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**Comprehensive Genomic and Transcriptomic Analysis of
Polycyclic Aromatic Hydrocarbon Degradation by a
Mycoremediation Fungus, *Dentipellis* sp. KUC8613**

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Abstract

The environmental accumulation of polycyclic aromatic hydrocarbons (PAHs) is of great concern due to potential carcinogenic and mutagenic risks, as well as their resistance to remediation. While many fungi have been reported to break down PAHs in environments, the details of gene-based metabolic pathways are not yet comprehensively understood. Specifically, the genome-scale transcriptional responses of fungal PAH-degradation have rarely been reported. In this study, we report the genomic and transcriptomic basis of PAH-bioremediation by a potent fungal degrader, *Dentipellis* sp. KUC8613. The genome size of this fungus was 36.71 Mbp long encoding 14,320 putative protein-coding genes. The strain efficiently removed more than 90 % of 100 mg/liter concentration of PAHs within ten days. The genomic and transcriptomic analysis of this white rot fungus highlights that the strain primarily utilized non-ligninolytic enzymes to remove various PAHs, rather than typical ligninolytic enzymes known for playing important roles in PAH-degradation. PAH-removal by non-ligninolytic enzymes was initiated by both different PAH-specific and common overexpression of P450s, followed by downstream PAH-transforming enzymes such as epoxide hydrolases, dehydrogenases, FAD-dependent monooxygenases, dioxygenases and glycosyl- or glutathione transferases. Among the various PAHs, phenanthrene induced a more dynamic transcriptomic response possibly due its greater cytotoxicity, leading to highly upregulated genes involved in the translocation of PAHs, a defense system against reactive oxygen species, and ATP synthesis. Our genomic and transcriptomic data provide a foundation of understanding regarding the mycoremediation of PAHs and the application of this strain for polluted environments.

65

66

67 **Keywords**

68 PAH (Polycyclic aromatic hydrocarbon), Mycoremediation, *Dentipellis* sp.

69 KUC8613, White rot fungus, Genomics, Transcriptomics

70 INTRODUCTION

71
72 Fungi play a major role as the decomposers of recalcitrant organic
73 matters in nature (de Boer et al. 2005). The potent degrading abilities of
74 fungi can be attributed to low specificity of catabolic enzymes and the
75 formation of mycelial networks that can enhance chemical bioavailability
76 (Harms, Schlosser and Wick 2011). Mycoremediation, which is based on
77 the use of fungi and mushrooms for the bioremediation of polluted areas,
78 is a promising method to remove many hazardous chemicals of
79 environmental and public health concern (Deshmukh, Khardenavis and
80 Purohit 2016).

81 Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals
82 with two or more fused aromatic rings of carbon and hydrogen atoms
83 (Kadri et al. 2017). Among the various toxic materials, PAHs has been one
84 of the major targets for mycoremediation due to their possible
85 carcinogenic and mutagenic risks (Mastrangelo, Fadda and Marzia 1996,
86 Schutzendubel et al. 1999). The high hydrophobicity and chemical stability
87 make them persistent in environments and cause bioaccumulation. While
88 PAHs can be naturally formed by forest fires or volcanic eruptions, the
89 incomplete combustion of organic materials during industrial and other
90 human activities account for the majority of PAH formation (Johansson and
91 van Bavel 2003). Several physical and chemical treatment methods
92 including incineration, UV oxidation, fixation, and solvent extraction have
93 been developed to remove PAHs, but they have many drawbacks since
94 they are not cost-effective and environment-friendly (Gan, Lau and Ng
95 2009). The biodegradation of PAHs using fungi and other microorganisms
96 has been regarded as an alternative method to remove PAHs without
97 causing significant ecological damages (Abe et al. 1995, Gran-Scheuch et
98 al. 2017).

99 It has been reported that many wood-degrading fungi can
100 efficiently degrade a wide variety of PAHs (Field et al. 1992). Both
101 ligninolytic and non-ligninolytic fungi can degrade PAHs with initial

oxidation of substrates but the metabolic pathways they use may differ (Pozdnyakova 2012, Marco-Urrea, Garcia-Romera and Aranda 2015). Ligninolytic fungi can produce extracellular ligninolytic enzymes including manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase to produce quinone intermediates. Non-ligninolytic degradation of PAHs may use intracellular cytochrome P450 monooxygenases (CYPs) to produce unstable arene oxides which can be subsequently converted into phenols or trans-dihydrodiols. It was previously reported that even some ligninolytic fungi, including *Phanerochaete* and *Pleurotus* can use CYPs instead of ligninolytic enzymes for PAH-degradation (Ghosal et al. 2016).

Although great efforts have been made to demonstrate PAH-degradation in wood-degrading fungi through proteomic and metabolic assays, whole genome and transcriptome studies have rarely been conducted. A genome scale survey of potential PAH-responsive genes and their regulation at transcriptional level can contribute to the comprehensive understanding of complex genetic networks in a fungal PAH-degradation system and identification of novel PAH-responsive genes encoded in the genome.

Dentipellis sp. KUC8613, one of white rot fungi in the order Russulales, has previously been screened by high tolerance to various PAHs and regarded as a potential host for mycoremediation (Lee et al. 2014). In this study, we report the efficient PAH-removal by the KUC8613 strain over various PAHs. In order to expand our knowledge of the genetic basis for the PAH-removal capability of this fungus, we sequenced and analyzed the genome of *Dentipellis* sp. KUC8613 as part of the 1000 Fungal Genomes Project (1k FGP) (Grigoriev et al. 2014) at the US Department of Energy Joint Genome Institute (JGI) (<http://jgi.doe.gov/fungi>). From the genome, we investigated genetic repertoires potentially involved in the metabolism of PAHs. We further performed transcriptomic analysis to observe differential expression of these genes by four different PAHs and to identify novel PAH-responsive genes. Our genomic and transcriptomic analysis to elucidate PAH-removal

134 by KUC8613 will give us a more comprehensive understanding of PAH-
135 degradation by wood-degrading fungi.

136

137

Materials and Methods

Fungal strains, chemicals, and media

Dentipellis sp. KUC8613 was originally isolated from South Korea and identified by the Korea University Culture collection (KUC). Anthracene (ANT), fluoranthene (FLU), phenanthrene (PHE), pyrene (PYR), and all solvents including ethyl acetate, chloroform, and acetonitrile were purchased from Sigma (Sigma-Aldrich). Malt extract (ME) medium contained 20 g malt extract for 1 liter distilled water. 15 g agar was additionally added for malt extract agar (MEA) medium.

Determination of tolerance to PAHs

Four different PAHs (ANT, FLU, PHE, and PYR) were used to determine tolerance of the fungus to PAHs. Fungal mycelia were cultured on MEA containing 100 mg/liter of individual PAH. PAHs were first dissolved in acetone prior to being added into the culture medium. Acetone was evaporated from the medium by letting it stand for a few days. An actively growing fungal disk was inoculated at the center of a culture plate and incubated at 25 °C. Each sample was prepared in triplicate and the growth rate of fungal mycelia was determined by measuring the radius of the mycelia at between 0 and 10 days.

Liquid culture condition and determination of mycelial growth

Fungal mycelium was grown on MEA at 25 °C and maintained for less than a week at 5 °C before use. Liquid ME containing 100 mg/liter of individual PAH was prepared. PAHs were first dissolved in acetone and added into ME medium. Acetone was evaporated from the medium by letting it stand for a few days before fungal inoculation. An actively growing mycelium disc (4 cm diameter) was homogenized for 10 seconds in a sterile blender cup, containing 50 ml of ME medium. 2 ml of the homogenate was used to inoculate 50 ml ME medium in a 250 ml Erlenmeyer flask. Mycelium was grown at 25 °C in a rotary shaker with

shaking at 180 rpm. Heat-killed mycelia (400 mg in dry weight) and cultures without fungal inoculation were also used for the determination of PAH-adsorption to mycelia and natural degradation. Cultures were incubated for between 0 and 10 days.

For the determination of mycelial growth during liquid culture, mycelium was harvested after incubation by centrifugation and washed twice with 50 ml distilled water to a final volume of 10 ml. The sample was then and filtered through pre-weighted dried filter paper filter in a vacuum filtration apparatus. The mycelium and filter paper were oven-dried at 65 °C for one day, and dry weight was measured.

HPLC analysis of PAH-removal

The dried extracts were dissolved in 1 ml chloroform. For the quantitative analysis of residual PAHs, Waters high-pressure liquid chromatography (HPLC) system (Milford, MA, USA) equipped with Waters 2487 UV detector was used. Data acquisition was carried out using the Waters Empower 2 software. Ten microliters of each extract was injected onto the Waters C18 column with a flow rate of 1 ml/min. The mobile phase was acetonitrile : water (80:20) and the PAHs were detected at 254 nm.

Genomic DNA extraction and sequencing

The fungus was cultured on solid MEA media at 25 °C in the dark. Genomic DNA was extracted from mycelium using a CTAB-based fungal DNA isolation protocol (Fulton, Chunwongse and Tanksley 1995). The concentration of prepared DNA was determined using Qubit fluorometer (Invitrogen). For the whole genome sequencing of *Dentipellis* sp. KUC8613, two Illumina libraries with insert sizes 5.5 kb and 370 bp were prepared and sequenced using 2 × 150 bp reads from HiSeq-1TB. The sequence data was filtered so as to remove low quality reads and subsequently assembled with AllPathsLG release version R44008 (Gnerre et al. 2011). The genome was annotated using the JGI Annotation Pipeline

(Grigoriev et al. 2014) and FunGAP (Min, Grigoriev and Choi 2017). From JGI-MycoCosm repository (<https://genome.jgi.doe.gov/Densp1/Densp1.home.html>), we could retrieve the genome wide annotation data such as InterPro, CAZy, KOG, and KEGG for functional analysis.

RNA extraction and mRNA sequencing

For transcriptomic analysis, RNA from 5 days of culture were used. The grown mycelium was harvested from the culture medium by centrifugation and washed twice with 50 ml distilled water. The mycelium pellet was immediately frozen under liquid nitrogen and homogenized using a mortar and a pestle. Total RNA was isolated using an RNeasy plus mini kit (Qiagen, the Netherlands) according to the manufacturer's protocol. RNA concentration was determined using Qubit fluorometer (Invitrogen). RNA library was constructed using TruSeq RNA sample preparation kit (Illumina, San Diego, CA, US). The sequencing of the RNA library was carried out in MiSeq (2 × 200 cycles). Low-quality reads were filtered using the Trim Galore program. The filtered RNA-seq data was mapped against the assembled genome using CLC Genomics Workbench software v.12.0 (Qiagen, the Netherlands). Read alignment was performed with the following parameters: minimum length fraction and minimum similarity fraction = 0.8, strand specific = both, maximum number of hits for a read = 3. Reads Per Kilobase of transcript per Million mapped reads (RPKM) value was generated using default settings of CLC genomics and further used for the analysis of differential expression of genes. To check if the RNA-seq data from different conditions correlate each other, a Principal Component Analysis (PCA) was performed by an internal routine of CLC Genomics Workbench.

Gene Ontology enrichment analysis of upregulated genes

Upregulated genes by PAHs were identified according to the following criteria: fold change > 2 and p-value < 0.01. To further reveal

234 the enriched functions among upregulated genes, the Gene Ontology (GO)
235 enrichment analyses of upregulated genes were performed using the R
236 package clusterProfiler (version 3.2.1) (Yu et al. 2012). The threshold for
237 the enrichment analysis was p-value < 0.01.

238

Results

PAH tolerance and removal capability by *Dentipellis* sp. KUC8613

We measured the PAH tolerance of the fungus according to the radial growth of mycelia in solid media (Fig. 1A). ANT, FLU, PHE, and PYR were used as the representative PAHs having two to four fused aromatic rings. The average growth rate of the fungus at 25 °C was 4.7 mm/day without PAH. When four different PAHs were individually added in the growth media, KUC8613 showed high tolerance to all four PAHs with different degrees. In more detail, no detectable growth inhibition by ANT, FLU, and PYR was observed at any time point within ten days. A moderate decrease (14 %) in growth was observed only in PHE-added media. No significant change in mycelial morphology or pigmentation by any of PAH was recognized (Data not shown).

In order to verify PAH-removal by this fungus, the removal of PAHs in liquid culture was examined (Fig. 2). HPLC-mediated quantification showed that 44 % (ANT), 49 % (FLU), 33 % (PHE), and 46 % (PYR) of PAHs were removed after five days. The removal of 90.1 % (ANT), 99 % (FLU), 94.3 % (PHE), and 94.4 % (PYR) of PAHs were observed after 10 days, while 17.4 % (ANT), 25 % (FLU), 24.4 % (PHE), and 27.7 % (PYR) were removed by autonomous chemical decomposition. We also observed that less than 10 % of PAHs could be removed by adsorption to heat killed mycelia. Fungal biomass (dry weight) in liquid culture was also measured (Fig. S1). Only PHE induced a moderate growth retardation, which was congruent with our observation in PAH tolerance test at solid media.

Genome properties of *Dentipellis* sp. KUC8613

In order to elucidate genetic contents for PAH-removal in this fungus, whole genome sequencing was carried out under the 1k FGP program. The genome sequence was 36.71 Mbp long, comprising 1,184 contigs and 425 scaffolds (Table 1). The GC content of the genome was 55.4 %. In total, 14,320 protein-coding genes were predicted in the

genome with an average gene length of 1,737 bp. Both the genome size and the number of gene models of KUC8613 were typical among the compared white rot fungi available from JGI mycocosm (Grigoriev et al. 2014). Among 14,320 gene models, 6,305 (44 %) and 7,538 (52.6 %) were assigned to different GO (Gene Ontology) terms and KOG (euKaryotic Ortholog Groups) classes, respectively.

A genomic repository of carbohydrate-active enzymes (CAZymes) was first surveyed in order to analyze the pattern of carbohydrate metabolism in this fungus. The genome harbored a total of 417 CAZymes including of 71 auxiliary activities (AA), 49 carbohydrate-binding modules (CBM), and 180 glycoside hydrolases (GH) (Table S1). The cellulolytic CAZyme composition in typical white rot fungi is generally represented by the presence of lytic polysaccharide monooxygenases (LMPOs) (AA9), cellobiohydrolases (GH6 and GH7), a single cellobiose dehydrogenase (AA3-1), frequent CBM1-containing proteins, and ligninolytic enzymes (Riley et al. 2014). Indeed, this strain revealed 11 copies of LMPOs that potentially carry out the oxidative cleavage of polysaccharide chains (Table S2). It also contained four cellobiohydrolases, 22 genes encoding proteins carrying a CBM1 family module, and a single gene encoding cellobiose dehydrogenase. The CAZyme-pattern suggests that *Dentipellis* sp. KUC8613 has the classical wood decay mode of previously well-characterized white rot fungi.

The genome of KUC8613 was annotated with 11 genes encoding ligninolytic enzymes, including seven fungal class II peroxidases (PODs; AA2) and four laccases (AA1_1). PODs were further classified into six MnPs and a single LiP by sequence comparison. When we compared the KUC8613 genome with eight other white rot fungal genomes, KUC8613 showed less than the average number of both PODs and laccase genes among the compared genomes (Fig. 3).

While only a limited number of genes encoding ligninolytic enzymes were found in the genome of KUC8613, we could instead identify other genes encoding non-ligninolytic type of enzymes that can potentially

mediate the initial oxidation of aromatic ring structures. The presence of 154 putative P450s were revealed from the fungal genome (Table S3). Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database analysis, we could further sort out 77 P450s potentially involved in the metabolism of xenobiotics, specifically those from CYP1, CYP2, and CYP3 families (Lewis 2003).

Additional genes that are potentially involved in the downstream steps for PAH-transformation were also investigated (Table S4). We identified 19 putative epoxide hydrolases that might catalyze a reaction to produce trans-dihydrodiols from arene oxides. Oxidoreductase enzymes including 16 alcohol dehydrogenases, 17 aldehyde dehydrogenases, one trans-1,2-dihydrobenzene-1,2-diol dehydrogenase, and 31 FAD-dependent monooxygenases may catalyze a series of oxidation reactions to produce metabolic intermediates. Multicomponent dioxygenases comprising eight ferredoxins, four ferredoxin reductases, two hydroxyquinol 1,2-dioxygenases, and two aromatic ring-opening dioxygenases were also found in the fungal genome. Aromatic intermediates formed by above enzymes then can act as substrates for additional ring cleavage or conjugation steps (Casillas et al. 1996, Habe and Omori 2003). Glutathione S-transferases (GSTs), sulfotransferases, or glycosyltransferases (GTFs) can catalyze the addition of glutathione, sulfate, or glycosyl donors to PAH molecules, respectively, thus making them less toxic and more water-soluble. In the genome of KUC8613, ten putative GSTs, two sulfotransferases, and 13 GTFs were encoded for the potential conjugation of PAH intermediates.

Genome-wide transcriptomic responses during PAH removal

In order to further investigate which of the potential genes are actually upregulated during PAH-removal in this fungus, a genome-wide transcriptomic analysis was performed. Mycelial discs grown for five days in liquid ME media supplemented with ANT, FLU, PHE, and PYR (100 mg/liter) were subjected to RNA-seq. Principal component analysis (PCA)

of the gene expression data clearly separated PAH-added groups from the control group (Fig. 4A). The PCA data also showed a separation within PAH samples. While the ANT, FLU, and PYR samples were clustered together, the PHE samples formed an independent group distinct from other PAH samples, suggesting that the gene expression profile of PHE sample is unlike those of other PAHs.

Of the 14,320 gene models predicted in the genome, we identified 1,922 genes whose expression was upregulated by at least one of four different PAHs (Fold change > 2 and P-value < 0.01) (Table S5). The numbers of total genes upregulated by ANT, FLU, PHE, and PYR were 915, 950, 1539, and 1,095, respectively (Fig. 4B). Among the upregulated genes, the molecular function of 772 genes could not be predicted. Upregulated genes were assigned to 23 specific KOG classes and dominantly (54.9 % of 1,041 KOG-assigned genes) categorized into five KOG classes including posttranslational modification, signal transduction, energy production and conversion, carbohydrate transport and metabolism, and transcription (Table S6). As illustrated by a Venn-diagram in Figure 4B, upregulated genes showed common and different PAH-specific overexpression during PAH-removal. A total of 550 genes were commonly overexpressed by all four PAHs. The numbers of PAH-specific genes by ANT, FLU, PHE, and PYR were 67, 72, 548, and 59, respectively. The number of PHE-induced genes were significantly higher than genes induced by other PAHs. A heatmap plotted by log₂RPKM of up-regulated gene expression showed a similar pattern as well (Fig. 4C). The majority of up-regulated genes were commonly overexpressed in all PAH-samples while PHE showed a distinct gene expression pattern compared to those of other three PAHs.

We first investigated the expression profiles of ligninolytic enzymes (LiP, MnP, and laccase) and P450s for initial aromatic-ring oxidation. No ligninolytic enzymes were upregulated during PAH-removal (Table S7). On the other hand, P450s showed both common and different PAH-specific overexpression. Among 154 putative P450 genes found in the KUC8613

367 genome, transcription of 15 genes was induced by one or more PAHs
368 (Table 2). Six P450 genes were constitutively upregulated by two or three
369 PAHs, while the remaining nine genes showed different PAH-specific
370 overexpression. These nine genes could be further categorized into six
371 PHE-inducible (ProtID 548471, 772453, 879478, 840982, 841965, and
372 873894) and one for each ANT-, FLU- or PYR-inducible genes (ProtID
373 861037, 798081, and 832704 for ANT, FLU, and PYR, respectively).

374 We also identified a total of 27 other PAH-responsive genes
375 potentially responsible for the downstream steps for PAH-transformation.
376 The expression patterns of these genes showed that most of them were
377 commonly overexpressed regardless of PAH-type (Fig. S2). These 27 PAH-
378 responsive genes were three epoxide hydrolases (ProtID 581771, 840197,
379 and 871191), three ferredoxins (ProtID 226033, 839077, and 887248), one
380 ferredoxin reductase (ProtID 832353), five alcohol dehydrogenases (ProtID
381 843873, 859290, 867273, 712202, and 886660), three aldehyde
382 dehydrogenases (ProtID 837250, 884278, and 857188), Five FAD-
383 dependent monooxygenases (ProtID 820609, 772612, 838248, 870155,
384 and 879414), two dioxygenases (ProtID 841503 and 842583), three GSTs
385 (ProtID 847321, 697729, and 830936), one GTF (ProtID 879011), and one
386 sulfotransferase (ProtID 838043).

387 In addition to the PAH-transforming genes, the 550 commonly
388 overexpressed genes by all four PAHs included 41 genes involved in lipid
389 transport and metabolism; these include a lipase (ProtID 835364), a sterol
390 desaturase (ProtID 864816), a methyltransferase (ProtID 833162), and a
391 phospholipid/glycerol acyltransferase (ProtID 39743) (Table S8). This
392 observation is consistent with previous studies in which the bioavailability
393 of hydrophobic PAHs can be enhanced by the cellular production of lipid
394 biosurfactants to emulsify PAHs and promote solubility (Cao et al. 2015).

395 Since our genomic and transcriptomic analysis suggests that this
396 fungus utilizes the intracellular non-ligninolytic type of enzymes instead of
397 extracellular ligninolytic enzymes for PAH-transformation, translocation of
398 PAHs and metabolic intermediates across the cell membranes and

organelles should be controlled by membrane bound transporters. It is well known that major facilitator superfamily (MFS) transporters and ATP-binding cassette (ABC) transporters are two major transporter families that mediate the import and export of drugs and xenobiotics (Jeong et al. 2017, Carmona et al. 2009). We observed the common upregulation of 14 MFS and five ABC transporters by all four PAHs (Table S9). The active transcription of MFS transporters was particularly noticeable by PHE. The more than ten-fold elevated expression of four MFS transporters (ProtID 787334, 857773, 800757, and 328771) by PHE suggests an important role of these transporters for cellular response to PHE.

While a significant number of genes were commonly overexpressed by all four PAHs, certain genes also showed PAH-specificity within different PAH-samples. Among four PAHs, PHE induced 35 % more genes than the average number of upregulated genes by other PAHs. This resulted in the PHE-specific overexpression of 548 genes (Fig. 4B). According to Gene Ontology (GO) functional enrichment analysis, enriched GO terms among PHE-specific genes were ATP synthesis coupled proton transport and different types of cytochrome c oxidases (Fig S3). We also identified 10 additional MFS and two ABC transporters, suggesting their restricted involvement in the translocation of PAHs other than PHE (Table S10). PAHs are known to increase the production of reactive oxygen species (ROS), leading to oxidative stress (Alkio et al. 2005). Since only PHE showed detectable cytotoxicity during fungal growth, significant upregulation of ROS scavenging enzymes is expected. Indeed, we observed the PHE-specific upregulation of genes such as manganese and iron superoxide dismutase (ProtID 648712, and 860732), and alkyl hydroperoxide reductase (ProtID 194063), which are potentially involved in enzymatic antioxidant defense mechanisms.

Discussion

PAH-transformation by wood-degrading fungi might be a ubiquitous phenomenon (Field et al. 1992, Mao and Guan 2016). In this study, we demonstrated that *Dentipellis* sp. KUC8613 have a high capability (> 90 % of 100 mg/L PAH) of removing four different types of PAHs within a short period of time. Our genomic and transcriptomic analysis to further elucidate PAH-transforming system encoded in the genome revealed a total of 1,922 upregulated genes many of which have unknown molecular functions. To our knowledge, genome-scale transcriptomic responses by a wood-degrading fungus during PAH-transformation have not been previously described.

While ligninolytic system in white rot fungi was often described as the key for PAH-transformation (Ghosal et al. 2016), our data showed that KUC8613 was capable of removing PAHs using P450s instead of ligninolytic enzymes. Ligninolytic enzymes may not be essential for PAH-transformation in this fungi, but they still may take part in PAH-transformation at certain growth conditions. It has been previously shown that the production of ligninolytic enzymes by white rot fungi can be dependent on carbon and nitrogen concentration in the growth media (Ben Hamman, de La Rubia and Martinez 1997, D'Souza, Merrit and Reddy 1999). It would be worthwhile to check PAH-removal pattern by this fungus under different conditions that favor the production of ligninolytic enzymes. Taken together, these data strongly suggest that the screening of strong mycoremediation hosts primarily based on ligninolytic enzyme activities should be reconsidered.

Our transcriptomic analysis revealed that P450s might be responsible for the initial oxidation of aromatic rings in this fungus. A previous genome-scale identification of P450 genes in *Phanerochaete chrysosporium* showed PAH-oxidizing activity of these enzymes with varying PAH specificity (Syed et al. 2010). Similarly, upregulated P450s in this fungus showed both PAH-specific and common overexpression patterns. PHE induced the highest number of P450 genes, suggesting

metabolic complexity of this compound compared to other three PAHs. In addition to P450s, we observed upregulation of many other genes encoding potential PAH-transforming activities such as epoxide hydrolase, alcohol dehydrogenase, aldehyde dehydrogenase, FAD-dependent monooxygenase, dioxygenase, GTF, GST and sulfotransferase. The biotransformation of PAHs by dioxygenases is mainly known to occur in many PAH-degrading bacteria (Peng et al. 2008), but in some *Trichoderma* species, involvement of dioxygenase was also reported during phenanthrene degradation (Hadibarata, Tachibana and Itoh 2007). The shared upregulation of many potential PAH-transforming genes suggests some overlap between the metabolic pathways of different PAHs. The elucidation of the exact molecular mechanisms associated with the key enzymes discovered in this study requires further study. In addition, upregulation of many genes with unknown molecular functions also gives a clue for future research into the identification of novel genes involved in PAH-transformation.

The distinct transcriptomic response induced by PHE in this fungus was explained by a large number of PHE-specific genes involved in translocation of PAHs, defense against ROS, and ATP synthesis. The reason why PHE induced the transcription of a significantly larger number of genes is not clear but our experimental evidences suggested that it might be associated with PHE-induced cellular cytotoxicity. A similar observation that PHE upregulated a larger number of genes than other PAHs was also previously reported in soil fungus (Gao et al. 2019).

Based on the global gene expression pattern, we constructed a tentative pathway for the transformation and detoxification of PAHs in *Dentipellis* sp. KUC8613 (Fig. 5). The pathway includes the initial ring-oxidation step followed by downstream transformation step as well as transporters for the translocation of PAHs and their metabolites, lipases for the enhanced solubility of PAHs, and antioxidant enzymes for reduced oxidative stress. Our data based on whole-genomic and transcriptomic analysis will provide a strong insight into the complex gene regulation of

492 wood-degrading fungi for PAH-degradation, and may serve as a guide for
493 the efficient screening and utilization of fungal hosts for mycoremediation.
494

Accession numbers and availability of the genome sequence and the strain

This whole genome sequence of *Dentipellis* sp. KUC8613 has been deposited at DDBJ/EMBL/GenBank under the accession number NSJX000000000. In addition, the genome assembly and annotation are available at DOE JGI Genome Portal MycoCosm (Grigoriev et al. 2014) (<http://genome.jgi.doe.gov/Densp1>). The strain is available from Korean Collection for Type Cultures (KCTC) with the accession number KCTC46678 (<http://kctc.kribb.re.kr/>)

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TABLES AND FIGURES LEGENDS

Table 1. Genome features of *Dentipellis* sp. KUC8613 compared with other white rot fungi

Table 2. Common and different PAH-specific upregulation of P450 genes during PAH-removal

Figure 1. Radial growth of the fungal mycelia at the presence of different PAHs. ANT, anthracene; FLU, fluoranthene; PHE, phenanthrene; PYR, pyrene.

Figure 2. PAH-removal efficiency by *Dentipellis* sp. KUC8613. The residual amount of PAH during the fungal culture was measured by HPLC. ANT, anthracene; FLU, fluoranthene; PHE, phenanthrene; PYR, pyrene; Control 1, 10 days of incubation without fungal inoculation; Control 2, 10 days of incubation with dead mycelia.

Figure 3. Comparison of relative tolerance to mixed PAHs (Lee et al., 2014) and the number of ligninolytic enzymes in white rot fungi. The species tree was built based on single-copy orthologs including *A. nidulans* as an out-group. The bootstrap-based branch supports and the scale bar that represents the mean number of amino acid substitutions per site are shown. Gene numbers are shaded white or red based on the Z-scores computed for each column.

Figure 4. Transcriptomic analysis during removal of four different PAHs. (A) Principal component analysis (PCA) of transcriptomics data. (B) Venn-diagram representing the number of upregulated genes by four different PAHs. (C) Heatmap showing transcription pattern ($\log_2$ RPKM) of 1,922 upregulated genes by four different PAHs. Columns represent different genes and rows represent different PAH-treated groups. ANT, anthracene; FLU, fluoranthene; PHE, phenanthrene; PYR, pyrene.

Figure 5. A schematic view of PAH-transformation in *Dentipellis* sp. KUC8613. Blue and Red arrows represent the common and PAH-specific

680 overexpression of PAH-transforming genes, respectively. The PAH-
681 transformation pathway was divided into 1) initial ring-oxidation, and 2)
682 downstream transformation steps based on key enzymatic reactions. ANT,
683 anthracene; FLU, fluoranthene; PHE, phenanthrene; PYR, pyrene.

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(Table 1)

Assembly statistics	<i>Dentipellis</i> sp. KUC8613	<i>H.</i> <i>annosum</i>	<i>T.</i> <i>versicolor</i>	<i>P. gigantea</i>	<i>B. adusta</i>	<i>P.</i> <i>brevispora</i> HHB-7030 SS6	<i>P.</i> <i>ostreatus</i> PC15	<i>S. paradoxa</i> KUC8140
Genome size (Mbp)	36.71	33.6	44.79	30.14	42.73	49.96	35.6	44.41
Number of contigs	1,184	630	1,443	1,195	1,263	3,178	3,272	1,374
Number of scaffolds	425	39	283	573	508	1,645	572	1,291
Annotation statistics								
Number of gene models	14,320	13,405	14,296	11,891	15,473	16,170	11,603	17,098
Average gene length (bp)	1,737	1,601	1,790	1,714	1,703	1,627	1,772	1,735
Average exon length (bp)	263	561	241	230	248	225	217	246
Average intron length (bp)	69	82	83	69	71	78	76	68
Average protein length (aa)	405	379	422	411	406	400	445	413
Number of exons per gene	5.47	5.39	5.81	6	5.59	5.66	6.4	5.78

(Table 2)

ProtID	Fold change ANT/ Control	P-value ANT/Contr ol	Fold change FLU/Contro l	P-value FLU/Contro l	Fold change PHE/Contr ol	P-value PHE/Contr ol	Fold change PYR/Contro l	P-value PYR/Contro l
859292	2.39*	1.00E-05	1.95	9.00E-04	2.59*	1.36E-06	2.63*	4.92E-07
882466	2.92*	8.40E-03	3.65*	1.30E-03	-	-	-	-
926550	-	-	2.13*	3.10E-03	-2.43	3.80E-03	2.03*	4.80E-03
833741	1.90	1.27E-05	2.13*	2.57E-07	2.78*	2.37E-12	2.05*	8.29E-07
818401	1.93	8.20E-03	3.04*	5.26E-06	2.62*	8.53E-05	2.27*	7.00E-04
875659	-	-	-	-	2.61*	1.99E-07	2.16*	2.54E-05
861037	2.19*	5.0E-04	1.91	5.10E-03	-	-	1.98	1.70E-03
798081	-	-	2.28*	3.30E-3	-	-	-	-
548471	-	-	-	-	2.55*	1.83E-11	-	-
772453	-	-	-	-	2.59*	3.60E-03	-	-
879478	1.73	2.60E-03	1.91	4.00E-04	3.17*	2.06E-10	1.91	4.00E-04
840982	-	-	-	-	2.69*	1.90E-03	-	-
841965	-	-	1.92	3.90E-3	2.99*	7.74E-07	-	-
873894	-	-	-	-	3.49*	9.90E-03	-	-
832704	-	-	1.73	2.00E-04	1.91	8.50E-06	2.07*	3.86E-07

688 Gene expression values with P-value > 0.01 were substituted by a dash (-). Fold changes of higher than 2 were bolded and denoted
689 with an asterisk (*).

(Fig. 1)

(Fig. 2)

(Fig. 3)

(Fig. 4)

(Fig. 5)