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Generation of a Transformant Showing Higher Manganese Peroxidase (Mnp) Activity by Overexpression of Mnp Gene in *Trametes versicolor*

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***Trametes versicolor* has a lignin degrading enzyme system, which is also involved in the degradation of diverse recalcitrant compounds. Manganese-dependent peroxidase (MnP) is one of the lignin degrading enzymes in *T. versicolor*. In this study, a cDNA clone of a putative MnP-coding gene was cloned and transferred into an expression vector (pBARGPE1) carrying a phosphinothricin resistance gene (*bar*) as a selectable marker to yield the expression vector, pBARTvMnP2. Transformants were generated through genetic transformation using pBARTvMnP2. The genomic integration of the MnP clone was confirmed by PCR with *bar*-specific primers. One transformant showed higher enzyme activity than the recipient strain did, and was genetically stable even after 10 consecutive transfers on non-selective medium.**

Keywords: gene expression, manganese-dependent peroxidase (MnP), *Trametes versicolor*

White-rot fungi, which have lignin degrading enzymes, play important roles in carbon recycling in nature, because lignin, next to cellulose, is the second most abundant organic carbon compound on earth. The white-rot fungi degrade lignins not only to use them as carbon sources but also to remove a physical barrier against cellulose utilization. Due to their powerful degrading capabilities towards various recalcitrant chemicals, white-rot fungi and their lignin degrading enzymes have long been studied for biotechnical applications such as biobleaching (Takano *et al.*, 2001), biodecolorization (Dias *et al.*, 2003) and bioremediation (Beltz *et al.*, 2001; Cheong *et al.*, 2006). The lignin degrading enzymes consist of laccase, lignin peroxidase, manganese peroxidase and H_2O_2-supplying glucose oxidase for the peroxidase reactions. Manganese-dependent peroxidase (MnP) oxidizes phenolic compounds in the presence of H_2O_2 and manganese. This enzyme oxidizes Mn(II) to Mn(III), and in turn oxidizes monomeric phenols (Wariishi *et al.*, 1988), phenolic lignin dimers (Wariishi *et al.*, 1989) and synthetic lignin (Wariishi *et al.*, 1991) via the formation of phenoxy radicals.

Phanerochaete chrysosporium is one of the most widely studied white-rot fungi with regards to lignin degrading enzymes (Tien and Tu, 1987), and its 4 MnP genes have been reported (Alic *et al.*, 1997). There are also many reports on the MnPs of additional white-rot fungi such as *Pleurotus ostreatus* (Kamitsuji *et al.*, 2004), *Trametes versicolor* (Johansson *et al.*, 2002), and others (Manubens *et al.*, 2003; Hakala *et al.*, 2006). MnPs in white-rot fungi have conserved amino acid sequences for metal binding regions, and the nucleotide sequences in those regions can be used as the PCR primers for gene cloning (Kim *et al.*, 2003; Kim *et al.*, 2005).

In order to get a fungal strain with high enzyme activity, there are several choices; selection of a high enzyme-producing strain from hundreds of wild type fungal isolates, or generation of a mutant strain through mutagen treatment. It is also possible to get a strain showing excellent enzyme activity by genetic engineering techniques. In this study, we cloned a full MnP cDNA from *T. versicolor*, and introduced an extra copy of the MnP gene under the control of a glyceraldehydes-3-phosphate dehydrogenase gene promoter from *Aspergillus nidulans*, into the genome of the wild type strain using a genetic transformation procedure.

Materials and Methods

Cloning of a full MnP cDNA from T. versicolor

T. versicolor monokaryon 9522-1 was grown as reported previously (Kim *et al.*, 2005; Cheong *et al.*, 2006). The genomic DNA and total RNA were isolated using the CTAB method and an RNeasy Plant Mini kit (Qiagene, USA), respectively (Cheong *et al.*, 2006). The first strand of cDNA was synthesized from 1 μg of total RNA using PowerScript Reverse Transcriptase (Promega, USA) by following the manufacturer's instructions. Two degenerated primers were used: a forward primer (F1); 5'-CACGACGCCATCGSCATCTC-3', and a reverse primer (R1); 5'-GTGCGASRCSAGMAGSGC AAC-3', which corresponded to the metal binding regions. In order to get 3'- and 5'-regions by RACE-PCR (rapid amplification of cDNA ends PCR), we used the forward primer (F2); 5'-ACGAGATCATCGGCGAGCAG-3', and the reverse primer (R2); 5'-AGGCGAGCAGGGCAACAAACC-3' which were derived from the specific sequences of the cloned region amplified by F1 and R1 primers. The amplified 5'- and 3'-regions of the cDNA were used to clone the full length cDNA by second-round PCR. The final amplified cDNA product was cloned into a pGEM-T vector for sequencing.

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(A)

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                                                                M  A
GGGGGATAGGGAGGCCAACGAGCTCTCTTCCTCCATCCCTCGCAATCCCAGGCAATGGCG    60

F  K  L  L  G  S  F  V  S  L  L  A  A  L  Q  V  A  N  G  A
TTCAAGCTCCTTGGTTCCTTCGTCTCCCTCCTCGCGGCCCTTCAGGTCGCTAACGGTGCC   120

L  T  R  R  V  T  C  A  T  G  Q  V  T  S  N  A  A  C  C  A
CTCACCCGCCGTGTGACGTGCGCCACCGGCCAGGTCACCTCGAACGCGGCCTGCTGCGCG   180

L  F  P  V  I  D  D  I  Q  T  N  L  F  D  G  G  E  C  G  E
CTCTTCCCCGTCATCGACGACATCCAGACGAACCTGTTCGACGGCGGCGAGTGCGGCGAG   240

E  V  H  E  S  L  R  L  T  F  H  D  A  I  G  I  S  P  A  I
GAGGTCCACGAGTCCCTCCGCCTCACCTTCCACGACGCCATCGGCATCTCGCCCGCCATC   300

A  K  T  G  V  F  G  G  G  G  A  D  G  S  I  A  I  F  A  D
GCCAAGACCGGTGTCTTCGGTGGTGGAGGCGCGGACGGCTCCATTGCCATCTTCGCCGAC   360

I  E  T  N  F  H  A  N  N  G  V  D  E  I  I  G  E  Q  A  P
ATCGAGACGAACTTCCACGCGAACAACGGTGTCGACGAGATCATCGGCGAGCAGGCGCCC   420

F  I  A  R  H  N  L  T  T  A  D  F  I  Q  L  A  G  A  I  G
TTCATCGCCCGCCACAACCTCACCACCGCCGACTTCATCCAGTTGGCCGGTGCCATCGGT   480

V  S  N  C  P  G  A  P  R  L  N  V  F  I  G  R  K  D  A  T
GTCTCCAACTGCCCTGGCGCGCCCCGCCTGAACGTCTTCATTGGCCGCAAGGACGCGACC   540

Q  P  A  P  D  L  T  V  P  E  P  F  D  D  V  T  K  I  L  A
CAGCCCGCTCCCGACCTGACGGTCCCCGAGCCCTTCGACGACGTCACCAAGATTCTTGCT   600

R  F  E  D  A  G  K  F  T  P  A  E  V  V  A  L  L  A  S  H
CGCTTCGAGGATGCCGGCAAGTTCACCCCCGCTGAGGTTGTTGCCCTGCTCGCCTCGCAC   660

T  I  A  A  A  D  H  V  D  P  T  I  P  G  T  P  F  D  S  T
ACGATCGCCGCTGCCGACCACGTCGACCCCACCATCCCGGGAACGCCCTTCGACTCCACC   720

P  E  L  F  D  T  Q  F  F  I  E  T  Q  L  R  G  T  L  F  P
CCCGAGCTGTTCGACACCCAGTTCTTCATCGAGACCCAGCTCCGCGGCACGCTCTTCCCC   780

G  N  G  S  N  Q  G  E  V  Q  S  P  L  G  G  E  L  R  L  Q
GGCAACGGCAGCAACCAGGGTGAGGTCCAGTCTCCCCTCGGCGGTGAGCTCCGTCTCCAG   840

S  D  G  L  L  A  R  D  Q  R  T  A  C  E  W  Q  S  F  V  N
TCCGACGGACTACTTGCCCGCGACCAGCGCACGGCCTGCGAGTGGCAGTCGTTCGTCAAC   900

N  Q  A  K  L  Q  S  A  F  K  A  A  F  A  R  M  T  V  L  G
AACCAGGCGAAGCTCCAGAGCGCGTTCAAGGCCGCGTTCGCGAGGATGACCGTGCTCGGC   960

Q  N  T  R  A  L  I  D  C  S  D  V  V  P  T  P  P  A  P  A
CAGAACACGCGCGCGCTCATCGACTGCTCGGACGTCGTCCCCACCCCGCCCGCCCCCGCC  1020

S  K  A  H  F  P  A  G  L  S  R  R  D  I  E  Q  A  C  R  A
AGCAAGGCACACTTCCCCGCCGGCCTCTCCCGCCGCGACATCGAGCAGGCGTGCCGCGCG  1080

T  P  F  P  T  L  P  T  D  P  G  P  V  T  T  V  A  P  V  P
ACGCCCTTCCCCACGCTCCCCACTGACCCCGGACCCGTTACCACCGTCGCCCCTGTCCCC  1140

P  S  *
CCGTCCTAAATGTTGCGCTGCGCAACGCTTGCATGTTATGCCACTAAGATTTTATTGGGA  1200

ATTGGGTCGCTTCACTAGAATGATGCCTTTGATAGTAATGCTGTCTTGAGTTGTTGAAAT  1260

GTGATGCTATTATTGGTTACGCGTCAAAAAAAAAAAAAAAAAAAAAAAAAAA          1313
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(B)

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1_TV_MnP        MAFKLLGSFVSLLAALQVANGALTRRVTCATGQVTSN-AACCALFPVIDDIQTNLFDGGE
2_TV_MnP[1]     MAFKLLASFVSVLAALQVANGAATRKVTCAGGQVTAN-AACCALFPVLEDIQTNLFDGGE
3_TV_MnP[2]     MAFKLLASFVSLLAALQVANGAATKKVTCASGQVTSN-AACCALFPVIDDIQANMFDGGG
4_TV_MnP[3]     MAFKLLASFVSLLAALQVANGAATKKVTCASGQVTSN-AACCALFPVIDDIQANMFDGGE
5_PR_MnP        ---MAFKQLLTAISIVSVANAALTRRVACPDGVNTATNAVCCSLFAVRDLIQDQLFDGGE
                            *** * *  * *  *  *   * ** ** *   **   ****

1_TV_MnP        CGEEVHESLRLTFHDAIGISPAIAKTGVFGGGGADGSIAIFADIETNFHANNGVDEIIGE
2_TV_MnP[1]     CGEEVHESLRLTFHDAIGISPAIARTGKFGGGGADGSIAIFADIETNFHANNGVDEIIGE
3_TV_MnP[2]     CNEDVHESLRLTFHDAIGISRKADKEGVFGGGGADGSIAIFANIETNFHANNGVDEIIDT
4_TV_MnP[3]     CNEDVHESLRLTFHDAIGISRKANKAGVFGGGGADGSIAIFADIETNFHANNGVDEIIDT
5_PR_MnP        CGEEVHESLRLTFHDAIGISPTIASTGVFGGGGADGSIAIFAEIETNFHANNGVDEIIGE
                * * ****************      * ************** ***************

1_TV_MnP        QAPFIARHNLTTADFIQLAGAIGVSNCPGAPRLNVFIGRKDATQPAPDLTVPEPFDDVTK
2_TV_MnP[1]     QAPFIARHNLTTADFIQFAGAIGVSNCPGAPRLNVFIGRKDATKPAPDLTVPEPFDDVTK
3_TV_MnP[2]     QAPIIARHNLTTADFIQFAGAIGASNCPGAPRLDVFLGRKDATQPAPDLTVPEPFDDVSK
4_TV_MnP[3]     QAPIIARHNLTTADFIQFAGAIGVSNCPGAPRLDVFLGRKDATQPAPDLTVPEPFDDVTK
5_PR_MnP        QAPFIQMTNMTTADFIQFAGAVGVSNCPGAPALPVFVGRPDATQPAPDKTVPEPFDTVDS
                *** *   * ******* *** * ******* * ** ** *** **** ******* *

1_TV_MnP        ILARFEDAGKFTPAEVVALLASHTIAAADHVDPTIPGTPFDSTPELFDTQFFIETQLRGT
2_TV_MnP[1]     ILARFEDAGKFTPAEVVALLASHTIAAADHVDPTIPGTPFDSTPELFDTQFFIETQLRGT
3_TV_MnP[2]     ILARFDDAGKFSSDEVVALLASHTIAAADHVDPTIPGTPFDSTPELFDTQFFIETQLRGT
4_TV_MnP[3]     ILARFDDAGKFSSDEVVALLVSHTIAAADHVDPTIPGTPFDSTPELFDTQFFIETQLQGT
5_PR_MnP        ILARFADAGGFSSAEVVALLASHTIAAADHVDPSIPGTPFDSTPEIFDTQFFIETQLRGI
                ***** *** *   ****** ************ *********** *********** *

1_TV_MnP        LFPGNGSNQGEVQSPLGGELRLQSDGLLARDQRTACEWQSFVNNQAKLQSAFKAAFARMT
2_TV_MnP[1]     LFPGNGSNQGEVMSPLHGELRLQSDAELARDARTACEWQSFVNNQAKLQSAFKAAFAKMT
3_TV_MnP[2]     LFPGNGSNQGEVMSPLHGELRLQSDAELARDARTACEWQSFVNNQAKLQSAFKAAFAKMT
4_TV_MnP[3]     LFPGNGSNQGEVMSPLRGEIRLQSDFLLARDSRTACEWQSFVNNQYKLQSAFKAAFRKMT
5_PR_MnP        LFPGTGGNQGEVESPLHGEIRLQSDSELARDSRTACEWQSFVNNQAKIQSAFKAAFRKMT
                **** * ***** *** ** *****  **** ************* * ********  **

1_TV_MnP        VLGQNTRALIDCSDVVPTPPAPASKAHFPAGLSRRDIEQACRATPFPTLPTDPGPVTTVA
2_TV_MnP[1]     VLGQNTRELIDCSDVVPTPPAPASAAHFPAGLTHRDVEQACRQTPFPALRTDPGPATSVA
3_TV_MnP[2]     VLGQNTRELIDCSDVVPTPPAPASAAHFPAGLTHRDVEQACRQTPFPALRTDPGPATSVV
4_TV_MnP[3]     ILGSKEHNLIDCSDVVPTPPAPASKAHLPAGLTRQDVQQACNKKAFPTLPTDPGPVTSVA
5_PR_MnP        ILGHSESSLIECSEVIQTPPALEGNAHLPAGQTMNDIEQACATTPFPSLSADPGPATSVA
                 **     ** ** *  ****    ** ***    *  ***    ** *  **** * *

1_TV_MnP        PVPPS
2_TV_MnP[1]     PVPPS
3_TV_MnP[2]     PVPPS
4_TV_MnP[3]     PVPPS
5_PR_MnP        PVPPS
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Fig. 1. (A) Nucleotide sequence of the cloned MnP cDNA and its deduced amino acid sequence. Single- line arrows represent the primers used in the first PCR for the cDNA fragment amplification. Double-line arrows represent the 5'- and 3'-RACE primers. The asterisk represents the stop codon. (B) Comparison of the amino acid sequence of the MnP cDNA with the reported MnP cDNA genes. Asterisks represent identical amino acids in all 5 genes. TV_MnP, cloned MnP gene in this research; TV_MnP[1], MnP of *Trametes versicolor* (Accession no. AAT90351) showing 92.3% identity; TV_MnP[2], MnP of *T. versicolor* (Accession no. AAT90350) showing 87.6% identity; TV_MnP[3], MnP of *T. versicolor* (Accession no. CAA91043) showing 86.2% identity; PR_MnP, MnP of *Phlebia radiata* (Accession no. CAC84573) showing 75.1% identity.

Construction of an expression vector for the MnP cDNA gene by homologous expression

The full length cDNA was amplified with two specific primers, 5'-AGGATCCGAGCTCTCTTCCTC-3' as the forward primer and 5'-GGATCCACAAACTCAAGACAGC-3' as the reverse primer, both of which had the *Bam*HI linkers. The amplified cDNA gene was cloned into a T&A vector, digested with *Bam*HI, and ligated with pBARGPE1 digested with the same enzyme in order to construct an expression clone designated as pBARTvMnP2. This vector had the phosphinothricin resistance gene as a selectable marker for fungal transformation, and the orientation of the inserted gene was confirmed through nucleotide sequencing using the sequencing primers in the vector.

Generation of transformant strains showing high enzyme activity

T. versicolor monokaryon was transformed using the expression clone pBARTvMnP2 as previously described (Kim *et al.*, 2002). Transformants showing normal growth on the phosphinothricin plate (300 μg/ml in CKMM medium; Leem *et al.*, 1999) were transferred to the same fresh plate. The transformants were then crossed with a mating partner strain (Kim *et al.*, 2002) to make sure the transformants were not contaminants, but had originated from *T. versicolor*. Genomic DNAs from the recipient strain and transformants were used as the template for PCR using two primers specific to the *trpC* promoter; 5'-GTCGACAGAAGATGATATT-3' as the forward primer and *bar*; 5'-AGTTAGACAACCTGAAG TCT-3' as the reverse primer for confirmation of the genomic integration of pBARTvMnP2. A peroxidase assay was performed by spectrophotometry with 3-amino-9-ethyl-carbazole (so-called carbazole) as the chromogenic substrate, along with H_2O_2 as the co-substrate (Kerby and Somerville, 1989). The decolorizing activity of a dye (remazole brilliant blue R: RBBR) was also carried out by plating the transformants on an RBBR-containing plate (Lee and Shin, 2000).

Determination of MnP expression in transformant by RT-PCR with MnP-specific primers

The recipient strain, and a transformant that showed higher enzyme activity as well as better decolorization than the recipient strain, were grown in complete YMG liquid medium, and their total RNAs were isolated from the 4 day-old cells as mentioned above. The first cDNA strand was synthesized from 1 μg of RNA using Powerscript RTase (Clontech) by following the manufacturer's instructions. RT-PCR was run using the cDNA strand as the template with two MnP-specific primers (forward primer; 5'-GTCTAGAAGCTCTCTT CCTCCA-3' and reverse primer; 5'-GGATCCACAACTCAA GACAGC-3'). The amplified bands were separated in 1% agarose gel.

Results and Discussion

Cloning of the full-length MnP cDNA clone

From the first cDNA fragment amplified by the F1 and R1 primers, the 5'- and 3'-region were extended using the RACE-PCR technique with the two specific primers (F2 and R2) and the RACE primers. The full-length cDNA clone of MnP2 (1,313 bp) and its deduced amino acid sequence (364 amino acids) were deposited in the EMBL Nucleotide Sequence Database (Accession no. AJ745879), as indicated in Fig. 1A. The putative MnP protein sequence showed high similarities (86.2-92.3%) with three known MnPs in *T. versicolor* (Accession no. CAA91043, AAT90350, and AAT90351), and also showed 75.1% similarity with the MnP from *Phlebia radiata* (Accession no. CAC84573) (Fig. 1B). With these results, the cloned cDNA was designated as an additional manganese peroxidase of *T. versicolor* (Accession no. CAG33918).

It is quite reasonable that *T. versicolor* has at least 4 MnP isogenes because this fungus shows high lignin-degrading activity (Johansson *et al.*, 2002), as well as degradation of various recalcitrant compounds such as phenanthrene (Han *et al.*, 2004), explosives (Cheong *et al.*, 2006), and other aromatic hydrocarbons (Song, 1997). *P. chrysosporium* also has 4 MnP isozyme genes, and they are differentially regulated at the transcriptional level (Alic *et al.*, 1997). In the case of the MnPs in *Physisporinus rivulosus*, two genes were shown to be regulated differently at the transcriptional level by manganese and veratryl alcohol (Hakala *et al.*, 2006). Therefore, it is necessary to determine how the 4 isogenes in this fungus are regulated.

Construction of an expression vector and the generation of transformant strains with high MnP activity

The expression clone (pBARTvMnP2) was inserted into the genome of the *T. versicolor* 9522-1 strain by genetic transformation. Several transformants were selected and vector integration was confirmed by PCR using *trpC* promoter-*bar* specific primers. A specific fragment (1.4 kb) was amplified from each transformant, while there was no band from the recipient strain (Fig. 2). When the general peroxidase activities of the transformants were analyzed with carbazole as the substrate, transformant TF6 showed an enzyme activity that was approximately 45% higher than that of the recipient strain (Fig. 3A). TF6 also showed better decolorizing activity when it was grown on an RBBR plate (Fig. 3B). The MnP expression of TF6 in an YMG liquid medium was compared with the recipient strain by RT-PCR. Both fungal strains showed high MnP expression since *T. versicolor* had its own MnP gene. However, TF6 showed an increased expression of MnP on day 4, resulting from the inserted MnP clone (Fig. 3C). Also, TF6 showed increased MnP activity even after 10 consecutive transfers on any complete medium, which meant this strain was genetically stable. We had many transformants that showed decreased MnP activities compared to the recipient stain, but this phenomenon is common in the transformation of filamentous fungi because the introduced foreign gene is inserted through random integration.

When *Coprinus cinereus* was transformed with a manganese peroxidase cDNA from *P. ostreatus* using a heterologous expression vector, two of the transformants showed higher lignin decolorizing activities (Ogawa *et al.*, 1998). A laccase gene (laccase IIIb) of *T. versicolor* was also expressed in the

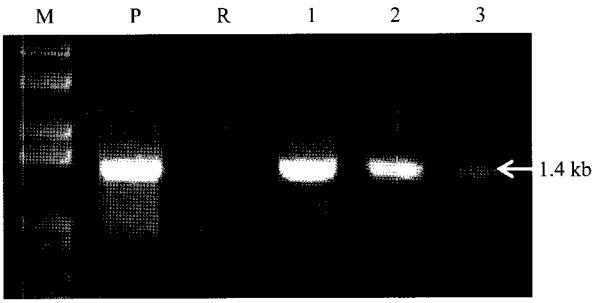

Fig. 2. Confirmation of the integration of the pBARTvMnP2 vector into transformant chromosomal DNAs by PCR with the *trpC* promoter-*bar* specific primers. M, molecular weight marker (1 kb ladder); P, positive control with the vector; R, negative control using the chromosomal DNA of the recipient strain; 1-3, chromosomal DNAs from the three different transformants showing the amplified bands (white arrow, 1.4 kb).

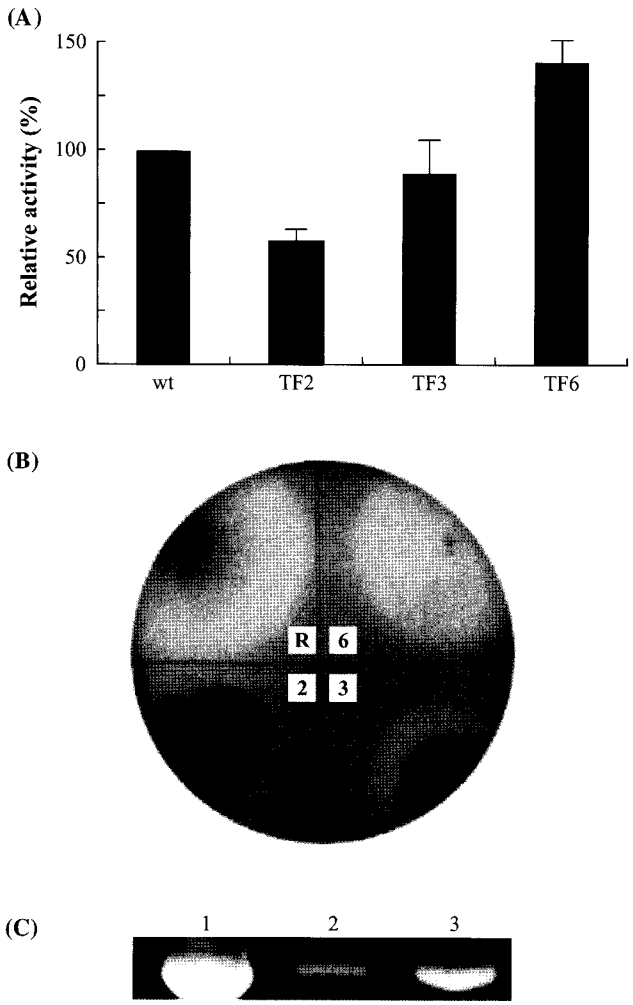

Fig. 3. (A) Determination of peroxidase with carbazole as the chromogenic substrate. Each experiment was run with triplicates and error bars are indicated. Wt, recipient strain (9522-1); TF2, TF3, and TF6 represent three different transformants, and TF6 shows 45% higher MnP activity than that of the recipient strain. (B) Comparison of decolorizing activity on RBBR-plate. R, recipient strain; 2, 3, and 6 represent the three different transformants. (C) Determination of MnP expression in the recipient and TF6 strains by RT-PCR using MnP-specific primers. 1, positive control with the pBARTvMnP2; 2, amplified RT-PCR product from the RNA of 4 day-old cells of the recipient strain; 3, same as in lane 2, but the cells of TF6.

yeast *Yarrowia lipolytica* for use in possible environmental applications (Jolivalt *et al.*, 2005). The technique described here is valuable for the generation of better strains, which may be useful in the biodegradation of recalcitrant compounds.

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References

Alic, M., L. Akileswaran, and M.M. Gold. 1997. Characterization of the genes encoding manganese peroxidase isozymes 3 from *Phanerochaete chrysosporium*. *Biochim. Biophys. Acta* 1338, 1-7.

Beltz, L.A., D. Neira, C. Axtell, S. Iverson, W. Deaton, T. Waldschmidt, J. Bumpus, and C. Johnston. 2001. Immunotoxicity of explosive-contaminated soil before and after bioremediation. *Arch. Environ. Contam. Toxicol.* 40, 311-317.

Cheong, S., S. Yeo, H.G. Song, and H. Choi. 2006. Determination of laccase gene expression during degradation of 2,4,6-trinitrotoluene and its catabolic intermediates in *Trametes versicolor*. *Microbiol. Res.* 161, 316-320.

Dias, A., R. Bezerra, P. Lemos, and A. Pereira. 2003. *In vivo* and laccase characterization of xenobiotic azo dyes by basidiomycetous fungus: characterization of its lignolytic system. *World J. Microbiol. Biotechnol.* 19, 969-975.

Hakala, T., K. Hilden, P. Maijala, C. Olsson, and A. Hadakka. 2006. Differential regulation of manganese peroxidases and characterization of two variable MnP encoding genes in the white-rot fungus *Physisporinus rivulosus*. *Appl. Microbiol. Biotechnol.* 73, 839-849.

Han, M., H. Choi, and H.G. Song. 2004. Degradation of phenanthrene by *Trametes versicolor* and its laccase. *J. Microbiol.* 42, 94-98.

Johansson, T., P. Nymann, and D. Cullen. 2002. Differential regulation of mnp2, a new manganese peroxidase encoding gene from the lignolytic fungus *Trametes versicolor* PRL572. *Appl. Environ. Microbiol.* 68, 2077-2080.

Jolivalt, C., C. Madzak, A. Brault, E. Caminade, C. Malosse, and C. Mougin. 2005. Expression of laccase IIIb from the white-rot fungus *Trametes versicolor* in the yeast *Yarrowia lipolytica* for environmental applications. *Environ. Biotechnol.* 66, 450-456.

Kamitsuji, H., Y. Honda, T. Watanabe, and M. Kuwahara. 2004. Production and induction of manganese peroxidase isozymes in a white-rot fungus *Pleurotus ostreatus*. *Appl. Microbiol. Biotechnol.* 65, 287-294.

Kerby, K. and S. Sommerville. 1989. Enhancement of specific intercellular peroxidases following inoculation of barley with *Erysiphe gaminis* f. sp. Hordei. *Physiol. Mol. Plant Pathol.* 35, 323-337.

Kim, K., Y. Leem, K. Kim, K. Kim, and H. Choi. 2002. Transformation of medicinal basidiomycete *Trametes versicolor* to hygromycin B resistance by restriction enzyme mediated integration. *FEMS Microbiol. Lett.* 209, 273-276.

Kim, Y., S. Cheong, S. Kim, H.-G. Song, and H. Choi. 2003. Partial cloning of genes for lignin degrading enzymes in *Trametes versicolor*. *Kor. J. Microbiol.* 39, 201-205.

Kim, Y., S. Yeo, J. Keum, H.G. Song, and H. Choi. 2005. Cloning of a manganese peroxidase cDNA gene repressed by manganese in *Trametes versicolor*. *J. Microbiol.* 43, 569-571.

Lee, Y.J. and K.S. Shin. 2000. Isolation and characterization of the second extracellular peroxidase of the white-rot fungus *Coriolus hirsutus*. *Mycologia* 96, 1057-1063.

Leem, Y., S. Kim, I. Ross, and H. Choi. 1999. Transformation and laccase mutant isolation in *Coprinus congregatus* by restriction enzyme-mediated integration. *FEMS Microbiol. Lett.* 172, 35-40.

Manubens, A., M. Avila, P. Canessa, and R. Vicuna. 2003. Differential regulation of genes encoding manganese peroxidase (MnP) in the basidiomycete *Ceriporiopsis subvermispora*. *Curr. Genet.* 43, 433-438.

Ogawa, K., T. Yamazaki, T. Hasebe, S. Kajiwara, A. Watanabe, Y. Asada, and K. Shishido. 1998. Molecular breeding of the basidiomycete *Coprinus cinereus* strains with high lignin-decolorization and -degradation activities using novel heterologous protein expression vectors. *Appl. Microbiol. Biotechnol.* 49,

285-289.

Song, H.G. 1997. Biodegradation of aromatic hydrocarbons by several white-rot fungi. *J. Microbiol.* 35, 66-71.

Takano, M., A. Nishida, and M. Nakamura. 2001. Screening of wood-rotting fungi for kraft pulp bleaching by the poly R decolorization test and biobleaching of hardwood kraft pulp by *Phanerochaete crassa* WD1694. *J. Wood Sci.* 47, 63-68.

Tien, M. and C.P.D. Tu. 1987. Cloning and sequencing of a cDNA for a lignase from *Phanerochaete chysosporium. Nature* 326, 520-523.

Wariishi, H., K. Valli, and M.H. Gold. 1989. Oxidative cleavage of a phenolic diarylpropane lignin model dimer by manganese peroxidase from *Phanerochaete chrysosporium. Biochem.* 28, 6017-6023.

Wariishi, H., K. Valli, and M.H. Gold. 1991. *In vitro* depolymerization of lignin by manganese peroxidase of *Phanerochaete chrysosporium. Biochem. Biophys. Res. Comm.* 176, 269-276.

Wariishi, H., L. Akileswaran, and M.H. Gold. 1988. Manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*: spectral characterization of the oxidized states and the catalytic cycle. *Biochem.* 27, 5365-5370.