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FULL PAPER

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# Molecular cloning of the promoter region of the glyceraldehyde-3-phosphate dehydrogenase gene that contributes to the construction of a new transformation system in *Coriolus versicolor*

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**Abstract** The white-rot basidiomycete *Coriolus versicolor* secretes several enzymes that participate in the degradation of lignin and various persistent organic pollutants. In this study, we attempted to establish a genetic transformation system with a homogenous promoter sequence for driving the gene for antibiotic resistance. We succeeded in cloning the promoter sequence of the gene for glyceraldehyde-3-phosphate dehydrogenase (*gpd*), which is expressed at high levels in *C. versicolor*. The expression vector pT7GPTHPT was constructed, which included a gene for resistance to hygromycin B under control of the *gpd* promoter. The successful selection of transformants on medium that contained hygromycin B indicated that the system should be useful not only for the genetic transformation of *C. versicolor*, but also for the overproduction of useful fungal enzymes such as laccase and peroxidase.

**Key words** *Coriolus versicolor* · Glyceraldehyde-3-phosphate dehydrogenase · Homologous transformation · Hygromycin B · Promoter

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## Introduction

White-rot fungi are able to degrade lignin (Higuchi 1971; Kirk and Farrell 1987) and various persistent organic pollutants (Fernando et al. 1990; Ullah et al. 2000). *Coriolus versicolor* is a white-rot basidiomycete that is known to secrete numerous enzymes that catalyze the cleavage of side chains and aromatic rings in lignin model compounds (Kawai et al. 1988). Laccases secreted from *C. versicolor* can catalyze the reduction of dioxygen (Reinhammer et al. 1980). In nature, however, fungi normally produce enzymes in limited amounts, which themselves are influenced by growth conditions (Berka et al. 1997; Aifa et al. 1999). It is likely that gene integration systems will prove useful in enhancing the production of fungal enzymes.

The transformation of *Schizophyllum commune*, *Lentinus edodes*, and *Pleurotus ostreatus* has been reported, with the resultant fungal transformants selected as a consequence of the integration of genes for resistance to phleomycin, carboxin, and hygromycin B, respectively (Schuren and Wessels 1994; Hirano et al. 2000; Honda et al. 2000). The integration using phleomycin and hygromycin B genes has been also reported in *C. versicolor*.

The gene for glyceraldehyde-3-phosphate dehydrogenase (*gpd*) is a key enzyme in the glycolytic pathway (Edens et al. 1984). Vectors carrying the promoter region of the *gpd* gene have been reported to be very efficient in directing the expression of heterologous genes (Bitter and Egan 1984). Furthermore, the regulatory sequences of *gpd* have been used as promoter sequences in vectors designed for the transformation of filamentous fungi, such as *S. commune* (Schuren and Wessels et al. 1994), *Aspergillus nidulans* (Hanegraaf et al. 1991), *Agaricus bisporus* (van den Rhee et al. 1996), and *Phanerochaete chrysosporium* (Mayfield et al. 1994). The *gpdA* promoter from *A. nidulans* has been used in the same microorganism for the strong and constitutive expression of homologous and heterologous extracellular proteins (Punt et al. 1991; Mayfield et al. 1994).

In the present study, we attempted to obtain a recombinant strain of a basidiomycete, *C. versicolor*, which

expressed a gene for resistance to hygromycin B under the control of a strong promoter. We found that the promoter of the *gpd* gene from the genomic DNA of *C. versicolor* could be used to regulate the expression of a foreign gene in this fungus.

## Materials and methods

### Strain and culture conditions

A dikaryotic strain of *C. versicolor* (IFO30340) was used as the recipient in transformation experiments. Mycelia of *C. versicolor* were grown on plates of potato dextrose agar (Kyokuto, Tokyo, Japan) or in GP-Cu liquid medium (30 g glucose, 10 g peptone, 1.5 g $KH_2PO_4$, 500 mg $MgSO_4 \cdot 7H_2O$, 2 mg thiamine hydrochloride, and 16 mg $CuSO_4 \cdot 5H_2O$ /l) at 28°C.

### Molecular techniques

mRNA was extracted from the mycelia of *C. versicolor* with a QuickPrep *Micro* mRNA Purification Kit (Pharmacia Biosciences, Tokyo, Japan). First-strand cDNA was synthesized from mRNA with a First-Strand cDNA Synthesis Kit (Pharmacia Biosciences). Total DNA was extracted from candidate transformed and wild-type mycelia, which had been cultured in GP-Cu liquid medium at 28°C for 7 days. DNA was isolated by the CTAB method (Rogers and Bendich 1985).

### Cloning of the promoter of the *gpd* gene

Degenerate oligonucleotide primers were designed based on the strongly conserved amino acid sequences of *gpd* genes previously characterized from other basidiomycetes (Harmsen et al. 1992): primer F1 (5′-GGAATYAACGGKTTCGGTCG-3′), which anneals to sequences coding for GINGFGR; and primer R1 (5′-GTGAASACACCGGTSGACTC-3′), which anneals to sequences coding for ESTGVFT. Using these primers and first-strand cDNA from mRNA of *C. versicolor* as the template, PCR amplification was performed using 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. The fragment obtained by polymerase chain reaction (PCR) was cloned in a pCR2.1 vector and then sequenced with an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA). To clone a DNA fragment containing the promoter region of the *gpd* gene that is adjacent to the amplified fragment, PCR amplification was performed with an in vitro cloning kit (Takara Shuzo, Kyoto, Japan) according to the manufacturer's instructions. After digestion of total DNA by *Hin*dIII, the *Hin*dIII cassette was ligated to the resultant DNA, and then PCR was performed with a cassette primer and two nested oligonucleotide primers (GPDP1, 5′-GGAGGACGATACGGCCGATAC-3′; GPDP2, 5′-ACGGCCGATACGACCGAAAC-3′) based on the amplified cDNA fragment. To construct the expression vector, a DNA fragment of the promoter region was amplified by PCR with a pair of primers: GPDSP2, 5′-ACAGGAGTGTGTAGGTGACAT-3; and GPDAP1, 5′-gatetcGTGGATGTGGTGGAT-3′, where small letters indicate the sequence of the restriction site, *Eco*RV. PCR amplification was performed using 30 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, with total DNA of *C. versicolor* as the template.

### Construction of expression vector for genomic transformation

The expression vector pT7GPTHPT was constructed for use in transformation experiments (Fig. 1). pT7GPTnos consisted of the *gpd* promoter (844 bp) from *C. versicolor* and a nopaline synthase (*nos*) terminator (267 bp) from pBI121 (BD Bioscience Clonetech, Tokyo, Japan), and was based on the pT7Blue T-vector (Novagen, San Diego, CA, USA).

The coding sequence of the *hph* gene, which encodes hygromycin B phosphotransferase, was inserted into the *Bam*HI site of pT7GPTnos. The resultant recombinant plasmid was designated pT7GPTHPT (Fig. 1).

### Transformation

Protoplasts of *C. versicolor* were prepared as described by Sato et al. (1998) with slight modifications. *C. versicolor* was cultivated at 28°C for 2 days in SM medium (1% sucrose and 1% malt extract). The mycelia were collected, washed

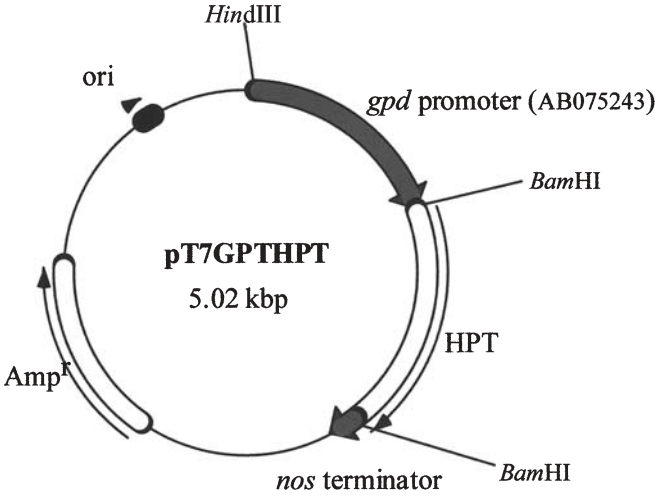


**Fig. 1.** Map of the vector pT7GPTHPT. The *gpd* promoter is an 844-bp sequence from the *gpd* gene of *Coriolus versicolor*. HPT is the coding sequence of the gene for resistance to hygromycin B obtained from *Escherichia coli*. The *nos* used as the terminator sequence was derived from the pBI121 vector. *Solid bold lines* show sequences derived from the pT7Blue T-vector. *Amp*$^r$ is an ampicillin resistance gene

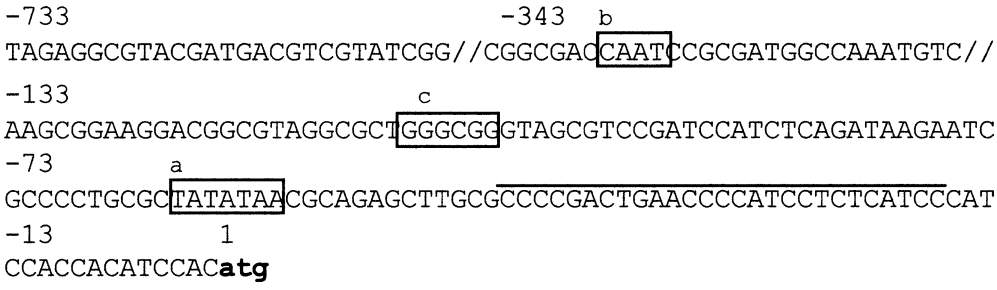


**Fig. 2.** The nucleotide sequence of the *gpd* promoter region from *C. versicolor*. The start codon is shown in *boldface*. A TATA-like sequence (*a*), a CAAT-like sequence (*b*), and a GC box (*c*) are indicated. a *single line above* the sequence indicates a pyrimidine-rich sequence; and the *numbers* indicate positions relative to the site of initiation of translation of the *gpd* gene

twice with M buffer [0.2% sodium malate (pH 5.5) and 0.6 M mannitol], and treated with a solution of enzyme [10 mg/ml Yatalase (Takara Shuzo), 10 min/ml cellulase Onozuka R-10 (Yakult Honsha, Tokyo, Japan), and 1 mg/ml chitinase (Sigma, St. Louis, MO, USA) in M buffer] at 30°C for 2–3 h with shaking at 50–60 rpm. After incubation, the protoplasts in suspension were collected by centrifugation at 2000 *g* for 20 min, and washed with STC buffer [0.01 M Tris-HCl (pH 7.5), 0.01 M $CaCl_2$, and 1.2 M sorbitol]. The mycelia and protoplasts of *C. versicolor* were plated on MS agar plates [2% malt extract, 0.6 M sucrose, 1.5% agar, and 0.7% low melting temperature agarose (BioWhittaker Molecular Applications, Rockland, MA, USA), respectively] with variable amounts of hygromycin B (0–200 μg/ml; Wako, Osaka, Japan). Transformations using polyethylene glycol (PEG) were performed as described by Yanai et al. (1996) with slight modifications. Protoplasts of *C. versicolor* were suspended in STC buffer at $10^7$–$10^8$ protoplasts/ml, and 100 μl suspension was mixed with 25 μg plasmid DNA. The mixture was kept on ice for 5 min, after which 25 μl PEG solution [60% (w/v) PEG4000 (Wako), 10 mM Tris-HCl (pH 7.5), and 10 mM $CaCl_2$] were added. The mixture was kept on ice for 20 min, mixed gently with 1.25 ml the PEG solution, kept at room temperature for 20 min, and finally mixed with 10 min STC buffer. After centrifugation of this suspension at 1000 *g* for 5 min, the pelleted protoplasts were suspended in 4 ml MS liquid medium and incubated at 28°C for 48 h. The mixture was then spread on an MS agar plate prepared with 30 μg/ml hygromycin B.

### Southern blot analysis

The integration of introduced DNA in the transformants was confirmed by Southern blot analysis (Southern et al. 1975). Total DNA (6 μg) was digested separately with *Bam*HI or *Hin*dIII, and each digest DNA was transferred to a Hybond N+ nylon membrane (Bio-Rad, Tokyo, Japan) after fractionation by electrophoresis. The *Bam*HI fragment of plasmid pT7GPTHPT containing the hygromycin B resistance gene was radioactively labeled with $^{32}$P-dCTP and used as a probe.

## Results

### Characterization of the *gpd* promoter and construction of the plasmid vector

To isolate partial cDNA for the *C. versicolor gpd* gene, two degenerate oligonucleotide primers were designed according to the deduced amino acid sequences of previously characterized *gpd* genes from other basidiomycetes, *S. commune* and *P. chrysosporium*. Using these primers, a fragment was amplified by PCR with first-strand cDNA from *C. versicolor* as the template. The homology between the amino acid sequence of the resultant 293-bp-long DNA fragment and the sequences of the other basidiomycete *gpd* proteins was quite high, 79.6% for *S. commune* and 86.7% for *P. chrysosporium*. We therefore concluded that the amplified fragment is a partial cDNA for the *C. versicolor gpd* gene.

For cloning the promoter sequence of *gpd* gene, we performed PCR with *Hin*dIII-digested and subsequent *Hin*dIII cassette-ligated total DNA as a template (see Materials and methods). In this procedure, two nested primers, designed according to the sequence information of the partial cDNA already described, and a cassette primer were used for amplification of the promoter sequence. After performing the nested PCR, we amplified a DNA fragment containing 913-bp *gpd* promoter regions (accession no. AB075243); the fragment contained a TATA-like sequence, a CAAT-like sequence, and a GC box (Fig. 2). It also contained a binding site for the GATA factor (Ko and Engel 1993), a recognition site for the cAMP-response-element-binding protein (CREB) (Brindle and Montminy 1992), and a characteristic pyrimidine-rich sequence specific to filamentous fungi (Gurr et al. 1988).

### Transformation of *C. versicolor*

We used the PCR-amplified 844-bp promoter fragment of the *C. versicolor gpd* gene to construct the expression vector pT7GPTHPT that contained a coding sequence for the

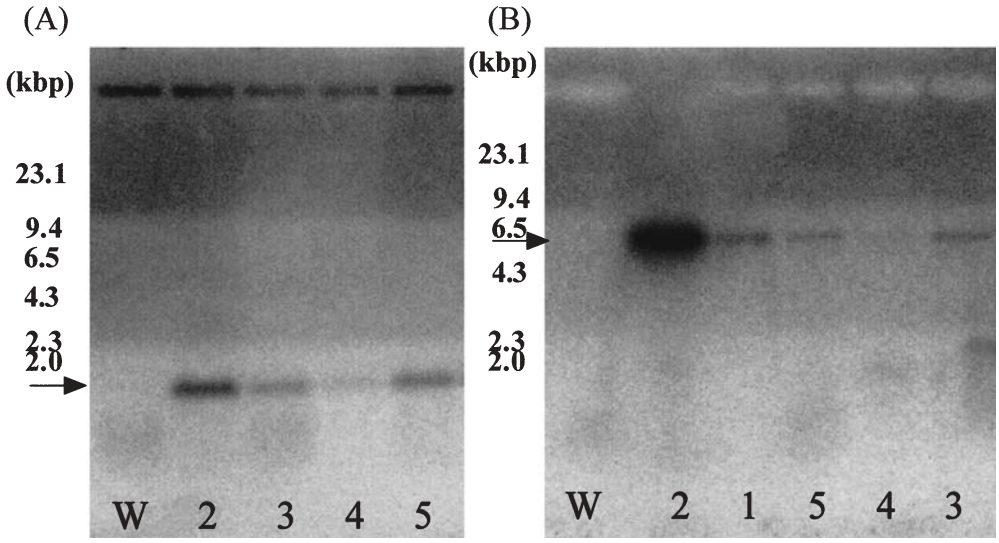


**Fig. 3.** Southern blot analysis of transformants. Restriction digests of DNA extracted from transformants were probed with $^{32}$P-labeled *hph* cDNA. *W*, wild-type *C. versicolor*; *lanes 1–5*, various lines of transformants. **A** *Bam*HI-digested DNA; **B** *Hin*dIII-digested total DNA. The *arrows* indicate specific fragments, as described in the text

hygromycin B resistance gene under control of the promoter. The construct was useful in the genetic transformation of *C. versicolor*, the efficiency of which was 0.04 transformants/μg of the vector DNA (data not shown). This transformation efficiency is similar to that of other filamentous fungi and basidiomycetes (Gouka et al. 1996; van den Rhee et al. 1996; Irie et al. 2001). All transformants had an apparently normal phenotype, and their growth characteristics were similar to those of the wild-type strain on the nonselective medium.

To examine the integration of plasmid DNA into genomic DNA of the transformants, we performed Southern blot analysis using a 1.0-kb fragment of the coding region of *hph* gene as the probe. Total DNA was prepared from the various transformants, and all samples of the DNA were digested with *Bam*HI or *Hin*dIII (Fig. 3). Specific of 1.0- and 5.0-kbp fragments were detected in all transformants of *Bam*HI- and *Hin*dIII-digested genomic DNA, respectively. All analyzed transformants contained one or more copies of the transforming vector pT7GPTHPT. These results indicate that *hph* genes were integrated into the genomic DNA of the transformants and that the gene was expressed stably under control of the *gpd* promoter in *C. versicolor*.

We tested the sensitivity of transformants to hygromycin B on media containing different amounts of the antibiotic (from 30 to 200 μg/ml). As indicated in Fig. 4, the growth rates of transformants varied on the 100 μg/ml medium, which suggests that the expression level of *hph* varied between the transformants.

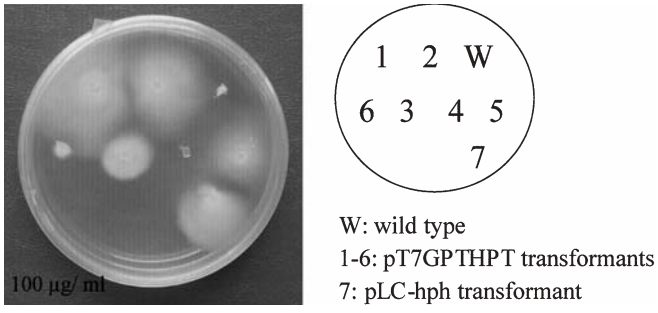


**Fig. 4.** Growth of various transformants of *C. versicolor* and wild-type *C. versicolor* in the presence of 100 μg/ml hygromycin B

## Discussion

In this study, we constructed a genetic transformation system of *C. versicolor* using a new expression vector. This vector was generated by isolating the promoter sequence of the *gpd* gene of *C. versicolor*. The *gpd* gene encodes one of the key enzymes in the glycolytic pathway and is expressed strongly in some filamentous fungi. The activity of the promoter of the *gpd* gene of *A. nidulans* (*gpdA*) has previously been investigated for the efficient production of both intracellular and extracellular proteins (Punt et al. 1991). The expression profiles of *gpd* promoters have been also reported in the basidiomycetes *S. commune* (Harmsen et al. 1992; Schuren and Wessels 1994), *P. chrysosporium* (Harmsen et al. 1992; Mayfield et al. 1994), *L. edodes* (Montminy and Bilezikjian 1987; Hirano et al. 2000), and *P. ostreatus* (Irie et al. 2001). Thus, we consider that the *gpd* gene was expressed as strongly in *C. versicolor*, as were the genes in the other filamentous fungi.

The isolated *gpd* gene promoter contained a TATA-like sequence, a CAAT-like sequence, and a GC box (see Fig. 2). *gpd*, *pgk*, *gut*, and *qa* boxes, which are conserved in *A. nidulans* and *A. niger* GPD promoter regions, were not observed in that of *C. versicolor*, as also reported for other basidiomycetes (Punt et al. 1990; Hirano et al. 1999). The *C. versicolor* promoter also contained a CREB-binding sequence that regulates transcription of the gene in re-

sponse to cAMP. The presence of a CREB site in the promoter sequence suggests that expression of the *gpd* gene is regulated by the intracellular concentration of cAMP. Because CREB is a protein–recognition sequence that regulates transcription in response to cAMP, it may be possible to control the gene expression by adding cAMP to the medium in which *C. versicolor* is cultured.

We succeeded in obtaining genetically transformed *C. versicolor* by introducing a new expression vector into the genome. The transformation efficiency of *C. versicolor* (0.04 transformants/μg of vector DNA) is similar to that of other filamentous fungi in which the expression vector is constructed with *gpd* (Mayfield et al. 1994; Yanai et al. 1996). Although the efficiency of transformation was not appreciably higher than that with other filamentous fungi, the resistance level to hygromycin B was sufficient for the successful selection of transformants. All the transformants tested could grow on selective medium that contained 100 μg/ml hygromycin B (Fig. 4), and three of them were resistant to hygromycin B at concentrations higher than 200 μg/ml.

The results from Southern blot analysis suggest that the signal from genomic DNA of the transformant digested by *Hin*dIII has an unique pattern among the transformants when the gene is introduced at any specific site in the genome. As shown in Fig. 3, strong hybridization signals were observed at about 5.0 kbp, which corresponds to the length of the transforming vector. It is likely that these signals reflect tandem introduced DNA, as has also been reported in the transformation of *S. commune* (van den Rhee et al. 1996). As indicated in Fig. 3, the intensities of the signals varied among the transformants, which may reflect the integration of different numbers of genes into the transformant genomes. Transformant 4, whose signal was fainter than those of other transformants in Southern blot analysis, grew slower than others on the medium containing 100 μg/ml hygromycin B (Figs. 3, 4). These results suggest that the sensitivity to hygromycin B of transformants was influenced by the number of introduced genes or the expression level of the genes. The specific signal remained detectable in Southern blot analysis after all the transformants had been cultured on medium without antibiotic for 2 months, indicating that the introduced genes were stably integrated into the genome of *C. versicolor* (data not shown).

Recently we have also succeeded in transforming *C. versicolor* using another vector, pLC-hph (no. 7, Fig. 4). This vector has been found to be useful in *P. ostreatus* (Mayfield et al. 1994) and *L. edodes* (Sato et al. 1998), and it contains the promoter sequence of the *L. edodes ras* gene and the terminator sequence of the *L. edodes priA* gene (Schuren and Wessels 1994; Irie et al. 2001). A cotransformation system combining both pT7GPTHPT and pLC-hph may contribute to the production of transformed *C. versicolor* that effectively overproduces enzymes for the enhanced degradation of lignins and aromatic pollutants. The gene expression system we developed in this study should also be useful for the production of various types of recombinant proteins in *C. versicolor*.

## References


Aifa MS, Sayadi S, Gargori A (1999) Heterologous expression of lignin peroxidase of *Phanerochaete chrysosporium* in *Aspergillus niger*. Biotech Lett 21:846–853

Berka RM, Schneider P, Golightly EJ, Brown SH, Madden M, Brown KM, Halkier T, Mondorf K, Xu F (1997) Characterization of the gene encoding an extracellular laccase of *Myceliophthora thermophila* and analysis of the recombinant enzyme expressed in *Aspergillus oryzae*. Appl Environ Microbiol 63:3151–3157

Bitter GA, Egan KM (1984) Expression of heterologous genes in *Saccharomyces cerevisiae* from vectors utilizing the glyceraldehyde-3-phosphate dehydrogenase gene promoter. Gene (Amst) 32:263–274

Brindle PK, Montminy MR (1992) The CREB family of transcription activators. Curr Opin Genet Dev 2:199–204

Edens LE, Bom J, Ledeboer AM, Maat J, Toonen MY, Visser C, Verrips CT (1984) Synthesis and processing of the plant protein thaumatin in yeast. Cell 37:629–633

Fernando T, Bumpus JA, Asut SD (1990) Biodegradation of TNT (2,4,6-trinitrotoluene) by *Phanerochaete chrysosporium*. Appl Environ Microbiol 56:1666–1671

Gouka RJ, Punt PJ, Hessing JGM, van den Hondel CAMJJ (1996) Analysis of heterologous protein production in defined recombinant *Aspergillus awamori* strains. Appl Environ Microbiol 62:1951–1957

Gurr SJ, Unkles SE, Kinghorn JR (1988) The structure and organization of nuclear genes in filamentous fungi. In: Kinghorn JR (ed) Gene structure in eukaryotic microbes. IRL Oxford, UK, pp 93–139

Hanegraaf PPF, Punt PJ, van den Hondel CAMJJ, Dekker J, Yap W, van Verseveld HW, Stouthamer AH (1991) Construction and physiological characterization of glyceraldehyde-3-phosphate dehydrogenase overproducing transformants of *Aspergillus nidulans*. Appl Microbiol Biotech 34:765–771

Harmsen MC, Schuren FHJ, Moukha SM, van Zuilen CM, Punt HP, Wessels JGH (1992) Sequence analysis of the glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycetes *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Agaricus bisporus*. Curr Genet 22:447–454

Higuchi T (1971) Formation and biological degradation of lignin. Adv Enzymol Relat Areas Mol Biol 34:270–283

Hirano T, Sato T, Ogawa K, Kanda K, Yaegashi K, Enei H (1999) Isolation and characterization of the glyceraldehyde-3-phosphate dehydrogenase gene of *Lentinus edodes*. Biosci Biotechnol Biochem 63:1223–1227

Hirano T, Sato T, Yaegashi K, Enei H (2000) Efficient transformation of the edible basidiomycete *Lentinus edodes* with a vector using a glyceraldehyde-3-phosphate dehydrogenase promoter to hygromycin B resistance. Mol Gen Genet 263:1047–1052

Honda Y, Matsuyama T, Irie T, Watanabe T, Kuwahara M (2000) Carboxin resistance transformation of the homobasidiomycete fungus *Pleurotus ostreatus*. Curr Genet 37:209–212

Irie T, Honda Y, Hirano T, Sato T, Enei H, Watanabe T, Kuwahara M (2001) Stable transformation of *Pleurotus ostreatus* to hygromycin B resistance using *Lentinus edodes* GPD expression signals. Appl Microbiol Biotechnol 56:707–709

Kawai S, Umezawa T, Higuchi T (1988) Degradation mechanisms of phenolic β-1 lignin substructure model compounds by laccase of *Coriolus versicolor*. Arch Biochem Biophys 262:99–110

Kirk TK, Farrell RL (1987) Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol 41:465–505

Ko LJ, Engel JD (1993) DNA-binding specificities of the GATA transcription factor family. Mol Cell Biol 13:4011–4022

Mayfield MB, Kishi K, Alic M, Gold MH (1994) Homologous expression of recombinant manganese peroxidase in *Phanerochaete chrysosporium*. Appl Environ Microbiol 60:4303–4309

Montminy MR, Bilezikjian LM (1987) Binding of nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature (Lond) 328:175–178

Punt PJ, Dingemanse MA, Kuyvenhoven A, Soede RDM, Pouwels PH, van den Hondel CAMJJ (1990) Functional elements in the promoter region of the *Aspergillus nidulans gpdA* gene encoding glyceraldehyde-3-phosphate dehydrogenase. Gene (Amst) 93:101–109

Punt PJ, Zegers ND, Busscher M, Pouwels PH, van den Hondel CAMJJ (1991) Intracellular and extracellular production of proteins

in *Aspergillus* under the control of expression signals of the highly expressed *Aspergillus nidulans gpdA* gene. J Biotechnol 17:19–34

Reinhammer B, Malkin R, Jensen P, Karlsson B, Andreasson AE, Aasa RV, Vänngård T, Malmstorm BG (1980) A new copper(II) electron paramagnetic resonance signal in two laccases and in cytochrome c oxidase. J Biol Chem 255:5000–5003

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5:69–76

Sato T, Yaegashi K, Ishii S, Hirano T, Kajiwara S, Shishido K, Enei H (1998) Transformation of the edible basidiomycete *Lentinus edodes* by restriction enzyme-mediated integration of plasmid DNA. Biosci Biotechnol Biochem 62:2346–2350

Schuren FHJ, Wessels JGH (1994) Highly-efficient transformation of the homobasidiomycete *Schizophyllum commune* to phleomycin resistance. Curr Genet 26:179–183

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Ullah MA, Bedford CT, Evans CS (2000) Reactions of pentachlorophenol with laccase from *Coriolus versicolor*. Appl Microbiol Biotechnol 53:230–234

van den Rhee M, Graca P, Huizing H, Mooibroek H (1996) Transformation of the cultivated mushroom, *Agaricus bisporus*, to hygromycin B resistance. Mol Gen Genet 250:252–258

Yanai K, Yonekura K, Usami H, Hirakawa M, Kajiwara S, Yamazaki T, Shishido K, Adachi T (1996) The integrative transformation of *Pleurotus ostreatus* using bialaphos resistance as a dominant selectable marker. Biosci Biotech Biochem 60:472–475