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Discovery and characterization of new *O*-methyltransferase from the genome of the lignin-degrading fungus *Phanerochaete chrysosporium* for enhanced lignin degradation

Le Thanh Mai Pham, Yong Hwan Kim *

Department of Chemical Engineering, Kwangwoon University, 447-1, Wolgye-Dong, Nowon-Gu, Seoul 139-701, Republic of Korea

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ABSTRACT

Using bioinformatic homology search tools, this study utilized sequence phylogeny, gene organization and conserved motifs to identify members of the family of *O*-methyltransferases from lignin-degrading fungus *Phanerochaete chrysosporium.* The heterologous expression and characterization of *O*-methyltransferases from *P. chrysosporium* were studied. The expressed protein utilized *S*-(5′-adenosyl)-L-methionine *p*-toluenesulfonate salt (SAM) and methylated various free-hydroxyl phenolic compounds at both *meta* and *para* site. In the same motif, *O*-methyltransferases were also identified in other white-rot fungi including *Bjerkandera adusta*, *Ceriporiopsis (Gelatoporia) subvermispora* B, and *Trametes versicolor.* As free-hydroxyl phenolic compounds have been known as inhibitors for lignin peroxidase, the presence of *O*-methyltransferases in white-rot fungi suggested their biological functions in accelerating lignin degradation in white-rot basidiomycetes by converting those inhibitory groups into non-toxic methylated phenolic ones.

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1. Introduction

White-rot fungi have their own systems for generating hydrogen peroxide and can completely degrade lignin into carbon dioxide and water under aerobic conditions. Lignin peroxidases and versatile peroxidases have been reported to be the key enzymes responsible for the degradation of lignin, whereas manganese peroxidases can oxidize small phenolic structures [1–5]. *Phanerochaete chrysosporium* (*P. chrysoporium*) is the most intensively studied white-rot fungus [6,7] which secreted the highly-active lignin-degrading peroxidase, LiPH8. However, it has become clear that the *in-vitro* degradation of lignin by peroxidases is slow and inefficient compared with the *in-vivo* degradation by white-rot fungi. A previous study reported that a deficient electron transfer between the surface active site Trp171 and the heme site is caused by an inhibitory interaction between free-hydroxyl phenolic groups and the surface active sites. This resulted in the slow and inefficient oxidation of lignin [8]. Replacing the free-hydroxyl phenolic group on monolignolic analogs with a methyl group can resolve this inhibitory interaction. Under *in-vitro* conditions, the engineered 4-*O*-methyltransferase from *Clarkia breweri* (*C. breweri*), known as Isoeugenol 4-*O*-methyltransferase (IEMT_CLABR), acts as an auxiliary enzyme that protects the surface active sites of LiPH8 from free-hydroxyl phenolic groups and helps retain its oxidative activity on veratryl alcohol. A new multistep, enzyme-catalyzed strategy has been suggested for utilization in highly effective lignin degradation in a biorefinery process [9].

Recently, intensive genome sequencing work has focused on this fungus and has predicted a comprehensive catalog of enzymes, metabolic processes, regulatory and secretory mechanisms [12–15]. In this study, by taking advantage of the genomic database, all of the *O*-methyltransferase genes from *P. chrysosporium* were comprehensively identified and analyzed using sequence phylogeny, gene organization, and conserved motifs. Annotation and functional analyses also revealed the presence of various *O*-methyltransferases, not only in *P. chrysosporium,* but also in other white-rot fungi, such as *Bjerkandera adusta* (*B. adusta*), *Ceriporiopsis (Gelatoporia) subvermispora B* (*C. subvermispora)* and *Trametes versicolor* (*T. versicolor*). In addition, the heterologous expression and characterization of the annotated *O*-methyltransferases found on the genome of *P. chrysosporium* fungus were studied. The presence of *O*-methyltransferases was suggested to remove the free-hydroxyl phenolic compound from lignin degradation which was known as inhibitor for lignin peroxidase. In other word, detoxifying toxic phenolic group may help

* Corresponding author. Fax: +82 2 941 1785.
E-mail address: metalkim@kw.ac.kr (Y.H. Kim).

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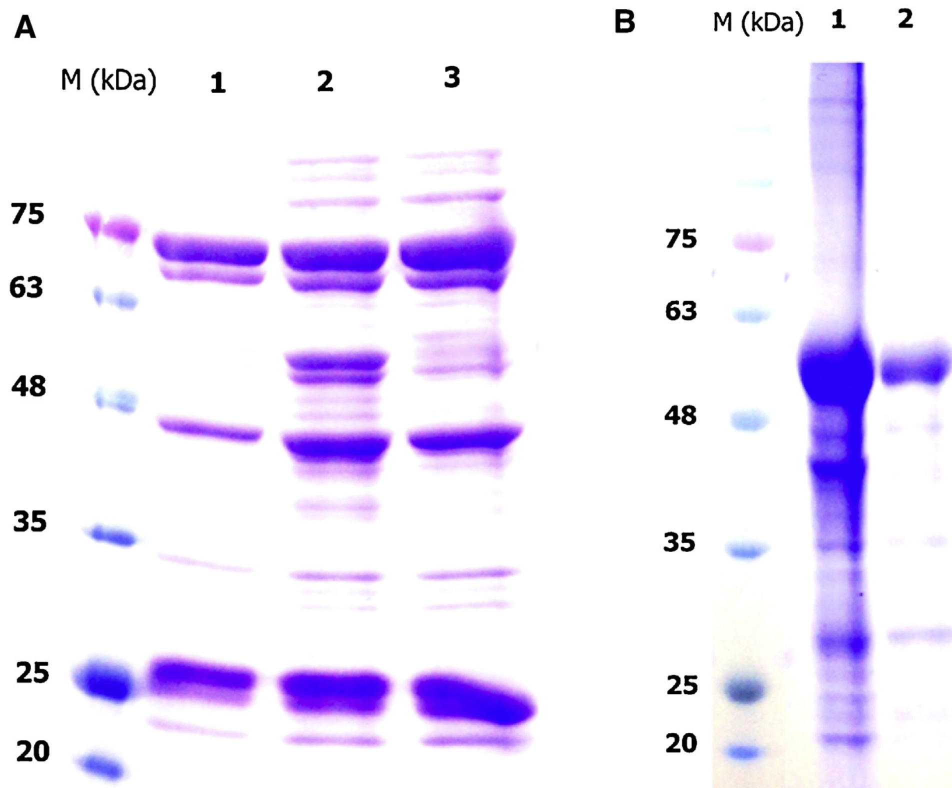

Fig. 1. Expression of pCold III DNA inserted Mtrase#1 and Mtrase#2 in *E. coli*. (A) Soluble protein fraction of recombinant *E. coli* cell—Lane M: protein marker (kDa), Lane 1: *E. coli* cell harboring pCold III DNA vector, Lane 2: *E. coli* cell harboring pCold III DNA vector inserted Mtrase#1 gene, Lane 3: *E. coli* cell harboring pCold III DNA vector inserted Mtrase#2 gene. (B) Insoluble expression and refolding of Mtrase#2—Lane M: protein marker (kDa), Lane 1: insoluble fraction of *E. coli* cell harboring pCold III DNA vector inserted Mtrase#2 gene, Lane 2: refolded protein fraction of *E. coli* cell harboring pCold III DNA vector inserted Mtrase#2 gene.

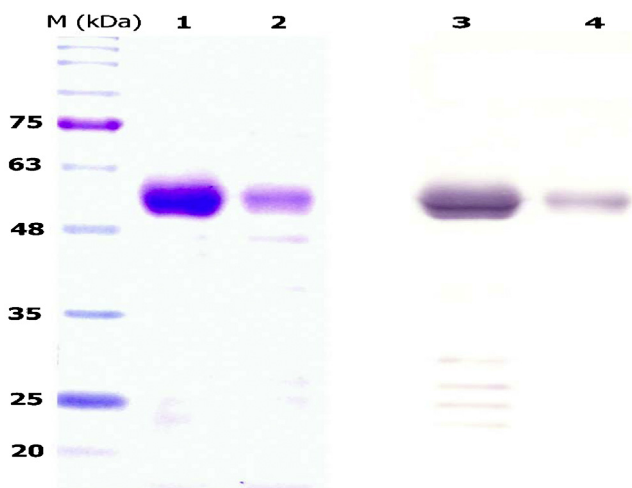

Fig. 2. (A) Purified fraction of Mtrase#1 (Lane 1) and Mtrase#2 (Lane 2), (B) western blotting of Mtrase#1 (Lane 3) and Mtrase#2 (Lane 4).

peroxidase activity maintain for a longer time, resulting in the acceleration of lignin degradation by white-rot fungi.

2. Materials and methods

2.1. Chemicals and microorganism

The hydrogen peroxide, ampicillin, isopropyl-*b*-D-thiogalactopyranoside (IPTG), guanidine hydrochloride, dibasic potassium phosphate, citric acid, trizma hydrochloride, Tris base, *S*-(5′-adenosyl)-L-methionine *p*-toluenesulfonate salt (SAM), *S*-(5′-adenosyl)-L-homocysteine (SAH), veratryl alcohol (VA) and various phenolic compounds that were used in this study were purchased from Sigma Chemical Co. (South Korea) and were used without any further purification. Bugbuster reagent was purchased from Novagen, and gene synthesis was conducted by Bioneer (South Korea). Vector pCold III DNA vector and competent *E. coli* BL21 (DE3) strain were purchased from Takara Clontech Co. (Japan). Ni-NTA agarose was purchased from Qiagen (Germany). Anti-6X His tag® antibody was from Abcam Co. (UK)

2.2. The genome screening and analysis of O-methyltransferases

The assembly and annotation of *P. chrysosporium* *O*-methyltransferase genes consisted of the following steps. (1) Screening of the automatically annotated genome using the Joint Genome Institute Portal was conducted with the BLASTP tool, and the BLOSUM62 scoring matrix was used as the default set. The genomic database sources for *P. chrysosporium v2.0, B. adusta v1.0, C. subvermispora* and *T. versicolor* were retrieved from MycoCosm (http://genome.jgi.doe.gov/programs/fungi/index.jsf) [16]. (2) Protein functions were identified by using InterProScan 5, which provided a functional analysis of the proteins by classifying them into families and predicting domains and domain sites (http://www.ebi.ac.uk/interpro/) [17] (3) Physical properties, such as isoelectric point (pI) and molecular weight (MW), were predicted using the ExPASy pI/Mw tool (http://web.expasy.org/compute_pi/) [18]. (4) A homology-based molecular model was generated using SWISS-MODEL (http://swissmodel.expasy.org/) [19], with the genome of the engineered 4-*O*-methyltranferase from *C. breweri* (PDB ID: 3TKY) as a template. Visualization of the substrate-binding sites was performed using PyMOL software. (5) Phylogenetic analysis of predicted proteins and identified *O*-

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