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Erratum

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Genomic organization of a cellulase gene family in *Phanerochaete chrysosporium*

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On page 409 of the paper starting on page 407 of Volume 22 there were unfortunately several errors in Fig. 2 of the following article. The figure is reprinted below.

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GATCACACCCTGGGGGTACGTTGATGAGCTTGGCATCAGGAGCCTTCGTC   50
TATTTCCTCCTTCGGGCCCGTGGGGCAGACGGTTCTCATATTCTCGGAAG   100
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CGTCGGCAGCCTGCACGAGGCGAGCACGGTCATGAGCACCGTGGATAAAC   250
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CGCTTCAGGGAGTGAGTATAAAGGCTGCATCCACAGCGCTGCTAATGACC   450
AGGTCGCTCCAAGCATCCTTCACAAGGTTACCCGGTCTACTGTACGCACT   500
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CATGTTCCGCGCCGCCGCACTCCTCGCTTTCACTTGTCTCGCCATGGTGT   600
 M  F  R  A  A  A  L  L  A  F  T  C  L  A  M  V  S
CTGGCCAACAGGCTGGCACCAACACGGCGGAGAACCACCCCCAGCTCCAG   650
  G  Q  Q  A  G  T  N  T  A  E  N  H  P  Q  L  Q
TCGCAGCAGTGCACGACGAGCGGCGGCTGCAAGCCGTTGAGCACGAAGGT   700
S  Q  Q  C  T  T  S  G  G  C  K  P  L  S  T  K  V
CGTCCTCGACTCGAACTGGCGCTGGGTCCACAGCACCTCGGGCTACACCA   750
 V  L  D  S  N  W  R  W  V  H  S  T  S  G  Y  T  N
ACTGCTACACCGGCAACGAGTGGGAC......(231)......TGCGGTC   800
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AAGTACCCCGGAAACAAGGCTGGTGCCAAGTACGGAACTGGTTACTGCGA   900
K  Y  P  G  N  K  A  G  A  K  Y  G  T  G  Y  C  D
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 S  Q  C  P  K  D  I  K  F  I  N  G  E
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                                             A  N
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V  G  N  W  T  E  T  G  S  N  T  G  T  G  S  Y  G
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 T  C  C  S  E  M  D  I  W  E  A  N  N  D  A  A  A
CTTTCACTCCCCACCCTTGCACCACCACCGGTCAGACCCGTTGCTCTGGG   1150
  F  T  P  H  P  C  T  T  T  G  Q  T  R  C  S  G
GATGACTGCGCGCGTAACACCGGTCTTTGCGACGGTGACGGCTGCGATTT   1200
D  D  C  A  R  N  T  G  L  C  D  G  D  G  C  D  F
CAACTCGTTCCGCATGGGTGACAAGACCTTCCTCGGCAAGGGGATGACCG   1250
 N  S  F  R  M  G  D  K  T  F  L  G  K  G  M  T  V
TCGACACCTCCAAGCCCTTCACCGTCGTCACCCAGTTCCTGACCAACGAC   1300
  D  T  S  K  P  F  T  V  V  T  Q  F  L  T  N  D
AACACCTCCACCGGCACGCTCTCTGAGATC  1330
N  T  S  T  G  T  L  S  E  I

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CCAAATTGTCGGCCCAAAATCGCCAGGGTGAAGTCGCTGTTTATCCGCTG   150
AACTCGTGCGCTGTCCACGAAGAGTGTCGCAGCGGGATGTCTCTGACATG   200
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AGGGTTACAGCTCAGAGCACCAGTACTCTCTTGCAAAGCAGCTCCCCCAG   300
AGCAACGGTCTCCCGGGTAAGTGTGTATTCGAGTGTAGCTATTACAATAC   350
TGATTTGGTCTCCAGCAAAATGTTCCGCGCTTCAGCACTCCTCGCGTTCT   400
                     M  F  R  A  S  A  L  L  A  F  S
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  F  A  A  I  A  Y  G  Q  Q  A  G  T  Q  T  A  E
ACCCACCCTCAGCTCACATCCCAGAAGTGCACGACCAGCGGCGGCTGCGT   500
T  H  P  Q  L  T  S  Q  K  C  T  T  S  G  G  C  V
CACCCAGGACACGACCGTCGTGCTCGACTCCAACTGGCGCTGGCTGCACT   550
 T  Q  D  T  T  V  V  L  D  S  N  W  R  W  L  H  S
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  V  Q  G  Y  T  N  C  Y  T  G  N  E  W  D
(273)......GACGGTGGCATGTCCAAGTTCCCCAACAACAAGGCTGGT   650
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 F  I  N  G  E

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                      A  N  V  E  G  W  N  A  T  S
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 A  N  A  G  T  G  N  Y  G  T  C  C  S  E  M  D  I
TCTGGGAGGCGAACAACGATGCTGCTGCCTTCACTCCCCACCCCTGCACC   900
  W  E  A  N  N  D  A  A  A  F  T  P  H  P  C  T
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T  D  G  Q  T  R  C  S  G  D  D  C  A  R  D  T  G
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 L  C  D  A  D  G  C  D  F  N  S  F  R  L  G  N  T
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  T  F  L  G  K  G  M  T  V  D  T  S  K  P  F  T
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V  V  T  Q  F  L  T  N  D  N  T  S  T  G  T  L  S
CGAGATC  1107
 E  I

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                M  F  R  K  V  A  L  I  A  F  T  L
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  N  T  K  I  V  L  D  A  N  W  R  W  L  H  S  T
TCGGGCTACACCAACTGCTACACTGGCAACGAGTGGGACGCGACGCTCTG   500
S  G  Y  T  N  C  Y  T  G  N  E  W  D  A  T  L  C
CCCCGACGGCGCGACGTGCGCGGCCAACTGCGCGCTCGACGGC.......   550
 P  D  G  A  T  C  A  A  N  C  A  L  D  G
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cagGCAAACGTCGAGGGATGGACTGGCACCAGCGCCAACGCAGGCACCGG   750
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ACGCCGCGGCGTTCACCCCGCACCCGTGCACGACCACCGGCCAGACGCGC   850
  A  A  A  F  T  P  H  P  C  T  T  T  G  Q  T  R
TGCTCGGGCGACGACTGCGCCCGGGACACCGGCCTGTGCGACGCCGACGG   900
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CTGCGACTTCAACTCCTTCCGCATGGGCAACCAGACGTTCCTCGGCAAGG   950
 C  D  F  N  S  F  R  M  G  N  Q  T  F  L  G  K  G
GCCTCACCGTTGACACCAGCAAGCCGTTCACGGTCGTCACCCAGTTCCTG   1000
  L  T  V  D  T  S  K  P  F  T  V  V  T  Q  F  L
ACGAACGACAACACGACGACGGGCACGCTCAGCGAGATC  1039
T  N  D  N  T  T  T  G  T  L  S  E  I
```

Fig. 2. Partial nucleotide and deduced amino-acid sequences of representatives of cosmid Groups 2b (*upper*), 3 (*middle*), and 4 ***(lower).*** *Dots* represent regions which have not been sequenced, and ***numbers*** *in parenthesis* indicate estimated distance in nucleotides between the two segments. Introns are in *lower case letters*. Putative promoter elements are *underlined.* Nucleotides 1067–1116, 844–933, and 776–825 of Groups 2b, 3 and 4, respectively, encode 17 amino acids which are indentical to the CBHI peptide sequence of Uzcategui et al. (1991). EMBL accession numbers are Z11733/Z11726 (2b); Z11730/Z11727 (3); and Z11731/Z11729 (4)

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Genomic organization of a cellulase gene family in *Phanerochaete chrysosporium*

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Summary. Southern blot and nucleotide sequence analysis of *Phanerochaete chrysosporium* BKM-F-1767 genomic clones indicate that this wood-degrading fungus contains at least six genes with significant homology to the *Trichoderma reesei* cellobiohydrolase I gene (*cbh1*). Using pulsed-field gel electrophoresis to separate *P. chrysosporium* chromosomes, the six cellulase genes were found to hybridize to at least three different chromosomes, one of which is dimorphic. The organization of these genes was similar in another *P. chrysosporium* strain, ME 446. It is clear that, unlike *T. reesei,* the most well-studied cellulolytic fungus, *P. chrysosporium* contains a complex, *cbh1*-like gene family.

Key words: Gene family – Cellulase genes – Cellobiohydrolases – Clamped homogeneous electric field

Introduction

The wood-rotting basidiomycete *Phanerochaete chrysosporium* has been studied widely because of its ability to degrade lignocellulose (reviewed by Kirk and Farrell 1987, Kirk 1988). Interest in *P. chrysosporium* has expanded due to potential applications of wood-degrading fungi and their extracellular enzymes in the paper industry (Kirk and Chang 1990), and in the detoxification of xenobiotics (Hammel 1989, 1992). The ability to control the cellulolytic activity of this fungus will play a pivotal role in the development of some of these processes (Eriksson and Kirk 1985; Kirk and Chang 1990).

Three classes of hydrolytic cellulases are secreted by *P. chrysosporium.* Five endoglucanases (E.C.3.2.1.4.) (Eriksson and Pettersson 1975a), as many as six cellobiohydrolases (E.C.3.2.1.91.) (Eriksson and Pettersson 1975b; Uzcategui et al. 1991; Uemura et al. 1992; Ishihara, Forestry and Forest Products Institute, Japan, personal communication), and two ß-glucosidases (E.C.3.2.1.21) (Deshpande et al. 1978) have been purified from *P. chrysosporium* cultures. Similar to other fungal cellulases, *P. chrysosporium* endoglucanases and cellobiohydrolases are reported to act synergistically on crystalline cellulose (Streamer et al. 1975; Uzcategui et al. 1991), and papain cleavage of cellobiohydrolases separates the catalytic domain from a cellulose-binding domain (Johansson et al. 1989; Uzcategui et al. 1991).

Compared to that of *T. reesei,* the molecular genetics of the *P. chrysosporium* cellulolytic system is poorly understood. Four cellulase genes, encoding cellobiohydrolases I and II (CBHI and CBHII) and endoglucanases I and III (EGI and EGIII), have been cloned and characterized from *T. reesei* (Shoemaker et al. 1983; Teeri et al. 1983; Penttila et al. 1986; Chen et al. 1987; Teeri et al. 1987; Van Arsdell et al. 1987; Saloheimo et al. 1988). Although the overall deduced amino-acid sequences of these genes are quite different, they all encode a highly conserved terminal domain (Teeri et al. 1987) that mediates the binding of cellulases to crystalline cellulose (Van Tilbeurgh et al. 1986; Stahlberg et al. 1988; Tomme et al. 1988; Johansson et al. 1989). Recently, three genes with significant homology to the *T. reesei* CBHI gene (*T.r.-cbh1*) have been cloned from *P. chrysosporium.* Designated *P.c.cbh1-1, P.c.cbh1-2* (Covert et al. 1992; GenBank X54411), and *P.c.cbh1-3* (Sims et al. 1988), these genes are clustered together within a 19 kb region (Covert 1990).

The role and interactions of individual *P. chrysosporium* genes in wood degradation are unknown. Toward a better understanding of this system, we have characterized a family of cellobiohydrolase genes and mapped their chromosomal locations.

Materials and methods

Isolation and characterization of cbh1-like clones. Standard Southern blot and cloning techniques were used throughout (Taub and

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Thompson 1982; Church and Gilbert 1984; Sambrook et al. 1989). Genomic DNA was isolated from *P. chrysosporium* BKM-F-1767 (ATCC 24725) and a genomic library composed of approximately 5700 members was constructed in cosmid pWE15 (Stratagene, La-Jolla, Calif.). The library was probed under conditions of low stringency (0.125 M Na_2HPO_4, 35% formamide, 7% sodium dodecyl sulfate, 1 mM EDTA at 37°C) with a 520 bp *Bam*HI fragment from *P.c.cbh1-1* (GenBank X54411). The probe was derived from a region of *P.c.cbh1-1* which is well-conserved relative to other *cbh1*-like genes. Positive clones were classified into related groups by probing with the *P.c.cbh1-1* fragment as well as a 700 bp *Eco*RI fragment from *T.r.cbh1* (provided by Genencor, South San Francisco, Calif.). The generation of end-specific RNA probes was as described (Wahl et al. 1987). Bluescript pKS (Stratagene) subclones of the cosmids were sequenced in their double-stranded form by the dideoxy chain-termination method (Sanger et al. 1977; Kraft et al. 1988).

Chromosomal location of cbh1-like genes. Clamped homogeneous electric field (CHEF) electrophoresis was performed on a BioRad CHEFII apparatus as described by Chu et al. (1986). Plug preparations, run conditions, and blotting were all as previously described (Gaskell et al. 1991). Cosmid clones were nick-translated to approximately 1×10^8 dpm/µpg. Hybridizations were at 48°C in 50% formamide, 7% sodium dodecyl sulfate, 0.125 M Na_2HPO_4, 1 mM EDTA. Washes were at 48°C in 0.125 M Na_2HPO_4, 2% sodium dodecyl sulfate. Allelic relationships of cloned genes were established by CHEF analysis of single-basidiospore cultures, which are homokaryotic (Alic et al. 1987; Thompson and Broda 1987). After fruiting (Gold and Cheng 1979) and germination, individual *P. chrysosporium* BKM-1767 basidiospores were isolated from agar plates using a needle and dissecting microscope. Single basidiospore cultures numbered 2 and 10 were previously used to identify lignin peroxidase alleles (Gaskell et al. 1991, 1992). *Aspergillus nidulans* strain 324 (*yA2; wA3; methH2, argB2, galA1, ivoA1, sC12*) DNA was used as a size marker (Brody and Carbon 1989) and *P. chrysosporium* ME 446 (ATCC 34541) DNA for comparision.

Results

Isolation and characterization of cbhl-like clones

When a P. chrysosporium library was probed at low stringency with a 520 bp *Bam*HI fragment from *P.c.cbh1-1*, 25 cosmids hybridized. One clone could not be recovered from the library and a second clone contained an insert composed of non-contiguous DNA. To distinguish the number of unique genes among the remaining 23 cosmids, a series of Southern blots using a variety of enzymes (*Bam*HI, *Eco*RI, *Not*I, *Hinc*II, *Pst*I, *Sau*3A and *Alu*I) were probed with either the 520 bp *Bam*HI fragment from *P.c.cbh1-1* or a 700 bp *Eco*RI fragment from *P.c.cbh1* (Shoemaker et al. 1983). As illustrated in Fig. 1, the cosmids could then be categorized into four groups of related clones, each appearing to represent a different genetic locus with homology to *P.c.cbh1*. ^{32}P-labeled transcripts were synthesized from the bacteriophage T3 and T7 promoters which flank cosmid pWE15 inserts (Wahl et al. 1987). These end-specific RNA probes were generated from representatives of each group and used to screen all 23 *cbh1*-like clones. The results supported the classification described in Fig. 1, i.e. probes from a Group 3 representative hybridized exclusively to other Group 3 members (data not shown). This indicates that the different groups do not represent closely linked or overlapping clones.

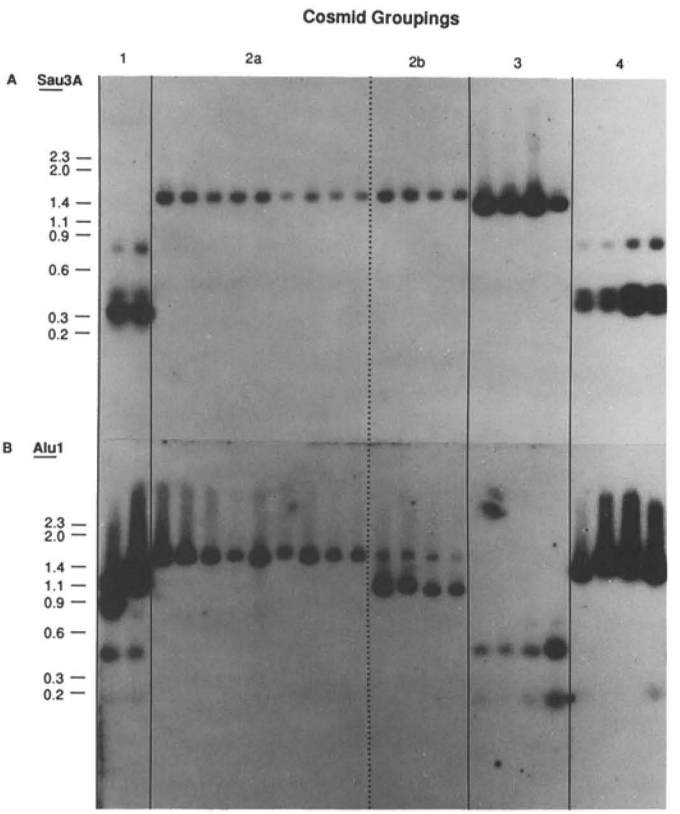

Fig. 1A, B. Twenty-three *P. chrysosporium* cosmid clones with homology to *T.r.cbh1* fall into four related groups. Southern blots of clones cut with either *Sau*3A *(panel A)* or *Alu*I (*panel B*) and probed with a 700 bp *Eco*RI fragment from *T.r.cbh1* at low stringency. *Panel A* was exposed for 8.5 h, *panel B* for 15 h. *Vertical lines* delineate related clones. *Number at top* denote group names. The two Group 1 cosmids give different patterns when digested with *Alu*I because the *cbh1*-like region on the second cosmid is at the extreme end of the insert and is therefore interrupted by the vector. The *upper band* in the Group 2b samples cut with *Alu*I is a partial digestion product. Size markers at left are in kilobase pairs

Group 2 was divided into two subgroups, 2a and 2b, on the basis of a polymorphism with *Alu*I (Fig. 1). This was the only restriction site polymorphism observed within the group and is likely to have resulted from allelic variation, not from two separate loci. Southern analysis of homokaryotic basidiospore DNA probed with Group 2a and 2b cosmids showed segregation of 2a and 2b loci into separate basidiospores thereby indicating allelism (data not shown).

When cut with *Bam*HI the two Group 1 clones resemble a previously isolated clone that contains two cbhl-like genes (*P.c.cbh1-1* and *P.c.cbh1-2*). Probing the Group 1 cosmids with a fragment from *P.c.cbh1-1* confirmed this relationship, but also indicated the presence of a third *P.c.cbh1*-like region (*P.c.cbh1-3*). A detailed analysis of the structure and transcriptional regulation of this cluster is presented elsewhere (Covert et al. 1992). The remaining three cosmid groups appear to contain only one cbhl-like region.

```
GATCACACCCTGGGGGTACGTTGATGAGCTTGGCATCAGGAGCCTTCGTC   50
TATTTCCTCCTTCGGGCCCGTGGGGCAGACGGTTCTCATATTCTCGGAAG  100
CCTCTTTCAACGACGGTCAGCACGTTTGCCTGGCTTGCGCGCGACGAGCC  150
GTTTCTCACTGTCAGGTTTACCCGCAGAAACGCAGGTGCGCGCTGCGCCT  200
CGTCGGCAGCCTGCACGAGGCGAGCACGGTCATGAGCACCGTGGATAAAC  250
ATTCCATTGGAGTACGAAGCTGCGCCCGGCCGCCTCGAAATTGTCGGGGC  300
AAGTACTCGGTGCAGCCGCTGCAATTGTCCGTCTTGGCTCATGGGACCAC  350
CTTCATCGCGACGGCGAGACACAGAGTAAAGCCGTGATGGTCGAACAGAC  400
CGCTTCAGGGAGTGAGTATAAAGGCTGCATCCACAGCGCTGCTAATGACC  450
AGGTCGCTCCAAGCATCCTTCACAAGGTTACCCGGTCTACTGTACGCACT  500
TCTACTCAGAGTTGGAATCTAGAACTGACGACTTGCCTTGCAGTTGTCCC  550
CATGTTCCGCGCCGCCGCACTCCTCGCTTTCACTTGTCTCGCCATGGTGT  600
 M  F  R  A  A  A  L  L  A  F  T  C  L  A  M  V  S
CTGGCCAACAGGCTGGCACCAACACGGCGGAGAACCACCCCCAGCTCCAG  650
 G  Q  Q  A  G  T  N  T  A  E  N  H  P  Q  L  Q
TCGCAGCAGTGCACGACGAGCGGCGGCTGCAAGCCGTTGAGCACGAAGGT  700
S  Q  Q  C  T  T  S  G  G  C  K  P  L  S  T  K  V
CGTCCTCGACTCGAACTGGCGCTGGGTCCACAGCACCTCGGGCTACACCA  750
 V  L  D  S  N  W  R  W  V  H  S  T  S  G  Y  T  N
ACTGCTACACCGGCAACGAGTGGGAC......(231)......TGCGGTC  800
  C  Y  T  G  N  E  W  D                 C  G  L
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AAGTACCCCGGAAACAAGGCTGGTGCCAAGTACGGAACTGGTTACTGCGA  900
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```

```
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AGGAACTGACGCGTCGCGACAGACACAAAAGGCCTCACTCGGGTCTCCCA  150
GGTCAACAAACGGTCACCGGTCAAAAACTTCACTGGTGAGTCCGCCATTG  200
TGCATGGCTGTCGGTCGACGTGCCGCTAACCGAGTTGGACGCACACTGGC  250
AGACGGTTGCAAAAATGTTCCGCAAGGTCGCACTCATCGCATTCACGCTG  300
                M  F  R  K  V  A  L  I  A  F  T  L
GCCGCCATGGCCGTCGGCCAGCAGGTCGGCACGAACACGGCCGAGAACCA  350
A  A  M  A  V  G  Q  Q  V  G  T  N  T  A  E  N  H
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 P  T  L  T  S  Q  K  C  T  T  A  G  G  C  V  S  Q
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TCGGGCTACACCAACTGCTACACTGGCAACGAGTGGGACGCGACGCTCTG  500
S  G  Y  T  N  C  Y  T  G  N  E  W  D  A  T  L  C
CCCCGACGGCGCGACGTGCGCGGCCAACTGCGCGCTCGACGGC.......  550
 P  D  G  A  T  C  A  A  N  C  A  L  D  G
```

```
GATCTTCTTCTACGCCTGCTGCAGCTCACGGGGCCATGAATTTCTAGTGC   50
TGTAGTGCTCGCGCGAGGCCTTCTATCCTCATCTGTGTCGTCAAGAGCCT  100
CCAAATTGTCGGCCCAAAATCGCCAGGGTGAAGTCGCTGTTTATCCGCTG  150
AACTCGTGCGCTGTCCACGAAGAGTGTCGCAGCGGGATGTCTCTGACATG  200
GGGGTAGATGTGCGGAGAATTGGCAAAGGTATAAAGGCTCTGGGTCGCCG  250
AGGGTTACAGCTCAGAGCACCAGTACTCTCTTGCAAAGCAGCTCCCCCAG  300
AGCAACGGTCTCCCGGGTAAGTGTGTATTCGAGTGTAGCTATTACAATAC  350
TGATTTGGTCTCCAGCAAAATGTTCCGCGCTTCAGCACTCCTCGCGTTCT  400
                M  F  R  A  S  A  L  L  A  F  S
CTTTCGCTGCCATCGCGTACGGCCAACAGGCCGGCACGCAAACAGCAGAG  450
  F  A  A  I  A  Y  G  Q  Q  A  G  T  Q  T  A  E
ACCCACCCTCAGCTCACATCCCAGAAGTGCACGACCAGCGGCGGCTGCGT  500
T  H  P  Q  L  T  S  Q  K  C  T  T  S  G  G  C  V
CACCCAGGACACGACCGTCGTGCTCGACTCCAACTGGCGCTGGCTGCACT  550
 T  Q  D  T  T  V  V  L  D  S  N  W  R  W  L  H  S
CCGTGCAGGGCTACACCAACTGCTACACCGGCAATGAGTGGGAC......  600
  V  Q  G  Y  T  N  C  Y  T  G  N  E  W  D
(273)......GACGGTGGCATGTCCAAGTTCCCCAACAACAAGGCTGGT  650
            D  G  G  M  S  K  F  P  N  N  K  A  G
GCCAAGTACGGCACTGGCTACTGCGACTCGCAGTGCCCGCGGGACATCAA  700
A  K  Y  G  T  G  Y  C  D  S  Q  C  P  R  D  I  K
GTTCATTAACGGCGAGgtaagcttggtcatgttggacaaatcagccagtg  750
 F  I  N  G  E
ctcatctcgcgcttcccgtagGCGAACGTCGAGGGCTGGAACGCCACCAG  800
                      A  N  V  E  G  W  N  A  T  S
CGCGAACGCGGGTACTGGTAACTACGGCACCTGCTGCAGCGAGATGGATA  850
 A  N  A  G  T  G  N  Y  G  T  C  C  S  E  M  D  I
TCTGGGAGGCGAACAACGATGCTGCTGCCTTCACTCCCCACCCCTGCACC  900
  W  E  A  N  N  D  A  A  A  F  T  P  H  P  C  T
ACCGATGGCCAGACTCGCTGCTCCGGTGACGACTGTGCCCGTGACACGGG  950
T  D  G  Q  T  R  C  S  G  D  D  C  A  R  D  T  G
TCTCTGCGACGCCGACGGTTGCGACTTCAACTCCTTCCGTCTCGGCAACA  1000
 L  C  D  A  D  G  C  D  F  N  S  F  R  L  G  N  T
CGACGTTCCTCGGCAAGGGCATGACCGTCGACACCAGCAAGCCCTTCACC  1050
  T  F  L  G  K  G  M  T  V  D  T  S  K  P  F  T
GTCGTCACGCAGTTCCTGACGAACGACAATACCTCGACGGGCACGCTCAG  1100
V  V  T  Q  F  L  T  N  D  N  T  S  T  G  T  L  S
CGAGATC  1107
 E  I
```

```
(255).....GGCGCCAAGTACGGCACTGGCTACTGCGACTCGCAGTGCC   600
          G  A  K  Y  G  T  G  Y  C  D  S  Q  C  P
CGAAGGACATCAAGTTCATCAACGGCGAGgtgcgtgcaggcgcgatcgtt   650
  K  D  I  K  F  I  N  G  E
cgattgggtgttatcccccttgctgactcgtccttcccccgcacatccg   700
cagGCAAACGTCGAGGGATGGACTGGCACCAGCGCCAACGCAGGCACCGG   750
   A  N  V  E  G  W  T  G  T  S  A  N  A  G  T  G
CACCTACGGCACCTGCTGCAACGAGATGGACATCTGGGAGGCGAACAACG   800
 T  Y  G  T  C  C  N  E  M  D  I  W  E  A  N  N  D
ACGCCGCGGCGTTCACCCCGCACCCGTGCACGACCACCGGCCAGACGCGC   850
  A  A  A  F  T  P  H  P  C  T  T  T  G  Q  T  R
TGCTCGGGCGACGACTGCGCCCGGGACACCGGCCTGTGCGACGCCGACGG   900
C  S  G  D  D  C  A  R  D  T  G  L  C  D  A  D  G
CTGCGACTTCAACTCCTTCCGCATGGGCAACCAGACGTTCCTCGGCAAGG   950
 C  D  F  N  S  F  R  M  G  N  Q  T  F  L  G  K  G
GCCTCACCGTTGACACCAGCAAGCCGTTCACGGTCGTCACCCAGTTCCTG   1000
  L  T  V  D  T  S  K  P  F  T  V  V  T  Q  F  L
ACGAACGACAACACGACGACGGGCACGCTCAGCGAGATC  1039
T  N  D  N  T  T  T  G  T  L  S  E  I
```

Fig. 2. Partial nucleotide and deduced amino-acid sequences of representatives of cosmid Groups 2b (*upper*), 3 (*middle*), and 4 (*lower*). *Dots* represent regions which have not been sequenced, and *numbers in parenthesis* indicate estimated distance in nucleotides between the two segments. Introns are in *lower case letters.* Putative promoter elements are *underlined.* Nucleotides 1067 – 1116. 844–933, and 776–825 of Groups 2b, 3 and 4, respectively, encode 17 amino acids which arc indentical to the CBHI peptide sequence of Uzcategui et al. (1991). EMBL accession numbers are Z11733/Z11726 (2b); 211727 (3): and 211731 211729 (4)

Nucleotide sequences of cbhl-like clones

Cosmids representing Groups 2, 3 and 4 were subcloned for sequencing. DNA was subcloned from representatives of both alleles of Group 2, but because of the high degree of identity between their sequences only one example (2b) is presented (Fig. 2, upper panel). Two regions of each gene were sequenced; the *5′* end of the gene including up to 550 bp of the *5′* untranslated region, and a highly conserved region within the core of the gene that is split by an intron (Fig. 2). All *cbh1*-like genes cloned and sequenced from *P. chrysosporium* have contained an intron at this position (Sims et al. 1988; GenBank X54411). The three sequences presented in Fig. 2, and thus the three groups of clones, appear to represent separate genes, because their *5′* untranslated sequences and intron sequences differ substantially.

The nucleotide and deduced amino-acid sequences of this internal region (minus the intron sequences) are compared to each other, to previously isolated *P.c.cbh1* clones, to *T.r.cbh1,* and to the *T. reesei* endoglucanase I gene (*Tr.egl1*) in Table 1. Within this region, four *cbh1*-like genes from *P. chrysosporium* (*P.c.ccbh1-3,* Y21, RR12, V88) are strikingly similar (83.2–87.4% nucleotide identity; 90.4–94.8% amino-acid homology; Table 1). With amino-acid homologies ranging from 59.1 to 63.0%, they are all clearly related to *T.r.cbh1.* As expected they also demonstrate homology to *T.r.egl1,* but these similarities are consistently lower, in large part due to deletions in *T.r.egl1* relative to the *P.c.cbh1* genes.

The same four *P. chrysosporium* genes share a similar codon bias. For six amino acids, Cys, Glu, His, Lys, Phe, and Tyr, the preference for one codon over the other is absolute. Similar to the lignin peroxidase genes of *P. chrysosporium* (de Boer et al. 1987; Tien and Tu 1987;

Table 1. Regional nucleotide identities and amino-acid homologies among *P. chrysosporium cbh1*-like and selected *T. reesei* cellulase genes

Clone/gene		Deduced amino-acid homologies[a]							
		Y21[b]	RR12[b]	V88[b]	*P.c. cbh1*-1[c]	*P.c. cbh1*-2[c]	*P.c. cbh1*-3[d]	*T.r.cbh1*[e]	*T.r.egl1*[f]
Nucleotide identities[g]	Y21		90.5	90.4	79.5	71.4	86.9	62.0	47.2
	RR12	83.9		94.8	80.3	74.1	91.8	63.0	42.9
	V88	83.2	87.4		77.8	74.0	90.4	61.4	43.7
	P.c.cbh1-1	74.3	76.6	75.8		79.5	82.0	58.5	45.3
	P.c.cbh1-2	68.4	72.8	74.7	72.9		75.8	50.9	41.0
	P.c.cbh1-3	83.6	85.9	83.7	77.4	71.5		62.0	45.3
	T.r.cbh1	59.5	61.2	61.4	60.1	53.5	63.1		43.6
	T.r.egl1	51.0	52.7	53.5	49.7	50.5	55.1	47.7	

[a] Percentage of amino-acid homology within overlapping alignments calculated using the Dayhoff Table (Schwartz and Dayhoff 1979), threshold = 0.8. Complete sequences were compared within a region corresponding to residues 153–313of the *P.c.cbh1-3* (Sims et al. 1988) protein

[b] Cosmid clones partially sequenced in this manuscript: Y21 =Group 2; RR12 = Group 3; and V88 = Group 4. Nucleotide sequence corresponding only to an internal portion of the coding region was used to calculate the values in this table. Sequence derived from the 5'-end of the genes was not included (see Fig. 2)

[c] Data from Covert et al. 1992

[d] Data from Sims et al. 1988

[e] Data from Shoemaker et al. 1983

[f] Data from Penttila et al. 1986

[g] Percentage of identical nucleotides within overlapping alignments (introns excluded) as determined by Wilbur and Lipman (1983) using K-tuple of 3, window size of 20 and gap penalty of 3. Complete sequences were compared within a region corresponding to nucleotides 866–1348of *P.c.cbh1-3* (Sims et al. 1988)

Table 2. Comparison of nucleotide sequences around start codons of *P.c.cbh1* genes indicating the presence of a conserved ATG sequence in all genes

Locus	Sequence surrounding start codon
P.c.cbh1-1[a]	TAAATGGTCGATAT
P.c.cbh1-2[a]	CCTATGAGGACCGC
P.c.cbh1-3[b]	ACAATGTTCCGCAC
Group 2	CCCATGTTCCGCGC
Group 3	AAAATGTTCCGCGC
Group 4	AAAATGTTCCGCAA

[a] Covert 1990

[b] Sims et al. 1988

Schalch et al. 1989), codons ending in G or C are preferred. This is in marked contrast to *P.c.cbh1-1* and *P.c. cbh1-2* which are much less biased in their use of codons. The only similarity in this regard between these two genes and the four other *P.c.cbh1* genes is that *P.c.cbh1-1* also has an absolute preference for the Glu codon GAG. Furthermore, *P.c.cbh1-1* and *P.c.cbh1-2* differ structurally from the other genes in that they both contain an intron in their signal sequence (Covert 1990; GenBank X54411) and they lack a 14 bp sequence that is well conserved at the start codon of the other *P.c.cbh1* genes (Table 2). Filamentous fungal genes tend to have an A at the -3 position (Gurr et al. 1987), but this is the case in only three of the *P.c.cbh1* genes.

To summarize the restriction and sequence analyses, the *P.c.cbh1* gene family contains six members. Three are clustered together on the Group 1 clones. The other three are on the clones in Groups 2, 3 and 4, none of which appear to be clustered within the length of a cosmid insert.

Chromosomal organization of cbhl-like clones

P. chrysosporium chromosomes were separated on CHEF gels and probed with representatives of each of the four cosmid groups (Fig. 3). The Group 1 clone hybridized to two bands approximately 4.3–4.8 megabases in size, the Group 2 and Group 3 clones hybridized to a band less than 2.9 megabases in size, and the Group 4 clone hybridized to a third band, approximately 3.5–3.8 megabases in size. These results were reproduced when CHEF gel Southerns were probed with other group representatives. Both Group 1 cosmids, four Group 2, four Group 3, and two Group 4 cosmids were used as probes (data not shown). Although the Group 2 and Group 3 cosmids appear to hybridize to the same chromosome, the possibility of two co-migrating chromosomes can not be ruled out. Based on its intensity in Fig. 3, the Group 4 chromosome appears to overlap with at least one other chromosome.

The finding that the Group 2 and Group 3 cosmids appear to reside on the same chromosome raises the possibility that they are alleles. However, the low levels of identity between their 5′ untranslated (30%) and intron sequences (34%) argue against this idea. Furthermore, both loci were found through Southern analysis to segregate together into homokaryotic basidiospores, thus confirming that they are not alleles (data not shown).

Figure 4 demonstrates that the pair of bands to which the Group 1 cosmids hybridized are homologues of a single chromosome. When probed with a Group 1 cosmid, both bands are present in the dikaryotic parent (BKM-F-1767), but single bands are observed in the two homokaryotic basidiospores (Fig. 4). This segregation into basidiospores indicates that the Group 1 chromosome is dimorphic in this strain of *P. chrysosporium.*

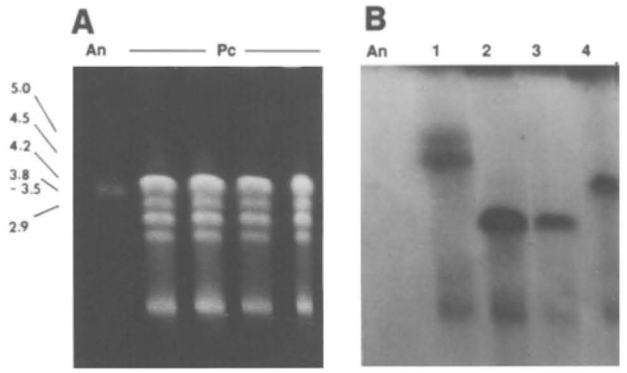

Fig. 3A,B. Chromosomal organization of *cbh1*-like genes in *P. chrysosporium, Panel A,* ethidium bromide-stained. CHEF gel of *A. nidulans* (*An*) and *P. chrysosporium* BKM-F-1767 (*Pc*) genomic DNA. The approximate sizes of the *A. nidulans* chromosomes are given in megabases (Brody and Carbon 1989). A Southern blot of this gel was cut into strips and probed with representatives of each cosmid group. An autoradiogram of the results is shown in *panel B. Numbers* indicate the groups to which the probes belong

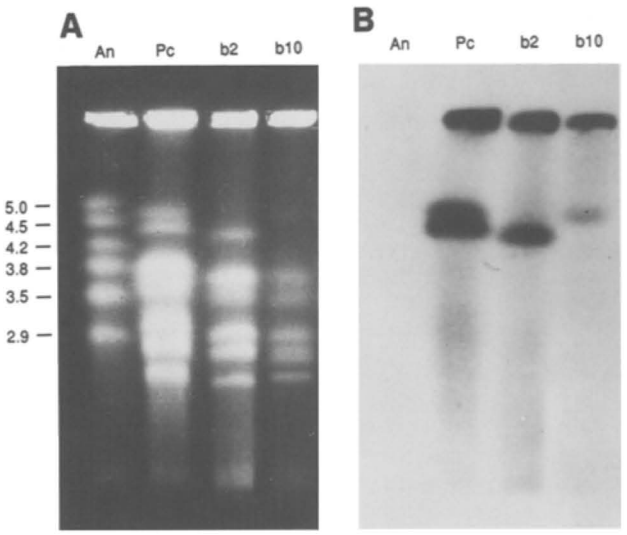

Fig. 4A,B. Group 1 cosmids are carried on a single dimorphic chromosome. *Panel A,* ethidium bromide-stained CHEF gel of DNA from *A. nidulans* (*An*), *P. chrysosporium* (*Pc*), and two homokaryotic strains derived from *P. chrysosporium* basidiospores (*b2 and b10*) *Panel B,* Southern blot of panel *A* probed with Group 1 cosmid O7. Approximate sizes of *A. nidulans* chromosomes are shown in megabases (Brody and Carbon 1989)

In order to determine if the *P.c.cbh1* gene family is organized similarly in a second strain of *P. chrysosporium,* DNA from strain ME 446 was separated on a CHEF gel and probed with representatives of each cosmid group. Figure 5 indicates that the electrophoretic karyotype of ME 446 as well as the chromosomal organization of the *P.c.cbh1* gene family in this strain, is very similar to that of BKM-F-1767. The only visible difference is that the Group 1 chromosome in ME446 is not dimorphic.

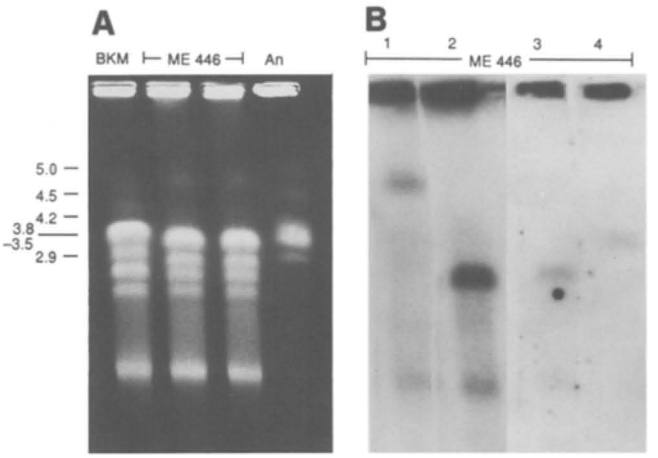

Fig. 5A, B. Chromosomal organization of *cbh1*-like genes in *P. chrysosporium* ME 446. *Panel A,* an ethidium bromide-stained CHEF gel of DNA from *P. chrysosporium* BKM-F-1767 (*BKM*), *P. chrysosporium* ME 446 (*ME 446*), and *A. nidulans* (*An*). Southern blots of lanes containing ME 446 DNA were cut into strips and probed with representatives of each cosmid group *Panel B,* the resulting autoradiogram. *Numbers* indicate the grpi[tp wjocj probes belong. signals in *lanes 3 and 4* are weaker than those of *1 and 2* because probes were held for 8 days aftger nick translation while blots were probed with Group 1 and Group 2 cosmids. Approximate sizes of *A. nidulans* chromosomes are shown in megabases (Brody and Carbon 1989)

Discussion

P. chrysosporium contains a family of cbh1-like genes

Our analyses indicate that *P. chrysosporium* contains at least six *cbh1*-like loci. three of these genes have been cloned previously and designated *P.c.cbh1-1, P.c.cbh1-2,* and *P.c.cbh1-3* in order to distinguish them from each other as well as *T.r.cbh1.* We propose to extend this nomenclature by designating the clones in Groups 2, 3 and 4 as *P.c.cbh1-4, P.c.cbh1-5,* and *P.c.cbh1-6,* respectively. The multiplicity of cbh1-like genes in P. chrysosporium may partly explain previous difficultirs in obtaining cellulase-negative mutants that retained the ability to degrade lignin efficiently (Johnsrud and Eriksson 1985; Kirk et al. 1986).

The *P.c.cbh1* gene family can be divided into two subfamilies. Relative to the four other *P.c.cbh1* genes, *P.c. cbh1-1* and *P.c.cbh1-2* are distinguished by several characteristics: their homology to other *cbh1*-like genes tends to be lower (Table 1), they are much less biased in terms of codon preference, they do not contain the conserved 14 bp consensus sequence surrounding their translational start condons (Table 2), and they contain an intron within their signal sequences (GenBank X54411). They are also very closely linked to each other (GenBank X54411).

The partial amino-acid sequences of three *P. chrysosporium* CBHs have been reported (Uzcategui et al. 1991). A 17 amino-acid peptide from the dominant enzyme, CBHI, is identical to the deduced amino-acid sequences of *P.c.cbh1-4, P.c.cbh1-5,* and *P.c.cbh1-6* (Fig. 1). It is possible, therefore, that CBHI is actually

encoded by these three closely related genes. A second CBHI-like protein appears to be encoded by *P.c.cbh1-3* and the third CBH is analogous in structure to CBHII of *T. reesei* (Uzcategui et al. 1991).

A variety of fungal degradative enzymes, including a-amylase in *Aspergillus oryzae* (Tsukagoshi et al. 1989) and *Aspergillus niger* var. *awamori* (Korman et al. 1990), lignin peroxidases in P. chrysosporium (de Boer et al. 1987; Brown et al. 1988; Tien and Tu 1987; Schalch et al. 1989; Huoponen et al. 1990; Gaskell et al. 1991) and pectin lyase in A. niger (Harmsen et al. 1990), are encoded by multi-gene families. The cbhi-like gene family described here, however, contrasts directly with the cellulolytic system of *T. reesei*, which contains only one *cbh1* gene. Although such a large family of related cellulase genes is without precedent among fungi, bacterial endoglucanases have been found to be encoded by many genes. For example, *Clostridium thermocellum* has at least 15 and *Ruminococcus albus* has up to ten endoglucanase genes (see Beguin 1990, for review). Relative to the P. chrysosporium *cbh1*-like genes, sequence conservation among the C. thermocellum genes is low (Knowles et al. 1987; Gilkes et al. 1991). However, hydrophobic cluster analysis, which detects relationships between sequences of very low sequence identity, revealed that three members of the *C. thermocellum* gene family are in fact related (Henrissat et al. 1989). It is not clear why cellulolytic organisms might require multiple copies of functionally related genes. Perhaps slight differences in sequence confer subtle differences in substrate specificity. As a result of the enumeration and characterization of the *P.c.cbh1* genes, it is now possible, through antisense suppression, gene disruptions, and/or heterologous expression, to evaluate the contribution of individual gene products in wood degradation.

The cbh1-like gene family is encoded on at least three chromosomes

The *P.c.cbh1*-like genes are encoded on at least three separate chromosomes, one of which is dimorphic in strain BKM-1767. The electrophoretic karyotypes of the two P. chrysosporium strains analysed here are very similar. This is somewhat surprising in the light of the known physiological and genetic differences between BKM-1767 and ME446 (Jäger et al. 1985; Kirk et al. 1986; Alic et al. 1987; Thompson and Broda 1987; Tonon et al. 1990), as well as the increasing body of literature demonstrating chromosome polymorphisms between strains of other fungi (Mills and McCluskey 1990). For example, two strains of another wood-degrading basidiomycete, *Schizophyllum commune*, were found to exhibit multiple chromosomal length polymorphisms (Horton and Raper 1991).

Recent data indicates that P. chrysosporium lignin peroxidase gene, *GLG4*, is located on the same dimorphic chromosome as the *P.c.cbh1-1/P.c.cbh1-2/P.c.cbh1-3* cluster (Stewart et al. 1992). This linkage is consistent with an RFLP map of ME446 which predicts two lignin peroxidase gene clusters, one of which is linked to *P.c.-cbh1-3* (Raeder et al. 1989). In contrast to that of other lignin peroxidase genes, *GLG4* transcription can be activated by carbon limitation, as can the transcription of *P.c.cbh1-3* (Covert et al. 1992). The relationship, if any, between genomic organization and regulation of these genes remains to be established. These investigations will be greatly facilitated by the construction of chromosome-specific libraries, now possible using CHEF separation of chromosomal DNA.

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