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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.

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INTRODUCTION

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

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

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

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102 oxidation of substrates but the metabolic pathways they use may differ

103 (Pozdnyakova 2012, Marco-Urrea, Garcia-Romera and Aranda 2015).

104 Ligninolytic fungi can produce extracellular ligninolytic enzymes including

105 manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase to

106 produce quinone intermediates. Non-ligninolytic degradation of PAHs may

107 use intracellular cytochrome P450 monooxygenases (CYPs) to produce

108 unstable arene oxides which can be subsequently converted into phenols

109 or trans-dihydrodiols. It was previously reported that even some

110 ligninolytic fungi, including *Phanerochaete* and *Pleurotus* can use CYPs

111 instead of ligninolytic enzymes for PAH-degradation (Ghosal et al. 2016).

112   Although great efforts have been made to demonstrate PAH-

113 degradation in wood-degrading fungi through proteomic and metabolic

114 assays, whole genome and transcriptome studies have rarely been

115 conducted. A genome scale survey of potential PAH-responsive genes and

116 their regulation at transcriptional level can contribute to the

117 comprehensive understanding of complex genetic networks in a fungal

118 PAH-degradation system and identification of novel PAH-responsive genes

119 encoded in the genome.

120   *Dentipellis* sp. KUC8613, one of white rot fungi in the order

121 Russulales, has previously been screened by high tolerance to various

122 PAHs and regarded as a potential host for mycoremediation *(Lee et al.*

123 *2014)*. In this study, we report the efficient PAH-removal by the KUC8613

124 strain over various PAHs. In order to expand our knowledge of the genetic

125 basis for the PAH-removal capability of this fungus, we sequenced and

126 analyzed the genome of *Dentipellis* sp. KUC8613 as part of the 1000

127 Fungal Genomes Project (1k FGP) (Grigoriev et al. 2014) at the US

128 Department of Energy Joint Genome Institute (JGI)

129 (http://jgi.doe.gov/fungi). From the genome, we investigated genetic

130 repertoires potentially involved in the metabolism of PAHs. We further

131 performed transcriptomic analysis to observe differential expression of

132 these genes by four different PAHs and to identify novel PAH-responsive

133 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.

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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

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

174   For the determination of mycelial growth during liquid culture,
175 mycelium was harvested after incubation by centrifugation and washed
176 twice with 50 ml distilled water to a final volume of 10 ml. The sample was
177 then and filtered through pre-weighted dried filter paper filter in a vacuum
178 filtration apparatus. The mycelium and filter paper were oven-dried at 65
179 °C for one day, and dry weight was measured.

180

181 **HPLC analysis of PAH-removal**

182   The dried extracts were dissolved in 1 ml chloroform. For the
183 quantitative analysis of residual PAHs, Waters high-pressure liquid
184 chromatography (HPLC) system (Milford, MA, USA) equipped with Waters
185 2487 UV detector was used. Data acquisition was carried out using the
186 Waters Empower 2 software. Ten microliters of each extract was injected
187 onto the Waters C18 column with a flow rate of 1 ml/min. The mobile
188 phase was acetonitrile : water (80:20) and the PAHs were detected at 254
189 nm.

190

191 **Genomic DNA extraction and sequencing**

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

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202 (Grigoriev et al. 2014) and FunGAP (Min, Grigoriev and Choi 2017). From
203 JGI-MycoCosm repository
204 (https://genome.jgi.doe.gov/Densp1/Densp1.home.html), we could
205 retrieve the genome wide annotation data such as InterPro, CAZy, KOG,
206 and KEGG for functional analysis.
207

RNA extraction and mRNA sequencing

209 For transcriptomic analysis, RNA from 5 days of culture were used.
210 The grown mycelium was harvested from the culture medium by
211 centrifugation and washed twice with 50 ml distilled water. The mycelium
212 pellet was immediately frozen under liquid nitrogen and homogenized
213 using a mortar and a pestle. Total RNA was isolated using an RNeasy plus
214 mini kit (Qiagen, the Netherlands) according to the manufacturer's
215 protocol. RNA concentration was determined using Qubit fluorometer
216 (Invitrogen). RNA library was constructed using TruSeq RNA sample
217 preparation kit (Illumina, San Diego, CA, US). The sequencing of the RNA
218 library was carried out in MiSeq (2 × 200 cycles). Low-quality reads were
219 filtered using the Trim Galore program. The filtered RNA-seq data was
220 mapped against the assembled genome using CLC Genomics Workbench
221 software v.12.0 (Qiagen, the Netherlands). Read alignment was performed
222 with the following parameters: minimum length fraction and minimum
223 similarity fraction = 0.8, strand specific = both, maximum number of hits
224 for a read = 3. Reads Per Kilobase of transcript per Million mapped reads
225 (RPKM) value was generated using default settings of CLC genomics and
226 further used for the analysis of differential expression of genes. To check if
227 the RNA-seq data from different conditions correlate each other, a
228 Principal Component Analysis (PCA) was performed by an internal routine
229 of CLC Genomics Workbench.
230

Gene Ontology enrichment analysis of upregulated genes

232 Upregulated genes by PAHs were identified according to the
233 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

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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

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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)

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335 of the gene expression data clearly separated PAH-added groups from the

336 control group (Fig. 4A). The PCA data also showed a separation within PAH

337 samples. While the ANT, FLU, and PYR samples were clustered together,

338 the PHE samples formed an independent group distinct from other PAH

339 samples, suggesting that the gene expression profile of PHE sample is

340 unlike those of other PAHs.

341   Of the 14,320 gene models predicted in the genome, we identified

342 1,922 genes whose expression was upregulated by at least one of four

343 different PAHs (Fold change > 2 and P-value < 0.01) (Table S5). The

344 numbers of total genes upregulated by ANT, FLU, PHE, and PYR were 915,

345 950, 1539, and 1,095, respectively (Fig. 4B). Among the upregulated

346 genes, the molecular function of 772 genes could not be predicted.

347 Upregulated genes were assigned to 23 specific KOG classes and

348 dominantly (54.9 % of 1,041 KOG-assigned genes) categorized into five

349 KOG classes including posttranslational modification, signal transduction,

350 energy production and conversion, carbohydrate transport and

351 metabolism, and transcription (Table S6). As illustrated by a Venn-diagram

352 in Figure 4B, upregulated genes showed common and different PAH-

353 specific overexpression during PAH-removal. A total of 550 genes were

354 commonly overexpressed by all four PAHs. The numbers of PAH-specific

355 genes by ANT, FLU, PHE, and PYR were 67, 72, 548, and 59, respectively.

356 The number of PHE-induced genes were significantly higher than genes

357 induced by other PAHs. A heatmap plotted by log_2RPKM of up-regulated

358 gene expression showed a similar pattern as well (Fig. 4C). The majority of

359 up-regulated genes were commonly overexpressed in all PAH-samples

360 while PHE showed a distinct gene expression pattern compared to those of

361 other three PAHs.

362   We first investigated the expression profiles of ligninolytic enzymes

363 (LiP, MnP, and laccase) and P450s for initial aromatic-ring oxidation. No

364 ligninolytic enzymes were upregulated during PAH-removal (Table S7). On

365 the other hand, P450s showed both common and different PAH-specific

366 overexpression. Among 154 putative P450 genes found in the KUC8613

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

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

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

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

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399 organelles should be controlled by membrane bound transporters. It is
400 well known that major facilitator superfamily (MFS) transporters and ATP-
401 binding cassette (ABC) transporters are two major transporter families
402 that mediate the import and export of drugs and xenobiotics (Jeong et al.
403 2017, Carmona et al. 2009). We observed the common upregulation of 14
404 MFS and five ABC transporters by all four PAHs (Table S9). The active
405 transcription of MFS transporters was particularly noticeable by PHE. The
406 more than ten-fold elevated expression of four MFS transporters (ProtID
407 787334, 857773, 800757, and 328771) by PHE suggests an important role
408 of these transporters for cellular response to PHE.

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

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Discussion

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

439   While ligninolytic system in white rot fungi was often described as
440 the key for PAH-transformation (Ghosal et al. 2016), our data showed that
441 KUC8613 was capable of removing PAHs using P450s instead of
442 ligninolytic enzymes. Ligninolytic enzymes may not be essential for PAH-
443 transformation in this fungi, but they still may take part in PAH-
444 transformation at certain growth conditions. It has been previously shown
445 that the production of ligninolytic enzymes by white rot fungi can be
446 dependent on carbon and nitrogen concentration in the growth media
447 (Ben Hamman, de La Rubia and Martinez 1997, D'Souza, Merrit and Reddy
448 1999). It would be worthwhile to check PAH-removal pattern by this
449 fungus under different conditions that favor the production of ligninolytic
450 enzymes. Taken together, these data strongly suggest that the screening
451 of strong mycoremdiation hosts primarily based on ligninolytic enzyme
452 activities should be reconsidered.

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

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

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

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

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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.

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Accession numbers and availability of the genome sequence and the strain

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497   This whole genome sequence of *Dentipellis* sp. KUC8613 has been
498 deposited at DDBJ/EMBL/GenBank under the accession number
499 NSJX00000000. In addition, the genome assembly and annotation are
500 available at DOE JGI Genome Portal MycoCosm (Grigoriev et al. 2014)
501 (http://genome.jgi.doe.gov/Densp1). The strain is available from Korean
502 Collection for Type Cultures (KCTC) with the accession number KCTC46678
503 (http://kctc.kribb.re.kr/)

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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,
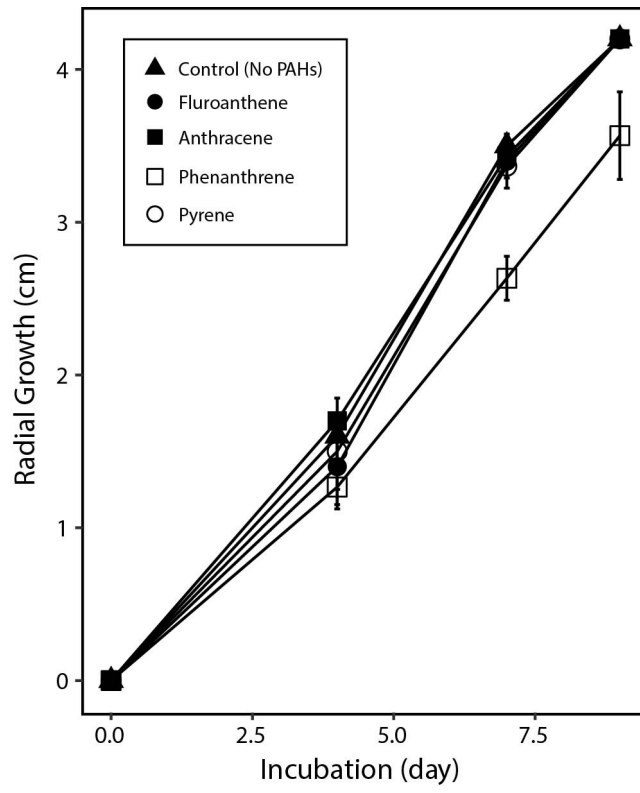683 anthracene; FLU, fluoranthene; PHE, phenanthrene; PYR, pyrene.

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(Table 1)

Assembly statistics	*Dentipellis* sp. **KUC8613**	*H. annosum*	*T. versicolor*	*P. gigantea*	*B. adusta*	*P. brevispora* HHB-7030 SS6	*P. ostreatus* PC15	*S. paradoxa* 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/Control	Fold change FLU/Control	P-value FLU/Control	Fold change PHE/Control	P-value PHE/Control	Fold change PYR/Control	P-value PYR/Control
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
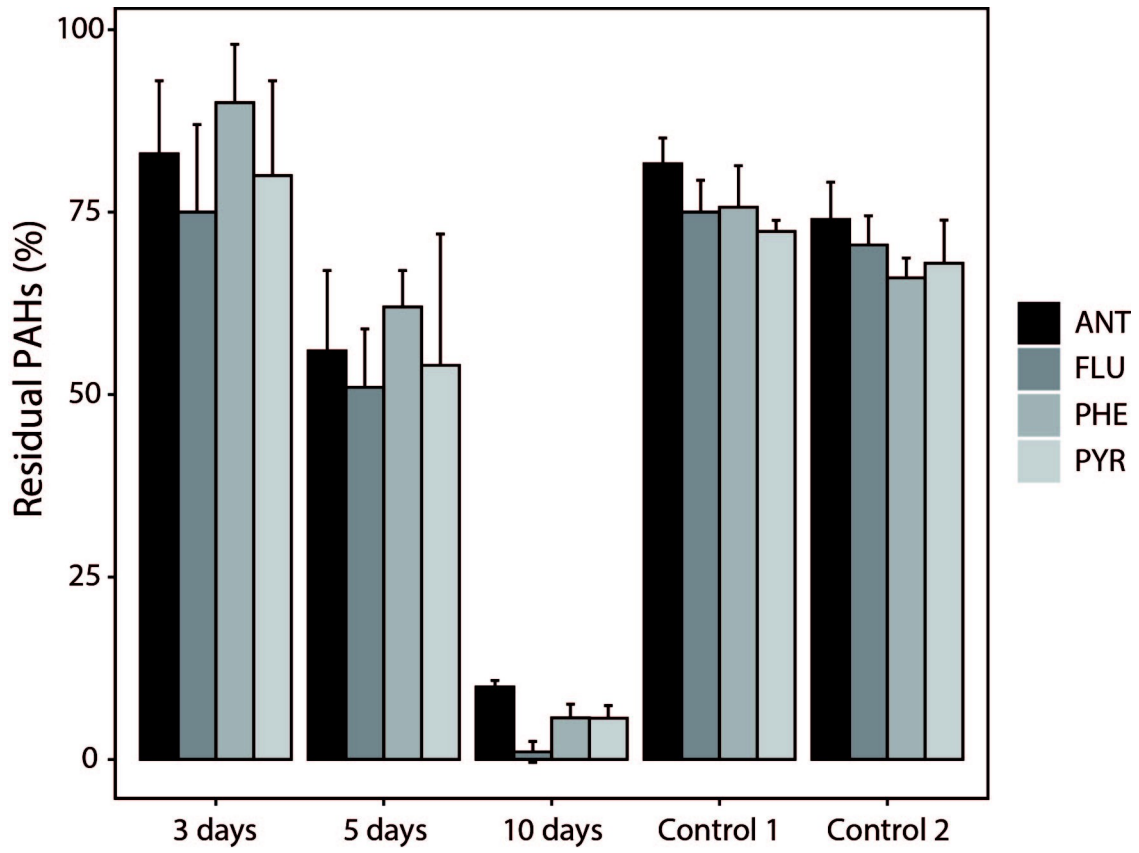689 with an asterisk (*).

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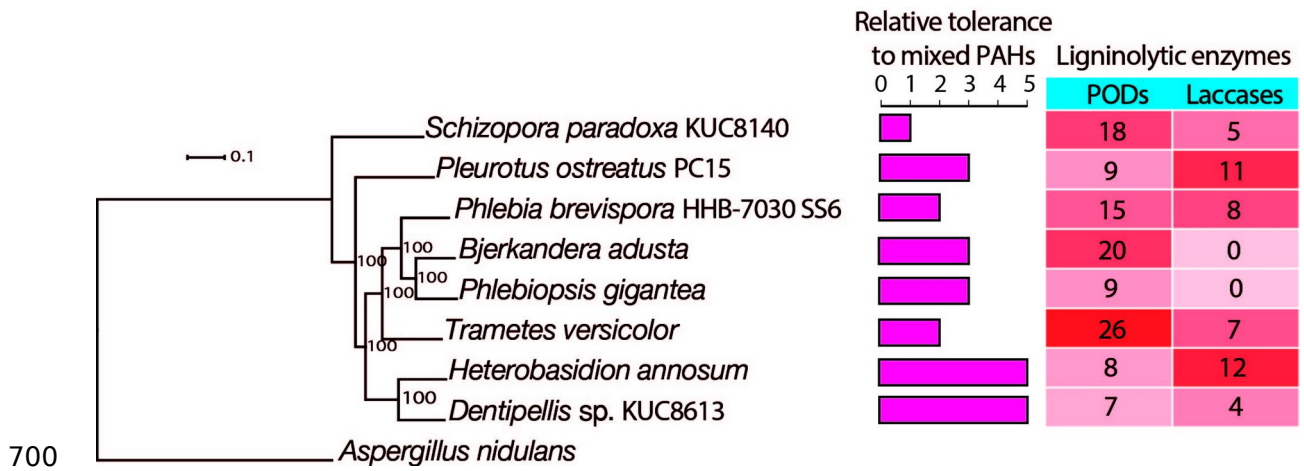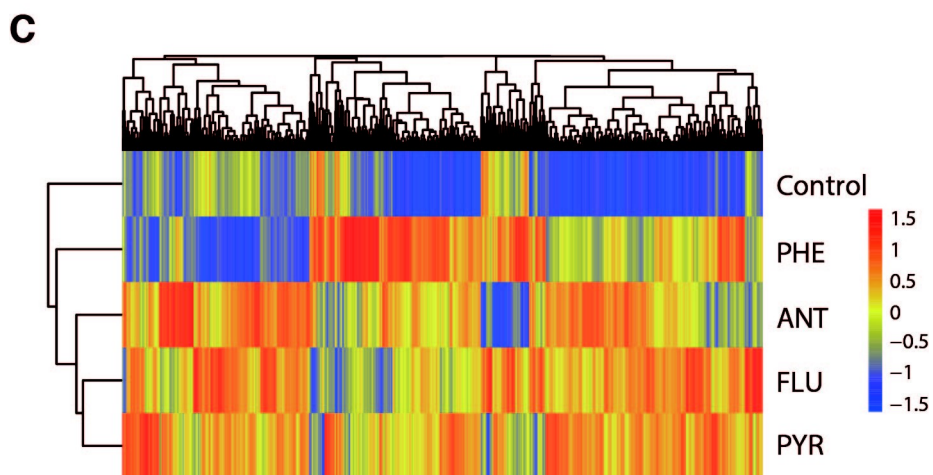(Fig. 1)

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(Fig. 2)

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(Fig. 3)

	Relative tolerance to mixed PAHs	Ligninolytic enzymes	
	0 1 2 3 4 5	PODs	Laccases
Schizopora paradoxa KUC8140		18	5
Pleurotus ostreatus PC15		9	11
Phlebia brevispora HHB-7030 SS6		15	8
Bjerkandera adusta		20	0
Phlebiopsis gigantea		9	0
Trametes versicolor		26	7
Heterobasidion annosum		8	12
Dentipellis sp. KUC8613		7	4
Aspergillus nidulans			

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704   (Fig. 4)

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Initial ring-oxidation

PAHs →[Lipase] Enhanced Solubility

Transporters

P450s → Arene oxides

Common
PAH-specific

Downstream transformation

Phenols →[GTF / GST / SULT]→ **Conjugates**

Oxidative stress →[SOD / AHP]→ Reduced cell damage

trans-dihydrodiols → Alcohol dehydrogenase / Aldehyde dehydrogenase / Monooxygenase / Dioxygenase → **Ring cleavage products**

Non-enzymatic

Epoxide hydrolase

705
706   (Fig. 5)
707