and exocellulase characteristics. The fungal Cel9A is apparently a membrane-bound protein, which is very unusual for microbial cellulases. Transcript levels of both genes were substantially higher in cellulose-grown cultures than in glucose-grown cultures. These results show that *P. chrysosporium* possesses a wide array of conventional and unconventional cellulase genes.**

Phanerochaete chrysosporium has served as a model system for investigating lignocellulose degradation. Components of the cellulolytic system include multiple exocellobiohydrolase I (CBHI) isozymes. as well as an exocellobiohydrolase II (CBHII) isozyme arid a b-glucosidase (for a review, see reference 20). The CBHIs are encoded by six structurally related genes (8, 9, 28, 38). Previously designated *cbh1-1* through *cbh1-6*, these have been renamed *cel7A* through *cel7F* in accordance with the glycoside hydrolase classification system of Henrissat and coworkers (16, 17). Single genes encode CBHII (*ce16A*) (33) and b-glucosidase (22).

Cellulolytic microbes typically feature endoglucanases that work in synergy with the exocellobiohydrolases (for a review, see reference 4) and both activities have been detected in *P. chrysosporium* cultures (12, 15, 34–36). However, as repeated attempts to clone endoglucanase genes have failed, it has been suggested that certain cellobiohydrolases might possess endoglucanase activity (5, 29). In short, the number, structure, and transcriptional regulation of *P. chrysosporium* endoglucanase genes have not been elucidated.

Cloning and characterization of *cel61A*. Expressed sequence tag libraries were prepared from wood chip cultures (1) inoculated with *P. chrysosporium* strain RP-78 (32). The PCR-amplified libraries were constructed from poly(A) RNA (18, 37) according to the manufacturer's recommendations (Smart PCR cDNA synthesis kit; Clontech, Palo Alto, Calif.). Approximately 3,000 clones were sequenced from plasmids (10) by using DYEnamic dye terminator chemistry (Amersham Biosciences) and Molecular Dynamics MegaBACE 1000 sequencers. Sequence quality was assessed by using Phred (13), and the sequence was edited (DNASTAR, Madison, Wis.) and functionally categorized (MyPipeOnline, Oklahoma State University Bioinformatics [http://wwv.bioinfo.okstate.edu/pipeonline/MyPOL.html]). Among 1,400 unique expressed sequence tag sequences, a single clone of approximately 650 nucleotides was found to have substantial similarity to other fungal endoglucanase genes. The genomic clone corresponding to the cDNA sequence was isolated by genome walking (Universal GenomeWalker kit; Clontech), and the sequence was recently verified by genome sequencing (http://ww.jgi.doe.gov/programs/whiterot/whiterot-mainpage.html). Intron positions were verified by reverse transcription (RT)-PCR amplification and cDNA sequencing. The catalytic domain of the predicted protein is 25 to 30% identical to glucoside hydrolase family 61 (http://www.afmb.cnrs-mrs.fr/~pedro/CAZY/ghf.html) from *Agaricus bisporus* CEL1 (2), *Aspergillus kawatchii* endoglucanase B (GenBank accession number AB055432), and *Trichoderma reesei* Cel61A (previously EGIV) (27). Accordingly, the gene was designated *cel61A*. The C-terminal cellulose-binding domain of *P. chrysosporium* Cel61A is clearly a member of the family 1 carbohydrate binding module. A putative secretion signal with cleavage between amino acid residues 23 and 24 was identified by using Signal P (http://www.cbs.dtu.dk/services/SignalP/). The comparison of cDNA and genomic sequences revealed several introns, which permitted competitive PCR (cPCR).

* Corresponding author. Mailing address: Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705. Phone: (608) 231-9468. Fax: (608) 231-9262. E-mail: dcullen@facstaff.wisc.edu.

† Present address: CSIRO Livestock Industries, Indooroopilly, Qld 4068, Australia.

‡ Present address: Department of Biomedical Engineering, University of Wisconsin—MadisonMadison, WI 53706.

§ Present address: Protometrix, Inc., Guilford, CT 06437.

TABLE 1. PCR primers and expected products

Target[a]	Oligonucleotide[b]	Product size (nt)[c]	
		Genomic	cDNA
Cel9A		636	479
Upstream	ATGATACCTCTCCGCTCTGC		
Downstream	CAGCGTGTTTGGGTCAGGATG		
Cbg1		483	263
Upstream	CCACCCGAACGTCACCGCTATT		
Downstream	AGGCCGAACCCGAACTC		
Cel7D		796	742
Upstream	AAGGTCGTCCTCGACTCGAA		
Downstream	CGACGGTGAAGGGCTTGGAG		
Cel61A		464	311
Upstream	GCCAGGCCAATATCTACTTCGTCA		
Downstream	AGGGGCGCTGCTACTTACACTGC		
EG44			
Upstream	TGGGGHCARTGYGGHGGHAT		
Downstream	ARTCVAGRTAYTGXXXCAT		

[a] Targets are specific genes for RT-cPCR or the gene encoding the endoglucanase designated EG44 (34).
[b] PCR primers are presented 5′ to 3′. Base assignments for degenerate primers are as follows: H. A/C/T; R, A/G; Y, C/T, V, A/C/G; X, A/C/G/T.
[c] Product lengths of competitive template (genomic) and cDNA targets.

Cloning and characterization of *cel9A*. Cloning of cel9A was serendipitous in that the degenerate primers (Table 1) were originally designed to PCR amplify a conventional endoglucanase, EG43 (34). Among several fragments that were isolated and sequenced, one showed significant similarity to the *Thermobifida fusca* E4 gene. Several rounds of genome walking and RT-PCR yielded genomic and cDNA clones, respectively. BLAST analysis of the Joint Genome Institute's White Rot database revealed a single genomic copy of *cel9A.* A comparison of full-length cDNA and genomic sequences identified seven introns.

Sequence analysis of the *P. chrysosporium cel9A* cDNA predicts a putative family 9 glycosyl hydrolase catalytic module of 590 amino acids. The Cel9A sequence has no obvious carbohydrate binding module, but, surprisingly, it contains a C-terminal *trans*-membrane region from amino acids 559 to 581 amino acids (Fig. 1) as predicted by the TMHMM version 2.0 server (http://www.cbs.dtu.dk/services/TMHMM-2.0/) (21).

The predicted family 9 catalytic module was most similar to that of *T. fusca* E4 (37% similarity) and clearly related to those of other members of family 9, which are derived from bacteria, insects, higher plants, or most recently, as a cellulosome component from the anaerobic fungus *Piromyces* (30). A putative secretion signal cleavage site was identified between residues 20 and 21 by using Signal P.

A multiple-sequence alignment of a selected set of family 9 glycosyl hydrolase catalytic domains clearly identifies the key catalytic residues and several regions of high conservation between the subclasses of family 9. One element of particular interest is the surface loop present at positions 245 to 255 in the bacterial family 9 cellulase E4; this loop generates an active-site topology, promoting processive action of the enzyme along a cellulose chain (25). A similar sequence insertion is evident in the *Arabidopsis thaliana* cell wall-bound family 9 enzyme, KORRIGAN (23), and in the fungal Cel9A discovered in the present study in *P. chrysosporium,* though it is not present in the true endo-acting enzymes in family 9.

To allow a rough, three-dimensional comparison of the soluble bacterial cellulases and the membrane-anchored enzymes, a homology model of *P. chrysosporium* Cel9A was created by using the Modeller version 4.0 package (26) and the *T. fusca* Cel9A (E4) structure as a template. As expected, the overall folds of the two enzymes were similar, and the residues involved in substrate binding in the active-site cleft (*T. fuscu* Cel9A residues Trp128 and Tyr420) were strictly conserved at the +1 and +2 subsite positions (data not shown). Also, the residues which coordinate a structurally important calcium ion **in** E4 and in CELD from *Clostridium thermocellum* (25), were strictly conserved. However. the key aromatic residues required for substrate binding at the −1 through −4subsites in E4 (Trp256, Trp209, and Trp313) were replaced mostly by alanines or serines in *P. chrysosporium* Cel9A. Interestingly. we observed a similar lack of conservation of the aromatic residues involved in substrate binding in KORRIGAN, the membrane-bound cellulase from *A. thaliana* (reference 23 and data not shown). In the case of the plant enzyme, this observation lends support to a recent suggestion that KORRIGAN may have a role in removing the sitosterol-b-glucoside precursor from the end of a growing glucan chain during cellulose synthesis in plants (24). Without further experiments, the specificity of the membrane-bound fungal Cel9A is more difficult to predict, but it is obvious that it is not a typical microbial cellulase.

Transcript analysis. RT coupled to cPCR (14), a technique well suited to analyzing closely related *P. chrysosporium* transcripts (6, 18, 31, 37), was used to assess transcript levels of *cel61A*, *cel9A*, and previously characterized genes encoding CBH1 (*cel7D*) and b-glucosidase (*cbg1*). Dramatic differences in transcript levels among the four genes (Fig. 2 and 3) were observed. Consistent with previous protein (35) and transcript (37, 38) analysis, *cel7D* transcript levels were relatively low in colonized wood (Fig. 3) and relatively high in Avicel-containing media.

In general, transcript levels of *cel9A* and *cbg1* were 10- to 100-fold lower than those of *cel7D* and *cel61A* when grown in defined media (11) containing 0.5% glucose and either 0.4% of a wood-derived crystalline cellulose (Avicel) (39) or 0.4% of a

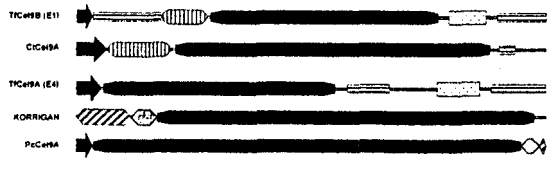

FIG. 1. Schematic representation of domain structures among selected family 9 glycosidases. TfCel9B, *T. fusca* Cel9B (previously E1): TfCel9A, *T. fusca* Cel9A (previously E4); CtCel9A, *C. thermocellum* Cel9A; KORRIGAN, *A. thaliana* Cel9; PcCel9A, *P. chrysosporium* Cel9A. Symbols: black arrow, signal peptide; horizontal striped box, carbohydrate binding module: vertical striped rounded box, immunoglobulin-like domain: black rounded box, = catalytic domain; stippled box, fibronectin; hexagon with diagonal lines, = cytoplasmic tail: gray hexagon, transmembrane region.

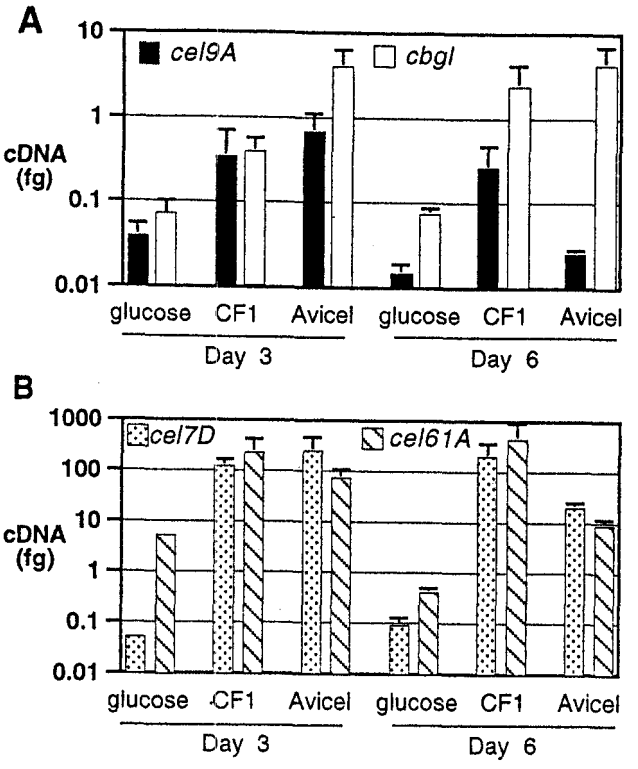

FIG. 2. Relative transcript levels of cellulase genes *cel9A* and *cbgI* (A) and *cel7D* and *cel61A* (B) in defined media containing 0.5% glucose and either Avicel or CF1 (see text for details). The ratios of adsorption, $(O/B)_{48}$, an indicator of pore structure and pore size population distribution (39), were 4.47 (Avicel) and 1.15 (CF1). [A larger $(O/B)_{48}$ correlates with a larger surface area and greater accessibility, while a smaller $(O/B)_{48}$ correlates with a smaller surface area and reduced accessibility (39)]. The cellulose azure test (7) indicated that cellulase activity began on day 3 in Avicel and CF1 cultures. Poly(A) RNA was isolated, and RT-cPCR was performed with competitive template amounts ranging from 10^{-11} to 10^{-20} g of plasmid/PCR, as described previously (31). The cDNA levels represent an indirect measure of transcripts in samples. For each gene and RNA sample combination, three separate cPCR dilution series were performed. Vertical error bars represent 1 standard deviation, and their absence indicates negligible variation.

more highly crystalline cellulose prepared from cotton linters (CF1) (Fig. 2) (3). Transcript patterns for *ce19A, cel7D,* and *cel61A* were roughly similar on media supplemented with CF1 or Avicel at day 3, suggesting that cellulose composition had relatively little effect on their regulation. With the exception of *cel9A* transcripts in the day 6 Avicel sample. the genes appeared to be induced in cellulose- as opposed to glucose-supplemented media (Fig. 2). The decrease in *cel9A* transcripts in the day 6 Avicel sample relative to the day 3 Avicel sample was also apparent for *cel7D* and *cel61A,* but not for *cbg1.* As is always the case when measuring steady-state levels, it is uncertain if the changes in transcript pattern reflect only substrate-dependent regulation or if differences in turnover rates play a role.

Transcript patterns on crystalline cellulose models show little resemblance to those on woody substrates (37), but the mechanisms of cellulose degradation and the identity of specific inducers are more readily elucidated in defined media. Consistent with an important role in cellulose degradation, *cel61A* transcript levels were relatively high in wood (Fig. 3), as well as in Avicel and CF1. However, in *T. reesei,* the corresponding *cel61A* gene encodes an enzyme (EGIV) with endoglucanase activity that is substantially lower than that of Cel7B (EGI), and the role of *T. reesei* Cel61A remains obscure (19). Future investigations will shed more light on the specific roles of these and possibly other as-yet-undiscovered cellulases in *Phanerochaete* spp. In particular. the recent completion of the mapping of the *P. chrysosporium* genome offers opportunities to dissect extracellular enzyme systems, both hydrolytic and oxidative, which are coordinately involved in lignocellulose degradation. In this connection, simple BLAST analyses of the genome reveal structurally related endoglucanase-like sequences distributed among several glycosyl hydrolase families (data not shown). In contrast. the *cel9A* sequence is unique. The increase in *cel9A* transcripts on cellulose and the similarity of its sequence to those of bacterial and insect endoglucanases suggest a role in cellulase degradation. However, the peculiar substrate binding site and the probable membrane association of *cel9A* imply that this enzyme has a very specialized role.

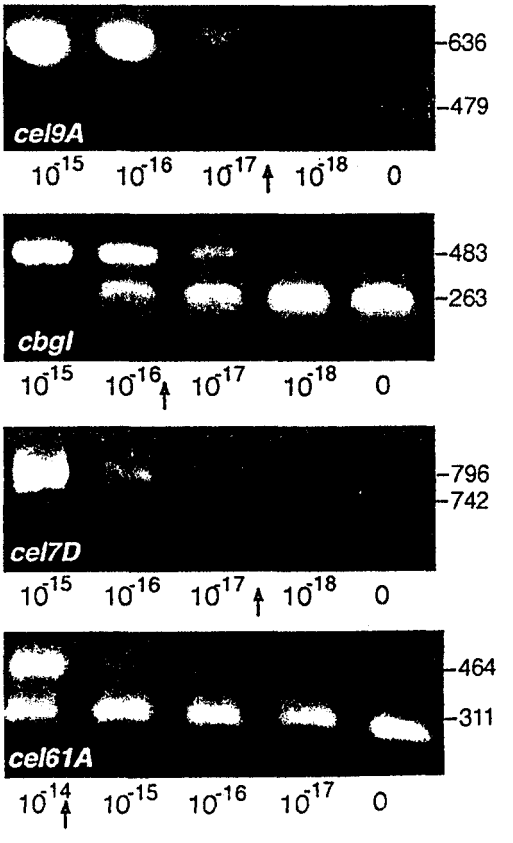

FIG. 3. RT-cPCR quantification of cDNAs from wood chips after 2 weeks of biopulping. cDNA levels (in grams) are indicated on the horizontal axis, and the lane labeled "0" was loaded with a PCR amplification mixture containing no competitive template. Vertical arrows show approximate equivalence points. Thus, *cel61A* cDNA levels are approximately 1,000-fold greater than *cel7D* cDNA levels. The sizes of competitive template and cDNA targets are indicated on the right in base pairs.

Nucleotide sequence accession numbers. The nucleotide sequences of *P. chrysosporium cel61A* and *cel9A* cDNAs have been assigned GenBank accession numbers AY094489 and AY094488, respectively.

This research was supported by U.S. Department of Energy grant DE-FG02-87ER13712 to D.C. and the Knut and Alice Wallenberg Foundation, Stockholm, Sweden (S.D. and U.R.). This work was also under the auspices of the U.S. Department of Energy, Office of Biological and Environmental Research; the University of California, Lawrence Livermore National Laboratory (contract no. W-7405-Eng-48); and the Lawrence Berkeley National Laboratory (contract no. DE-AC03-76SF00098).

We thank David Wilson for useful comments on the manuscript.

REFERENCES

1. **Akhtar, M.** April 1997. Method for enhancing biopulping efficacy. U.S. patent 5,620,564.
2. **Armesilla, A. L., C. F. Thurston, and E. Yague.** 1994. CEL1: a novel cellulose binding protein secreted by *Agaricus bisporus* during growth on crystalline cellulose. FEMS Microbiol. Lett. **116:**293–299.
3. **Atalla, R. H., and J. C. Gast.** 1980. CNMR spectra of cellulose polymorphs. J. Am. Chem. Soc. **102:**3249–3251.
4. **Beguin, P., and J. P. Aubert.** 1994. The biological degradation of cellulose. FEMS Microbiol. Rev. **13:**25–58.
5. **Birch, P. R. J., P. F. G. Sims, and P. Broda.** 1995. Substrate-dependent differential splicing of introns in the regions encoding the cellulose binding domains of two exocellobiohydrolase I-like genes in *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **61:**3741–3744.
6. **Bogan, B. W., B. Schoenike, R. T. Lamar, and D. Cullen.** 1996. Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **62:**2381–2386.
7. **Broda, P., P. R. J. Birch, P. R. Brooks, and P. F. G. Sims.** 1995. PCR-mediated analysis of lignocellulolytic gene transcription by *Phanerochaete chrysosporium*: substrate-dependent differential expression within gene families. Appl. Environ. Microbiol. **61:**2358–2364.
8. **Covert, S., A. Vanden Wymelenberg, and D. Cullen.** 1992. Structure, organization and transcription of a cellobiohydrolase gene cluster from *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **58:**2168–2175.
9. **Covert, S. F., J. Bolduc, and D. Cullen.** 1992. Genomic organization of a cellulase gene family in *Phanerochaete chrysosporium*. Curr. Genet. **22:**407–413.
10. **Elkin, C. J., P. M. Richardson, H. M. Fourcade, N. M. Hammon, M. J. Pollard, P. Predki, T. Glavina, and T. L. Hawkins.** 2001. High-throughput plasmid purification for capillary sequencing. Genome Res. **11:**1269–1274.
11. **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.
12. **Eriksson, K.-E., and B. Pettersson.** 1975. Extracellular enzyme system utilized by the fungus *Sporotrichum pulverulentum (Chrysosporium lignorum)* for the breakdown of cellulose. 1. Separation, purification, and physio-chemical characterization of five endo-1,4-β-glucanases. Eur. J. Biochem. **51:**193–206.
13. **Ewing, B., L. Hillier, M. Wendl, and P. Green.** 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res. **8:**175–185.
14. **Gilliland, G., S. Perrin, and H. Bunn.** 1990. Competitive PCR for quantitation of mRNA, p. 60–69. *In* M. Innis, D. Gelfand, J. Sninsky, and T. White (ed.), PCR protocols. Academic Press, Inc., New York, N.Y.
15. **Henriksson, G., A. Nutt, H. Henriksson, B. Pettersson, J. Stahlberg, G. Johansson, and G. Pettersson.** 1999. Endoglucanase 28 (Cel12A), a new *Phanerochaete chrysosporium* cellulase. Eur. J. Biochem. **259:**88–95.
16. **Henrissat, B., and A. Bairoch.** 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. **293:**781–788.
17. **Henrissat, B., T. T. Teeri, and R. A. Warren.** 1998. A scheme for designating enzymes that hydrolyse the polysaccharides in the cell wall of plants. FEBS Lett. **425:**352–354.
18. **Janse, B. J. H., J. Gaskell, M. Ahktar, and D. Cullen.** 1998. Expression of *Phanerochaete chrysosporium* genes encoding lignin peroxidases, manganese peroxidases, and glyoxal oxidase in wood. Appl. Environ. Microbiol. **64:**3536–3538.
19. **Karlsson, J., M. Saloheimo, M. Siika-Aho, M. Tenkanen, M. Penttila, and F. Tjerneld.** 2001. Homologous expression and characterization of Cel61A (EG IV) of *Trichoderma reesei*. Eur. J. Biochem. **268:**6498–6507.
20. **Kirk, T. K., and D. Cullen.** 1998. Enzymology and molecular genetics of wood degradation by white-rot fungi, p. 273–308. *In* R. A. Young and M. Akhtar (ed.), Environmentally friendly technologies for the pulp and paper industry. John Wiley and Sons, New York, N.Y.
21. **Krogh, A., B. Larsson, G. von Heijne, and E. L. L. Sonnhammer.** 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. **305:**567–580.
22. **Li, B., and V. Renganathan.** 1998. Gene cloning and characterization of a novel cellulose-binding β-glucosidase from *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **64:**2748–2754.
23. **Nicol, F., I. His, A. Jauneau, S. Vernhettes, H. Canut, and H. Hofte.** 1998. A plasma membrane-bound endo-1–4-β-glucanase is required for normal cell wall assembly and cell elongation in *Arabidopsis*. EMBO J. **19:**5563–5576.
24. **Peng, L., Y. Kawagoe, P. Hogan, and D. Delmer.** 2001. Sitosterol-β-glucoside as primer for cellulose synthesis in plants. Science **295:**147–150.
25. **Sakon, J., D. Irwin, D. Wilson, and P. A. Karplus.** 1997. Structure and mechanism of endo/exocellulase E4 from *Thermomonospora fusca*. Nat. Struct. Biol. **4:**810–818.
26. **Sali, A., and T. L. Blundell.** 1993. Comparative protein modelling by satisfaction of spatial restraints. J. Mol. Biol. **234:**779–815.
27. **Saloheimo, M., T. Nakari-Stala, M. Tenkanen, and M. Penttila.** 1997. cDNA cloning of a *Trichoderma reesei* cellulase and demonstration of endoglucanase activity by expression in yeast. Eur. J. Biochem. **249:**584–591.
28. **Sims, P., C. James, and P. Broda.** 1988. The identification, molecular cloning and characterisation of a gene from *Phanerochaete chrysosporium* that shows strong homology to the exo-cellobiohydrolase I gene from *Trichoderma reesei*. Gene **74:**411–422.
29. **Sims, P., M. Soares-Felipe, Q. Wang, M. Gent, C. Tempelaars, and P. Broda.** 1994. Differential expression of multiple exo-cellobiohydrolase I-like genes in the lignin-degrading fungus *Phanerochaete chrysosporium*. Mol. Microbiol. **12:**209–216.
30. **Steenbakkers, P. J., W. Ubhayasekera, H. J. Goossen, E. M. Van Lierop, C. Van Der Drift, G. D. Vogels, S. L. Mowbray, and H. J. Op Den Camp.** 2002. An intron-containing glycoside hydrolase family 9 cellulase gene encodes the dominant 90 kDa component of the cellulosome of the anaerobic fungus Piromyces sp. strain E2. Biochem. J. **365:**193–204.
31. **Stewart, P., and D. Cullen.** 1999. Organization and differential regulation of a cluster of lignin peroxidase genes of *Phanerochaete chrysosporium*. J. Bacteriol. **181:**3427–3432.
32. **Stewart, P., J. Gaskell, and D. Cullen.** 2000. A homokaryotic derivative of a *Phanerochaete chrysosporium* strain and its use in genomic analysis of repetitive elements. Appl. Environ. Microbiol. **66:**1629–1633.
33. **Tempelaars, C. A. M., P. R. J. Birch, P. F. G. Sims, and P. Broda.** 1994. Isolation, characterization, and analysis of the expression of the *cbhII* gene of *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **60:**4387–4393.
34. **Uzcategui, E., G. Johansson, B. Ek, and G. Pettersson.** 1991. The 1,4-D-glucan glucanohydrolases from *Phanerochaete chrysosporium*. Re-assessment of their significance in cellulose degradation mechanisms. J. Biotechnol. **21:**143–160.
35. **Uzcategui, E., M. Raices, R. Montesino, G. Johansson, G. Pettersson, and K.-E. Eriksson.** 1991. Pilot-scale production and purification of the cellulolytic enzyme system from the white-rot fungus *Phanerochaete chrysosporium*. Biotechnol. Appl. Biochem. **13:**323–334.
36. **Uzcategui, E., A. Ruiz, R. Montesino, G. Johansson, and G. Pettersson.** 1991. The 1,4-β-D-glucan cellobiohydrolase from *Phanerochaete chrysosporium*. I. A system of synergistically acting enzymes homologous to *Trichoderma reesei*. J. Biotechnol. **19:**271–286.
37. **Vallim, M. A., B. J. H. Janse, J. Gaskell, A. A. Pizzirani-Kleiner, and D. Cullen.** 1998. *Phanerochaete chrysosporium* cellobiohydrolase and cellobiose dehydrogenase transcripts in wood. Appl. Environ. Microbiol. **64:**1924–1928.
38. **Vanden Wymelenberg, A., S. Covert, and D. Cullen.** 1993. Identification of the gene encoding the major cellobiohydrolase of the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **59:**3492–3497.
39. **Yu, X., and R. H. Atalla.** 1998. A staining technique for evaluating the pore structure variations of microcrystalline cellulose powders. Powder Technol. **98:**135–138.