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Original Paper

Molecular cloning of cDNAs encoding two glycoside hydrolase family 7 cellobiohydrolases from the basidiomycete *Flammulina velutipes*

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Abstract The basidiomycete *Flammulina velutipes* is one of the most popular edible mushrooms in Japan, and has the ability to grow on cellulosic biomass as a carbon source. In this study, we have isolated two enzymes belonging to glycoside hydrolase (GH) family 7 (*Fv*Cel7A and *Fv*Cel7B) from the cellulose-grown culture of the fungus, and cloned cDNAs encoding these enzymes by utilizing a transcriptomic database of this fungus. Although both enzymes contain a catalytic domain belonging to GH family 7, only *Fv*Cel7A has the family 1 carbohydrate-binding module at the C-terminal. Sequence comparison indicated that *Fv*Cel7A and *Fv*Cel7B have a similar pattern of disulfide bonds and similar active site architecture to other fungal GH family 7 enzymes, but show small differences at loop regions covering the active site, which may affect the reactivity of cellulosic substrates.

Key words: Biomass utilization, consolidated bioprocess, *Flammulina velutipes*, glycoside hydrolase.

The basidiomycete *Flammulina velutipes,* generally called Enoki-take or winter mushroom, is one of the most popular edible mushrooms in East Asian countries, and its production is over 100,000 tons a year (Chang 1993). This mushroom exhibits greater adaptability than other edible mushrooms for various kinds of substrates in artificial cultivation media (Royse 1996), suggesting that it may be useful in the conversion of various types of biomass. Furthermore, some basidiomycetes, including *F. velutipes,* produce alcohol dehydrogenase, and can be used to produce wine (Okamura et al. 2001). Therefore, since basidiomycetes have both enzyme production and alcohol fermentation ability, they are candidates for use in a consolidated bioprocess (CBP) to produce ethanol directly by fermentation following saccharification of cellulosic biomass. Indeed, it has been reported that *F. velutipes* utilizes not only glucose, but also cellobiose (β-D-glucosyl-1,4-D-glucose) as a fermentable sugar (Mizuno et al., 2009b). Effective production of cellulolytic and hemicellulolytic enzymes is required to develop a CBP for generating ethanol from cellulosic biomass (Mizuno et al. 2009a).

Commercial preparation of cellulases for the saccharification of cellulosic biomass has so far mainly utilized the cellulolytic ascomycete *Trichoderma reesei* (syn. *Hypocrea jecorina*) (Seidl et al. 2008; Viikari et al. 2007). In the cellulase cocktail obtained from the fungus, glycoside hydrolase (GH) family 7 cellobiohydrolase (*Tr*Cel7A), formerly known as cellobiohydrolase (CBH) I, accounts for more than 50% of total protein (Bhikhabhai et al., 1984). GH family 7 also includes endo-glucanase (EG)-type enzymes, and EG from *T. reesei* is named *Tr*Cel7B (formerly known as EGI). These enzymes have quite different hydrolytic features, i.e. *Tr*Cel7A effectively hydrolyzes crystalline cellulose (Teeri et al. 1998), whereas *Tr*Cel7B acts preferentially on amorphous or soluble substrates (Shoemaker and Brown 1978a; Shoemaker and Brown 1978b). However, when chromophoric disaccharides, such as *p*-nitrophenyl-β-D-cellobioside and *p*-nitrophenyl-β-D-

Abbreviations: AEC, anion-exchanging column; CBD, cellulose-binding domain; CBH, cellobiohydrolase; CBP, consolidated bioprocess; *Fv*, *Flammulina velutipes*; DEAE, diethylaminoethyl; GH, glycoside hydrolase; HIC, hydrophobic interaction column; *Pc*, *Phanerochaete chrysosporium*; *p*NPL, *p*-nitrophenyl-β-D-lactoside; RACE, rapid amplification of cDNA ends; *Tr*, *Trichoderma reesei*; YP, yeast extract and polypeptone

This article can be found at http://www.jspcmb.jp/

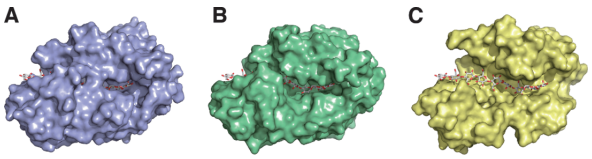


Figure 1. Structural comparison among GH family 7 cellulases. A, *Tr*Cel7A (PDB: 8CEL) (Divne et al. 1998); B, *Pc*Cel7D (PDB: 1GPI) (Muñoz et al. 2001); C, *Tr*Cel7B (PDB: 1EG1) (Kleywegt et al. 1997). The structures were superimposed and visualized using PyMol (ver. 0.99, DeLano Scientific LLC)

lactoside are used as substrates, both CBH- and EG-type enzymes in GH family 7 hydrolyze the glycosidic bond between the sugar and the chromophore (Deshpande et al. 1984), suggesting that these compounds can be used as general substrates of this enzyme family.

The crystal structures of several GH family 7 cellulases have been solved, and the difference of catalytic properties between CBH and EG has been discussed in terms of the different architectures of the catalytic sites (Divne et al. 1994; Divne et al. 1998; Kleywegt et al. 1997; Muñoz et al. 2001). Although the structure of the protein backbone is mostly conserved, the numbers and/or lengths of the loops covering the active site are quite different. *Tr*Cel7A has a rather closed tunnel-like active site, while *Tr*Cel7B lacks the loop over the active site, which consequently resembles a cleft, and the subsites are exposed to the solvent. Interestingly, GH family 7 cellulase from the basidiomycete *Phanerochaete chrysosporium* (*Pc*Cel7D) represents a hybrid form in which a half of the active site is covered by the loop peptides, as shown in Figure 1.

In the present study, we have examined the production of GH family 7 cellulases by *F. velutipes*, purified two Cel7s, cloned cDNAs encoding these enzymes, and compared the molecular architectures of *F. velutipes* GH family 7 enzymes and other fungal Cel7s.

Materials and methods

Cultivation of F. velutipes

F. velutipes wild-type strain Fv-1 was maintained on a yeast extract and polypeptone (YP)-based medium with trace elements (2.0 g L^{-1} polypeptone, 1.0 g L^{-1} yeast extract, 1.0 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 74 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 10 mg L^{-1} $FeSO_4 \cdot 7H_2O$, 5.0 mg L^{-1} $MnSO_4 \cdot 7H_2O$, 5.0 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 1.0 mg L^{-1} $CoCl_2 \cdot 6H_2O$, pH 5.0) containing 0.2% glucose and 1.5% agar at 26.5°C for 7 days. The mycelium on the plate was punched out and inoculated into 30 ml of YP-based medium containing 0.2% glycerol in a 500 ml Erlenmeyer flask. This was incubated as a pre-culture at 26.5°C for 7 days, and then the mycelium was homogenized using a Waring blender and inoculated into 200 ml of YP-based medium containing 2.0% cellulose powder (CF11; Whatman, Fairfield, NJ) in a 500 ml Erlenmeyer flask. The medium was incubated at 26.5°C with rotary shaking (ϕ108 mm, 150 rpm). After 7 days of cultivation, culture filtrate and mycelia were separated using a glass filter membrane (Advantec GA-100; Toyo Roshi Kaisha, Tokyo, Japan).

Isolation and identification of GH family 7 cellulases

The culture filtrate was concentrated 20-fold by using a stirred ultrafiltration device (Model 8050; Millipore, Billerica, MA) with a PM-10 ultrafiltration membrane (Millipore), and dialyzed against 20 mM potassium phosphate buffer, pH 7.0. After centrifugation at 5,000 *g*, the supernatant was applied to a DEAE-Toyopearl 650S column (7.5×170 mm; Tosoh, Tokyo, Japan) equilibrated with the same buffer. The protein was eluted with a linear gradient of KCl from 0 to 0.3 M in a volume of 1500 ml. Fractions were assayed for *p*-nitrophenyl-β-D-lactopyranoside (*p*NPL; Sigma-Aldrich) hydrolyzing activity (Igarashi et al. 1998). The active fractions were pooled and concentrated in a stirred ultrafiltration device (Millipore). The concentrated enzyme solution was dialyzed against 20 mM potassium phosphate buffer pH 7.0 containing 1.0 M ammonium sulfate, and applied to a Phenyl-Toyopearl 650S column (11×210 mm; Tosoh) equilibrated with the same buffer. The protein was eluted with a reversed gradient of ammonium sulfate from 1.0 to 0 M in a volume of 250 ml.

The protein concentration of the fractions containing *p*NPL-hydrolyzing activity was estimated using Protein Assay (Bio-Rad, Hercules, CA), and the purity of Cel7s was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12% polyacrylamide gel (Mini-Protean II; Bio-Rad). For determination of partial internal amino acid sequences, protein from each SDS-PAGE band was digested in the gel with *Staphylococcus aureus* V8 protease, and peptides were transferred to polyvinylidene difluoride membrane (Millipore) using a Trans-Blot SD cell (Bio-Rad). Peptide sequences were determined with a protein sequencer (model 491 cLC; Applied Biosystems, Foster City, CA) as described previously (Igarashi et al. 2003). Amino acid sequences thus obtained were subjected to BLAST search using the BLASTP program (version 2.2.22+) with the default settings.

Construction of F. velutipes *total cDNA database*

Homogenized *F. velutipes* mycelia obtained as described above were inoculated into 200 ml of YP-based media containing 2.0% rice straw powder, sugarcane bagasse powder, potato pulp, sugar beet pulp, or soft wood (*Cryptomeria japonica*, Japanese cedar) powder. The mycelia were also inoculated into YP-based media containing 0.2% hemicellulose (xylan from birch wood (Sigma-Aldrich, St. Louis, MO), xylan from larch wood (Sigma-Aldrich), xylan from rice straw, glucomannan from konjac tuber (Wako Pure Chemical Industries, Osaka, Japan), galactomannan from locust bean (Sigma-Aldrich), xyloglucan from tamarind seed (Tokyo Chemical Industry Co., Tokyo, Japan) or arabinogalactan prepared from larch wood according to Takabatake et al. 1994) in addition to 2.0% cellulose powder (Whatman). Each culture was incubated at 26.5°C with rotary shaking (ϕ108 mm, 150 rpm). After 3 days of cultivation, mycelia were collected using a glass filter membrane (Toyo Roshi Kaisha), and total RNA was extracted from approximately 100 mg of the frozen mycelia using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's

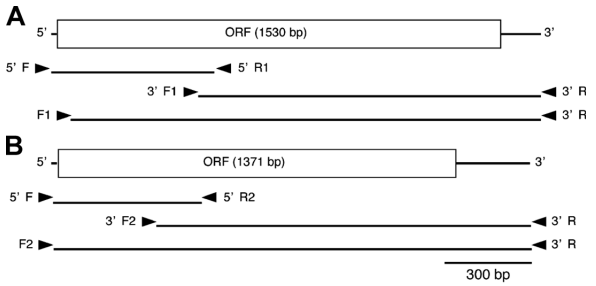
instruction. The total RNA thus obtained was applied to *de novo* Transcript Sequencing at Agencourt Bioscience Corporation (Beverly, MA). The results of a detailed analysis of the transcriptomic database will be reported elsewhere.

Cloning of cDNAs encoding Fv*Cel7A and* Fv*Cel7B*

First-strand cDNA was synthesized from mRNA, which was purified from 1 μg of total RNA obtained as described above on Oligotex-dT30 Super (Takara Bio, Shiga, Japan), using a GeneRacer^{TM} Kit with SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The oligonucleotide primers for amplification of a cDNA fragment encoding *Fv*Cel7A and *Fv*Cel7B were designed based on the total cDNA sequence as illustrated in Figure 2, and PCR was performed using KOD-Plus (version 2; Toyobo, Osaka, Japan) according to the manufacturer's instruction. Since abridged universal amplification primer was used as the reverse primer, the coding region and 3' untranslated region were amplified at the same time. The polynucleotides at the 5′ untranslated region were amplified using the gene-specific primers illustrated in Figure 2. All PCR products were cloned by using a Zero Blunt TOPO PCR cloning kit (Invitrogen) and *E. coli* JM109 and were sequenced by using a Thermo Sequenase primer cycle sequencing kit (GE Healthcare, Buckinghamshire, United Kingdom) with DNA sequencer model SQ5500E (Hitachi High Technologies, Tokyo, Japan). The nucleotide sequences have been submitted to the DDBJ/EMBL/GeneBank databases under accession numbers AB540999 and AB541000.

Sequence analysis

The presence of signal peptides and *N*-glycosylation and *O*-glycosylation sites was assessed using the SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/) (Bendtsen et al. 2004), NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/), and NetOGlyc 3.1 server (http://www.cbs.dtu.dk/services/NetOGlyc/) at the Center for Biological Sequence Analysis. Multiple alignment was performed with the aid of MAFFT (version 6; http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) (Katoh et al. 2005; Katoh et al. 2002) with the L-INS-i algorithm, and the sequence alignment with secondary structural information was constructed by use of the ESPript (http://espript.ibcp.fr/ESPript/ESPript/) (Gouet et al. 1999) with the crystal structure of *Tr*Cel7A (PDB: 1CEL) (Divne et al. 1994) as a template.



Primer	Sequence	Direction
5' F	5' -CGACTGGAGCACGAGGACACTGA- 3'	F
5' R1	5' -GAGTCGCAGTATCCGGTGCCGTA- 3'	R
3' F1	5' -TGAACGGCGCTCTGTACTTTGTGGA- 3'	F
3' R	5' -GCTGTCAACGATACGCTACGTAACG- 3'	R
F1	5' -CATGTTTAACAAAGTCGCCCTCCTC- 3'	F
5' R2	5' -GCCTCCGTCCTTGTCCATCTCGA- 3'	R
3' F2	5' -AGCGAAACATACGGCATCACCACCA- 3'	F
F2	5' -ATCGACGCCGAACGCAAACATGTTC- 3'	F

Figure 2. Schematic diagram of cDNAs encoding *Fv*Cel7A (A) and *Fv*Cel7B (B), showing locations and nucleotide sequences of primers used for PCR. F and R indicate the forward and reverse directions, respectively.

Results and discussion

F. velutipes is an edible mushroom widely cultivated in East Asian countries, including Japan. This fungus might be suitable for use in a consolidated bioprocess (CBP) to produce ethanol directly by fermentation following the production of enzymes and the saccharification of biomass, since it can utilize not only glucose, but also cellobiose as a fermentable sugar (Mizuno et al. 2009b). However, little is known about the cellulose-degrading enzymes produced by *F. velutipes*, which should be

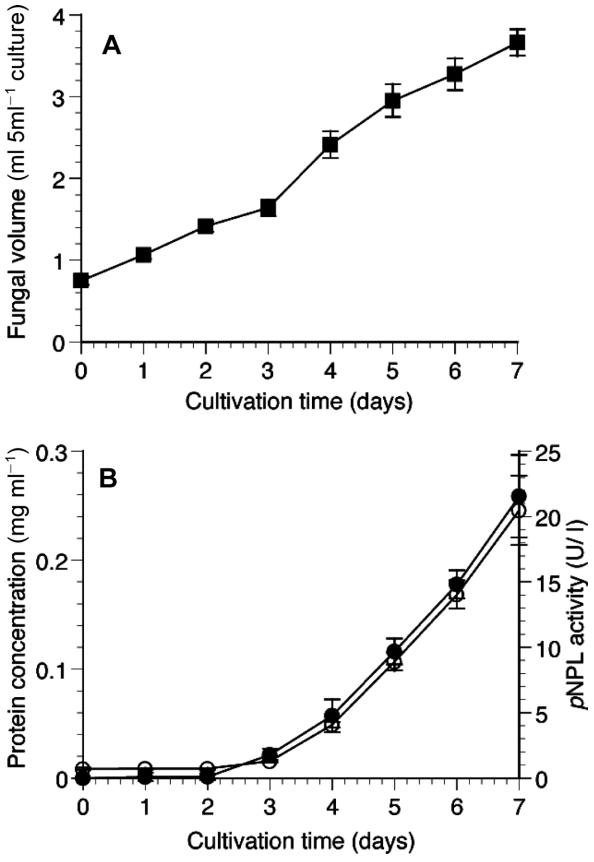


Figure 3. Time course of fungal growth (A), and protein concentration and *p*NPL-hydrolyzing activity (B) in cellulose-degrading culture of *F. velutipes*. The fungal volume represents the amount of mycelium and cellulose complex after sedimentation from 5 ml of culture. Filled square, fungal volume; filled circles, protein concentration; open circles, *p*NPL-hydrolyzing activity. One unit of the enzyme activity is defined as the amount of enzyme that produced one μmole of *p*-nitrophenol equivalent per min under the assay conditions.

characterized in detail to reach a better understanding of the extracellular cellulolytic system of this fungus. In the present study, we have isolated two cellulases belonging to GH family 7 and cloned cDNAs encoding these enzymes.

Purification of proteins having p*NPL-hydrolyzing activity*

GH family 7 includes both endo- and exo-type cellulases, which hydrolyze β-1,4-linkages randomly and processively, as listed in the Carbohydrate-Active enZymes (CAZy) server (Henrissat 1991; Henrissat and Bairoch 1993). Since the enzymes share common features, including hydrolysis of *p*NP-lactoside and cellobioside as model substrates, these activities can be used to confirm the presence of GH family 7 cellulases. Therefore, in the present study, we examined the time courses of fungal growth, protein concentration, and *p*NPL-hydrolyzing activity in the cellulose-degrading culture of *F. velutipes*. The results are shown in Figure 3. The fungus grew effectively on cellulose and secreted up to 250 mg L^{-1} extracellular proteins by day 7. Apparent *p*NPL-hydrolyzing activity was detected after day 3 of cultivation, and the level of the activity was well correlated with the protein concentration. SDS-PAGE of the culture filtrate after 7 days of cultivation (Figure 4) revealed three major proteins with molecular masses of 60, 50, and 37 kDa. In order to separate these proteins, the filtrate of a cellulose-grown culture was fractionated by anion-exchange chromatography (AEC) and then further fractionated by hydrophobic interaction chromatography (HIC), as shown in Figure 5. Although two major protein peaks were observed in AEC, only the latter peak had *p*NPL-hydrolyzing activity. The protein eluted in the void fractions showed a molecular weight of 37 kDa, and might belong to GH family 6, based on an internal amino acid sequence (data not shown), while the latter active fractions contained two major proteins with molecular weights of 60 and 50 kDa. The *p*NPL-hydrolyzing activity was separated into two major peaks by HIC, as shown in Figure 5B. The molecular weights of the proteins in fractions 11 and 31 were 50 and 60 kDa, respectively. Internal sequences of these two proteins were obtained using the Cleveland method, which yielded sequences of GAEYAKTYGITTSGD/YCSAQKTLFGDTNSF for the 50 kDa protein and TYGITTSGNALTLKF/SICDKDGCDFNS for the 60 kDa protein. BLASTP search showed that these fragments have high homology with GH family 7 cellulases from filamentous fungi. Therefore, the proteins with molecular masses of 60 and 50 kDa were named *Fv*Cel7A and *Fv*Cel7B, respectively.

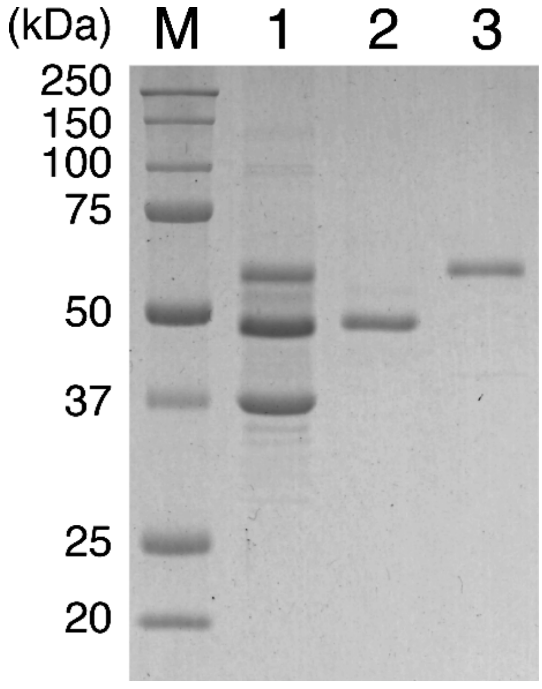


Figure 4. SDS-PAGE of *Fv*Cel7A and *Fv*Cel7B. Lane 1, filtrate of cellulose-grown culture of *F. velutipes*; lanes 2 and 3 correspond to the activity peaks of fractions 11 and 31, respectively, obtained from hydrophobic interaction chromatography; M, molecular weight standards.

Cloning of cDNAs encoding Fv*Cel7A and FvCel7B*

F. velutipes was cultivated in 12 different media and the total RNA obtained from these cultures was mixed and applied to *de novo* Transcript Sequencing to construct a transcriptomic database. Such a database is preferable to a genomic database because it theoretically does not include sequences from introns, and sequences can be directly used for protein search without annotation. Moreover, since we used media including various biomasses in addition to cellulose- and/or hemicellulose-degrading cultures, the transcriptomic database should contains cDNA sequences encoding major enzymes related to biomass degradation. Using the database, we have succeeded in determining contigs encoding *Fv*Cel7A (Contig No. 04714, 00194 and 00193) and *Fv*Cel7B (Contig No. 04321, 00180 and 19555), which enabled us to design primers to clone full-length cDNAs encoding these enzymes.

RT-PCR was carried out using oligonucleotide primers designed from the nucleotide sequence around the determined region and a 3′ RACE primer, and a fragment encoding the protein was cloned. After 5′ RACE, cDNA encoding the whole protein was obtained, as shown in Figure 6. The cDNA encoding *Fv*Cel7A contains an open reading frame (1,530 bp) encoding 510 amino acids. Analysis of the deduced amino acid sequence indicated that the first 18 amino acid residues in the N-terminal region is a signal peptide, and two possible *N*-glycosylation sites and seven possible *O*-

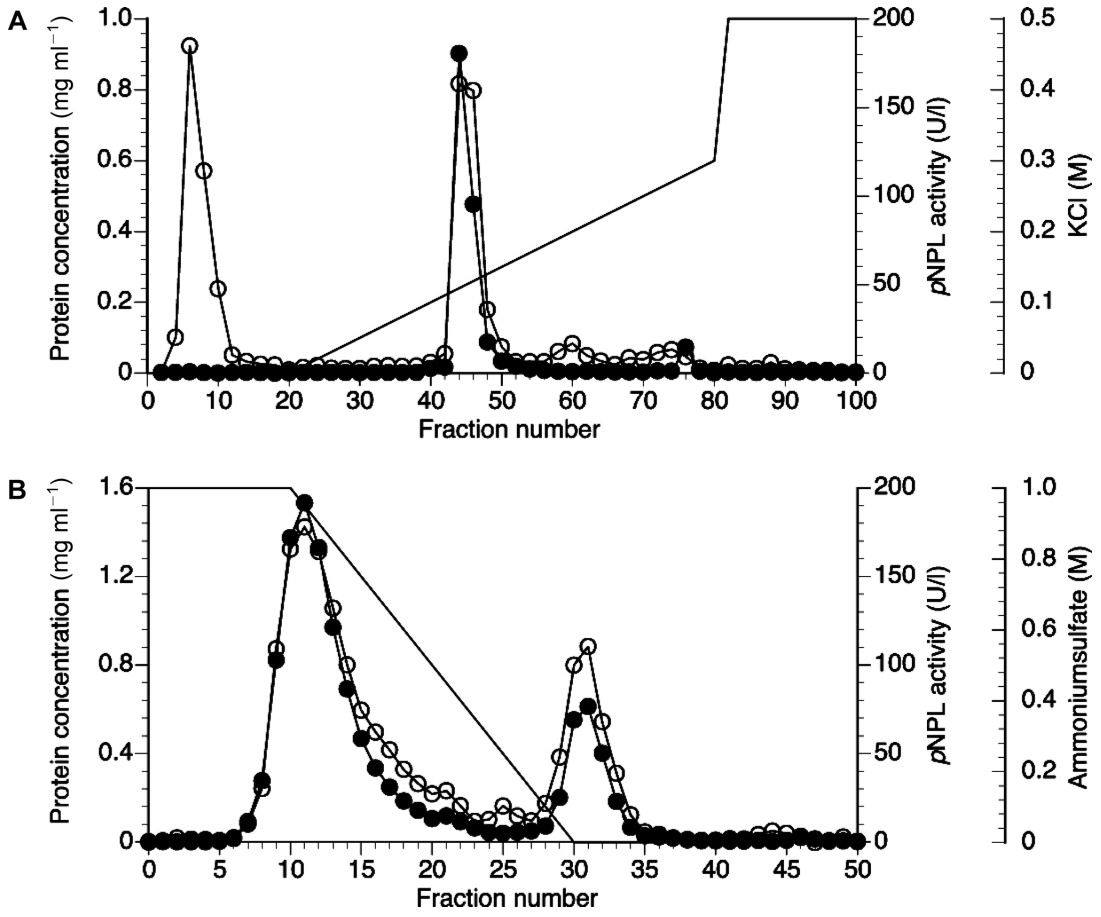


Figure 5. Fractionation of *Fv*Cel7A and *Fv*Cel7B by anion exchange chromatography (AEC) followed by hydrophobic interaction chromatography (HIC). (A) Separation of the concentrated culture filtrate of *F. velutipes* grown on cellulose by DEAE-Toyopearl 650S column. (B) Separation of fractions 43–47 obtained from AEC on a Phenyl-Toyopearl 650S column. Open circles, protein concentration; filled circles, *p*NPL-hydrolyzing activity.

glycosylation sites were predicted (Figure 6A). In addition to an N-terminal GH family 7 catalytic domain, a cellulose-binding domain (CBD) belonging to carbohydrate-binding module family 1 was located at the C-terminal region, with a Ser/Thr-rich linker peptide between the two domains. On the other hand, the cDNA encoding *Fv*Cel7B consisted of 1,371 bp, suggesting a slightly shorter protein (457 amino acids) compared with *Fv*Cel7A. As in *Fv*Cel7A, the N-terminal 18 amino acid residues, which appeared to be a signal peptide, are directly connected to a catalytic domain belonging to GH family 7, but no putative CBD was found at the C-terminal region. As shown in Figure 6B, there were three predicted *N*-glycosylation sites, but only one *O*-glycosylation site in *Fv*Cel7B, mainly because of the lack of a linker peptide. Since the calculated masses of the two enzymes are 52.4 (*Fv*Cel7A) and 47.9 kDa (*Fv*Cel7B), the higher actual molecular mass of *Fv*Cel7A than *Fv*Cel7B determined by SDS-PAGE is due to both the extra protein domain and glycan modifications.

The crystal structures of several fungal CBHs and EGs of GH family 7 have been solved and structural differences of the catalytic domain among *Tr*Cel7A, *Tr*Cel7B, and *Pc*Cel7D have been extensively discussed (Divne et al. 1994; Divne et al. 1998; Kleywegt et al. 1997; Muñoz et al. 2001). Therefore, the amino acid sequences of *Fv*Cel7A and *Fv*Cel7B were aligned to those data, and the predicted secondary structures were compared, as shown in Figure 7. GH family 7 cellobiohydrolases are known to have large numbers of disulfide bonds; for example, *Tr*Cel7A has 10 disulfide bonds in the catalytic domain. In both *Fv*Cel7A and *Fv*Cel7B, 9 disulfide bonds of the 10 are conserved, as is also the case in *Pc*Cel7D. Moreover, since the catalytic mechanism of GH family 7 cellulases is retaining hydrolysis (double-displacement mechanism), *Fv*Cel7A and *Fv*Cel7B should contain two catalytic acidic amino acids (proton donor and nucleophile). Among *Tr*Cel7A, *Pc*Cel7D, and *Tr*Cel7B, these amino acids are well conserved (arrowed in Figure 7; proton donor/nucleophile E217/E212 for *Tr*Cel7A, E212/E207 for *Pc*Cel7D, and E202/E197 for *Tr*Cel7B). Amino acid alignment of Cel7s from *F. velutipes* indicates that possible catalytic amino acids, E209/E214 for *Fv*Cel7A

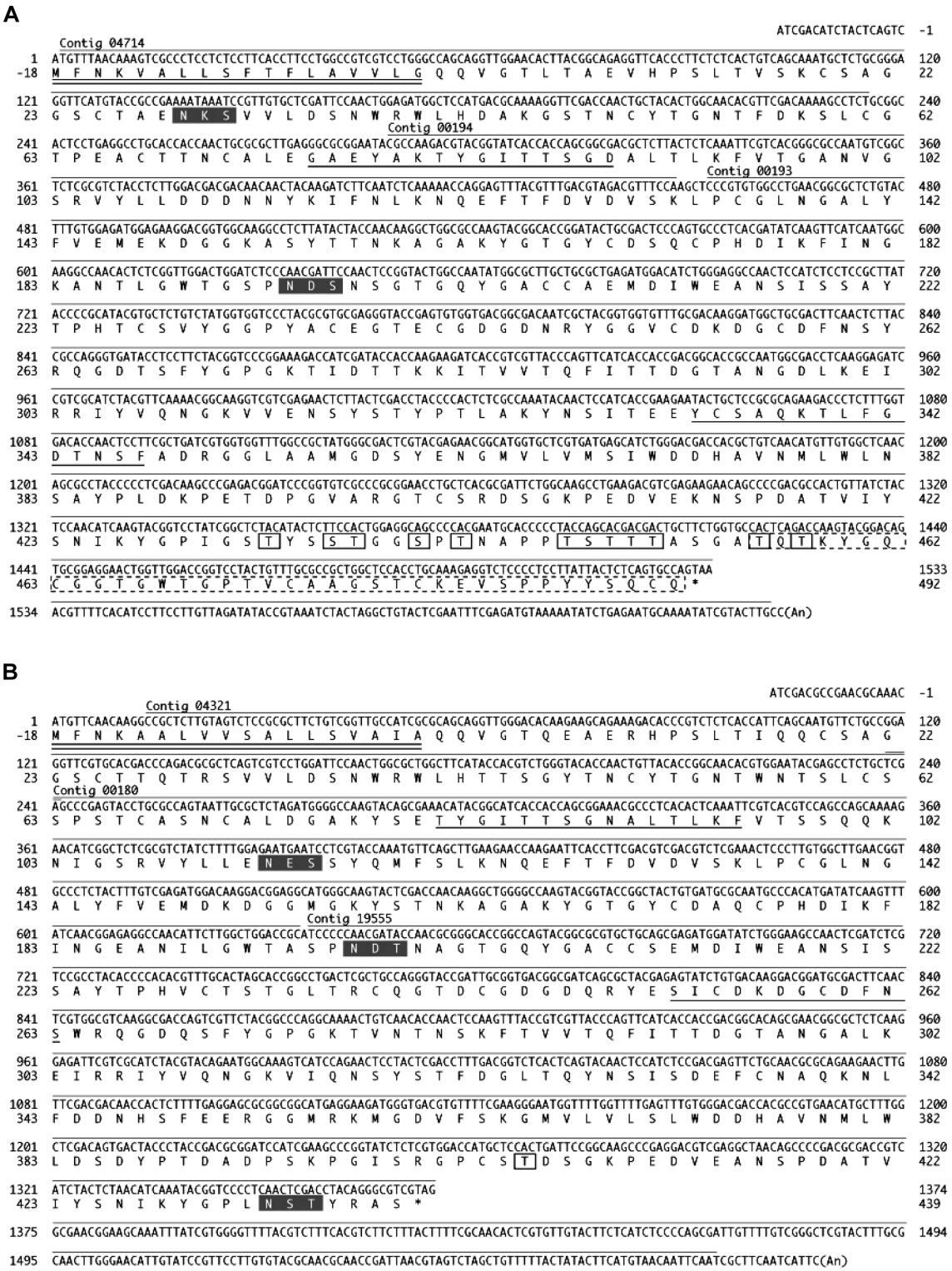


Figure 6. The nucleotide and deduced amino acid sequences of cDNAs encoding *Fv*Cel7A (A) and *Fv*Cel7B (B). The potential signal peptide sequence is double-underlined. The *N*- and *O*-glycosylation sites predicted by the NetNGlyc and NetOGlyc servers are highlighted and boxed, respectively. Predicted CBD in *Fv*Cel7A is boxed by dotted line and the regions matched to the contigs of transcriptomic database is numbered and lined.

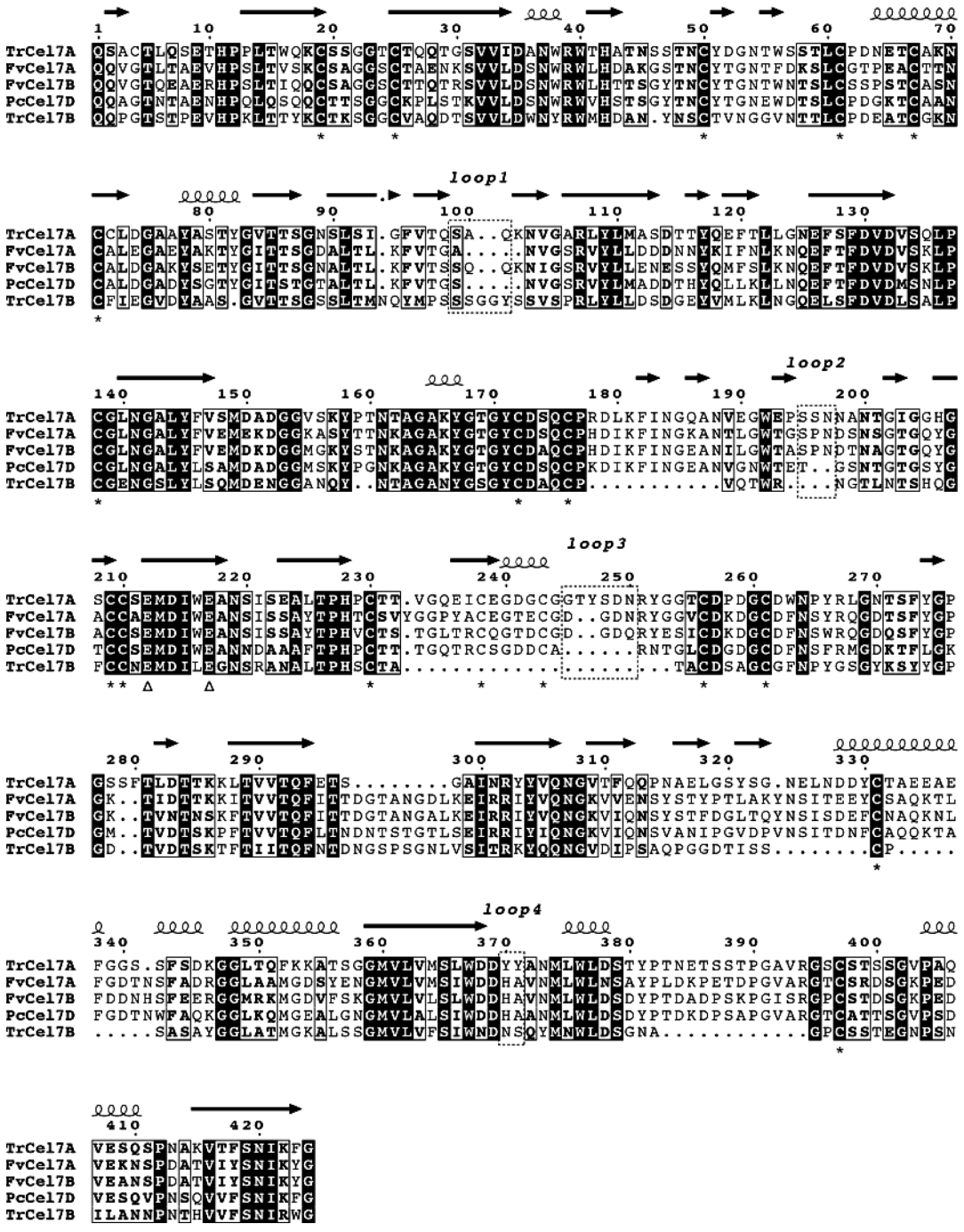


Figure 7. Amino acid alignments of *Fv*Cel7A and *Fv*Cel7B with *Tr*Cel7A, *Pc*Cel7D, and *Tr*Cel7B. Possible catalytic amino acids are indicated by arrowheads, loops variable among Cel7s are boxed by dotted line, and conserved Cys residues are shown with asterisks. Secondary structures were predicted based on the 3-dimensional structure of *Tr*Cel7A (PDB: 1CEL) (Divne et al. 1994) using ESPript (http://espript.ibcp.fr/ESPript/ESPript/).

and E212/E217 for *Fv*Cel7B, are highly conserved. Muñoz and coworkers reported that structural comparison of *Tr*Cel7A and *Pc*Cel7D revealed differences of three loops (loops 1–3 in Figure 7) covering the active site, possibly affecting the processivity of cellulose degradation (Muñoz et al. 2001). Although the structure of loop 1 is completely conserved in *Fv*Cel7B, as in *Tr*Cel7A, this loop is missing in *Fv*Cel7A, as in *Pc*Cel7D. Moreover, both *Fv*Cel7A and *Fv*Cel7B have a longer loop 2, which is identical to that in *Tr*Cel7A, but not *Pc*Cel7D. At loop 3, where active sites -2 and -1 are tightly fixed in *Tr*Cel7A (von Ossowski et al. 2003), five residues are missing in *Pc*Cel7D, whereas only two residues are skipped in both *Fv*Cel7A and *Fv*Cel7B. In *Fv*Cel7s, loop 4, the counterpart of loop 2, consists of amino acids with small

side chains (His-Ala), as in *Pc*Cel7D, but unlike *Tr*Cel7A (Tyr-Tyr). These structural differences can be interpreted as indicating that the Cel7s from *F. velutipes* contain a mixture of the structural features of *Tr*Cel7A and *Pc*Cel7D.

Conclusion

We have isolated two enzymes hydrolyzing *p*NPL (*Fv*Cel7A and *Fv*Cel7B) from cellulose-grown culture of the edible mushroom *F. velutipes*, and cloned cDNAs encoding these enzymes with the aid of a transcriptomic database of this fungus. Both enzymes appear to have a catalytic domain belonging to GH family 7, whereas only *Fv*Cel7A has CBD at the C-terminal. Sequence comparisons with fungal Cel7s of known structure suggest that both *Fv*Cel7A and *Fv*Cel7B share a similar pattern of disulfide bonds and active site architecture, but exhibit apparent differences at loop regions, as compared with other Cel7s. Further biochemical and molecular biological studies of the cellulolytic system of this fungus are needed to establish its suitability for enzyme production for biomass conversion.

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