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# Expression Pattern of Entire Cytochrome P450 Genes and Response of Defensomes in the Benzo[*a*]pyrene-Exposed Monogonont Rotifer *Brachionus koreanus*

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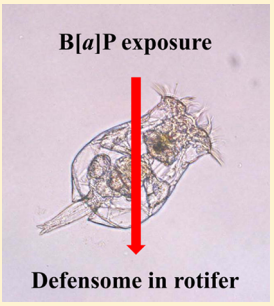
Supporting Information

**ABSTRACT:** Cytochrome P450 (*CYP*) proteins are involved in the first line of detoxification mechanism against diverse polycyclic aromatic hydrocarbons (PAHs) including benzo[*a*]pyrene (B[*a*]P). In aquatic invertebrates, there is still a lack of knowledge on the *CYP* genes involved in the molecular response to B[*a*]P exposure due to limited gene information. In this study, we cloned the entire 25 *CYP* genes in the monogonont rotifer *Brachionus koreanus* with the aid of next generation sequencing (NGS) technologies and analyzed their transcript profiles with a real-time RT-PCR array to better understand B[*a*]P-triggered molecular response over different time courses. As a result, B[*a*]P exposure induced *CYP2/3*-involved detoxification mechanisms and defensome, including phase II detoxification and antioxidant systems with a modulation of the chaperone heat shock protein (*hsp*) expression but did not change expression of other *CYP* clans in *B. koreanus*. Therefore, we found that B[*a*]P induced a strong detoxification mechanism to overcome detrimental effects of B[*a*]P associated with B[*a*]P-induced growth retardation as a trade-off in fitness costs. Also, this approach revealed that the entire *CYP* profiling can be a way of providing a better understanding on the mode of action of B[*a*]P in *B. koreanus* with respect to molecular defense metabolism.




## INTRODUCTION

Xenobiotic detoxification metabolism of chemicals is mediated by specialized enzyme systems as a chemical defensome that are classified into three phases, i.e., modification such as oxidation, conjugation, and excretion in living organisms.[1,2] With phase I biotransformation enzymes, ubiquitous cytochrome P450 (CYP) enzymes have important roles in the metabolism of endogenous and exogenous chemicals.[3,4] PAHs including B[*a*]P are well known as lipophilic and hydrophobic compounds that can directly accumulate in fatty tissues of aquatic animals as toxic components.[5] Although they can be found in natural conditions, recent environmental concentrations of PAHs have been increased by anthropogenic activities. Environmental aspects of B[*a*]P are particularly important in the trophic transfer of toxic metabolites that are potentially harmful to higher trophic levels. Several reports showed detrimental effects of dietary transfer of PAHs and their metabolites because the biotransformed metabolites could be further oxidized by a series of detoxification systems at higher tropic levels, resulting in growth retardation, physiological changes, or DNA adduct formation in predator organisms.[6−10] These results indicate that inefficient biotransformed PAH metabolites would have detrimental effects on different trophic levels within aquatic ecosystems. Analysis of the biotransformation capacity of primary producers is important for better understanding of the kinetics of PAHs including B[*a*]P.

B[*a*]P is metabolized through the cellular phase I and phase II detoxification systems in all animals, and the hazardous potential of B[*a*]P is strongly associated with activities of phase I enzymes including the CYP system. In marine invertebrates, activities of phase I enzymes are known to be varied,[11] and therefore, analysis of the CYP system may be able to provide the mechanistic understanding of the modulation of the detoxification system as a crucial role of *CYP* genes in the overall biotransformation in organisms. To date, the in vivo and/or in vitro mechanisms of effect of B[*a*]P have been relatively poorly investigated in aquatic invertebrates. For examples, several studies on the PAHs in aquatic invertebrates showed tissue accumulation, biomarker responses, metabolic change, oxidative DNA damage, immunomodulation, and toxicity.[12−20]

The *CYP* superfamily is composed of multigene subfamilies of the combined members of which can have a metabolic role toward diverse chemicals.[21−23] The *CYP* superfamily has been initially identified from several organisms including marine invertebrates such as *Mytilus* (mussel) and *Crassostrea* (oyster) species and insects[24,25] and also has been regularly charac-

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terized through NGS technology. However, until now, most studies on *CYP* superfamilies from several insect species were focused primarily on identification without gene expression analysis and/or functional characterization. Moreover, an ecotoxicogenomic approach has not conducted using aquatic invertebrates as yet due to the absence of gene mining and annotation of *CYP* superfamily genes. Therefore, the profiling of the entire *CYP*s of rotifer can be a useful approach to anticipate the *CYP* function in diverse research areas such as in the interaction of *CYP*s with potential substrates to better understand the mode of action of pollutants and toxicants in the aquatic ecosystem. To validate the usefulness of whole *CYP* profiling for an ecotoxicogenomic approach, we first cloned all the *CYP* genes in the genome of the monogonont rotifer *Brachionus koreanus* and analyzed the expression pattern of all of the *CYP* spectrum upon B[*a*]P exposure as an indicator of marine environmental pollution.

The monogonont rotifer (*B. koreanus* was used in this study) is widely distributed along coastal lines and has several promising advantages for ecotoxicological research such as small size, short generation cycle (≈ 24 h), simple structure, genetic homogeneity, high fecundity, and ease of maintenance in the laboratory. As an important producer and secondary consumer, rotifers play a role in bridging as an energy transmitter as well as a toxicant transporter in aquatic food webs. On the basis of these characteristics, rotifers are considered to be a potential model species in diverse aquatic research areas such as aquaculture, ecophysiology, ecotoxicology, and environmental genomics.[27,28] In addition, for a better use of the monogonont rotifer *B. koreanus* as a model species, we recently employed several NGS technologies to obtain whole genome information and RNA-Seq and effectively assembled its genome along with transcriptomes and annotated gene information.[29] In this context, we investigated all the defensome genes after exposure to PAH and tested its potential efficacy for environmental pollution monitoring in the monogonont rotifer *B. koreanus*.

As we emphasized earlier, the mode of action and the toxic effects of B[*a*]P are widely studied in vertebrates but not in invertebrates, and thus, the exposure study of this well-characterized chemical will be useful for a comparative understanding of the molecular responses of rotifers. In this study, we checked the usefulness of transcript profiling of whole *B. koreanus CYP*s (*Bk-CYP*s) upon exposure to B[*a*]P with expression profiles of all the defensome genes and enzymatic activities of several oxidative stress-related proteins. The specific objectives of this study were (1) to identify the entire *Bk-CYP* superfamily as a first report in Rotifera, (2) to examine the spectrum of *Bk-CYP* gene expression after B[*a*]P exposure and the relevant detoxification mechanism of *B. koreanus*, and (3) to suggest the specificity of the *Bk-CYP*s involved in the molecular response as a reliable potential molecular biomarker for environmental risk assessment.

## MATERIALS AND METHODS

**Rotifer Culture and Maintenance.** The monogonont rotifer *B. koreanus* (Figure S1, Supporting Information) was collected at Uljin (36°58′43.01″ N, 129°24′28.40″ E) in South Korea. A single individual was isolated, reared, and maintained in 0.2 μm-filtered artificial seawater (TetraMarine Salt Pro, Tetra, OH) adjusted to 25 °C under a LD 12:12 h photoperiod with 15 practical salinity units (psu) of salinity. The green algae *Chlorella* sp. was used as a live diet (approximately $6 \times 10^4$ cells/mL). Species identification was confirmed with the morphological characteristics and the mitochondrial genome analysis.[29−31]

**Retrieval and Annotation of the Entire Complement of *CYP* Genes.** Genomic DNA of *B. koreanus* was sequenced using a GS-FLX-titanium DNA sequencer (Roche Diagnostics, Mannheim, Germany) and SOLEXA sequencer (Illumina, CA, U.S.A.) and then assembled with the software NGS Cell (Ver. 4.06 beta 67189, CLC Bio, MA, U.S.A.) and Velvet (EMBL-EBI, U.K.). To obtain the sequence information of all the *CYP* genes, we carried out both local blast analysis using the assembled contigs and clones and also BLAST analysis of those clones to the nonredundant (NR) database at GenBank, respectively. To obtain full length *CYP* genes, some *CYP* sequences were subjected to 5′- and 3′-RACEs, and then we confirmed the exon/intron boundaries according to manufacturer's protocol (Invitrogen, Carlsbad, CA). Annotation and nomenclature of all *Bk-CYP* genes were done according to David R. Nelson's method based on their sequence similarities compared to other invertebrate *CYP*s.[25]

**Phylogenetic Analysis.** To analyze the *Bk-CYP*s' clusters on the phylogenetic tree, we aligned them with those of other species at the level of the deduced amino acid sequence by ClustalX 1.83. Gaps and missing data matrix were excluded from the analysis. The generated data matrix was converted to the nexus format using Mesquite (ver 2.0), and the data matrix was analyzed with Mr. Bayes v3.1.2 program using the general time-reversible (GTR) model. A total of 1,000,000 generations were conducted, and the sampling frequency was assigned as every 100 generations. After analysis, the first 10,000 generations were deleted as the burn-in process, and the consensus tree was constructed and then visualized with TreeView software of PHYLIP. The amino acid sequences used in phylogenetic analysis were adopted from a previous study.[32]

**B[*a*]P Exposure and Experimental Design.** B[*a*]P was purchased from Sigma (B1760; Sigma-Aldrich, Inc., St. Louis, MO, U.S.A.; purity >96%; MW 252.31) and was dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich, Inc., St. Louis, MO, U.S.A.). To check growth retardation of *B. koreanus* after B[*a*]P exposure, we counted the number of rotifers at 0, 1, 10, and 100 μg/L (0, 4, 40, and 400 nM) of B[*a*]P over 10 days (Table S1, Supporting Information). The concentrations of B[*a*]P were chosen within ranges that caused no coagulation in salt water (15 psu). During the experiment, we supplied an algal diet of *Chlorella* sp. (approximately $6 \times 10^4$ cells/mL) every 24 h without B[*a*]P treatment.

To examine the mode of the molecular response of the *Bk-CYP* genes, we exposed the rotifers to two concentrations (10 and 100 μg/L) of B[*a*]P over time (0, 6, 12, and 24 h). Each concentration was predetermined based on growth curves upon B[*a*]P exposure. For the control group, 0.01% DMSO was used after being administered to the exposed B[*a*]P at the same solvent concentration. The DMSO concentrations in control and treatment groups were maintained at a concentration of less than 0.01%. Approximately 7000 rotifer were exposed to 10 and 100 μg/L of B[*a*]P for 24 h. Because of the short life cycle (24 h from egg to adult) of rotifer, we tested all gene expression within 24 h.

**RNA Extraction, cDNA Synthesis, Real-Time RT-PCR Array, Measurement of Enzyme Activities, and Statistical Analysis.** A detailed description for all materials and methods was incorporated in the supplementary file as described in our previous studies.[33,34]

## RESULTS

**In Vivo Effects of B[*a*]P Exposure.** Different concentrations of B[*a*]P exposure (1, 10, and 100 μg/L) for 24 h did not show mortality in *B. koreanus* (unpublished data). We could not measure the exact no observed effect concentration (NOEC) and lethal concentration (LC) value of B[*a*]P for 24 h, as the rotifer did not shown mortality at even 1 mg/L of B[*a*]P exposure and due to minor aggregation at high concentrations over 2 mg/L in artificial seawater (15 psu). However, 100 μg/L of B[*a*]P caused gradual growth retardation of *B. koreanus* over 10 days (Figure 1; Table S1, Supporting Information).

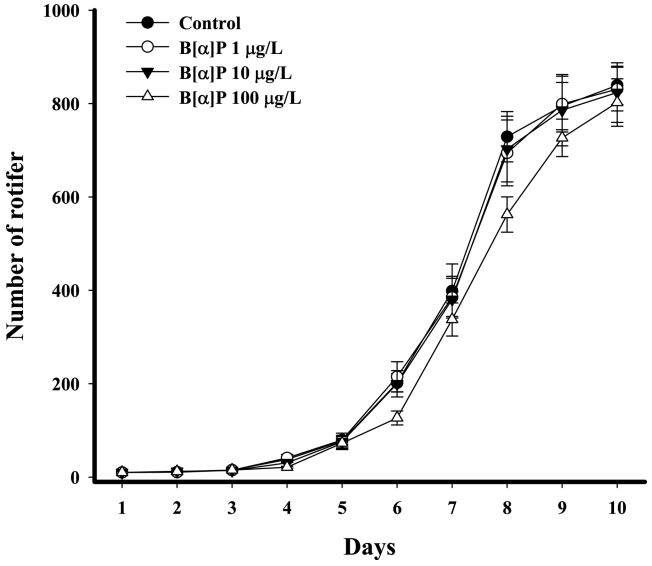


**Figure 1.** Effect of B[*a*]P on *B. koreanus* growth rate at the level of fecundity for 10 days. Four of the *B. koreanus* groups were exposed to 0, 1, 10, and 100 μg/L. Error bars indicate means ± SD.

**Annotation and Nomenclature of Whole *CYP* Genes in the Monogonont Rotifer, *B. koreanus*.** A total of 25 *Bk-CYP* genes were obtained by extensive NR blast search and local blast analysis in the monogonont rotifer, *B. koreanus* genome and RNA-Seq databases. Whole *Bk-CYP* genes were registered at the GenBank database (Table S2, Supporting Information). Twenty five *Bk-CYP* genes were separated with five distinct clans such as clan 2, clan 3, clan 4, clan 46 as a sister clade of the clan 4 and mitochondrial (MT) clan. A description of different *CYP* clans followed the criteria for invertebrates.[25,35] When the composition of the number of genes in each of the *CYP* clans was compared to that of other whole *CYP* genes, very similar patterns were observed in several invertebrates such as fruitfly, silkmoth, and waterflea (Figure S2, Supporting Information).[32] In addition, phylogenetic analysis fully supported the annotation of the rotifer whole *CYP* genes through congruent separations between different clans of input species (Figure S3−S7, Supporting Information). Additional descriptions on *B. koreanus*'s entire *CYPs* and their evolutionary significance were incorporated in the Supporting Information.

**Transcript Profiling of the Entire *Bk-CYP* Genes in Response to B[*a*]P Exposure.** To characterize 25 whole *Bk-CYP* expressions upon B[*a*]P exposure, we employed a time course analysis over 24 h (Figure 2; Table S3, Supporting Information). In the 10 μg/L B[*a*]P-exposed group, 5 genes were upregulated. Of them, 3 genes and 2 genes belonged to clan 2 and clan 3, respectively. Of down-regulated genes, 1 and 3 genes were separated into clan 3 and clan MT, respectively. Overall transcript profiles in the 100 μg/L of B[*a*]P exposure revealed that 11 *Bk-CYP* genes, involving in clans 2 and 3, were upregulated within a range of significant fold change (2-fold) with a $p$ value cutoff ($P < 0.05$), while 7 *Bk-CYP* genes were down-regulated over 24 h. Most down-regulated genes belonged to clans 4, 46, and MT, but 1 gene (*Bk-CYP3045B1*) exceptionally belonged to clan 2. Of 25 *CYP* genes, *Bk-CYP3042A1* and *Bk-CYP3043A1* genes, belonging to

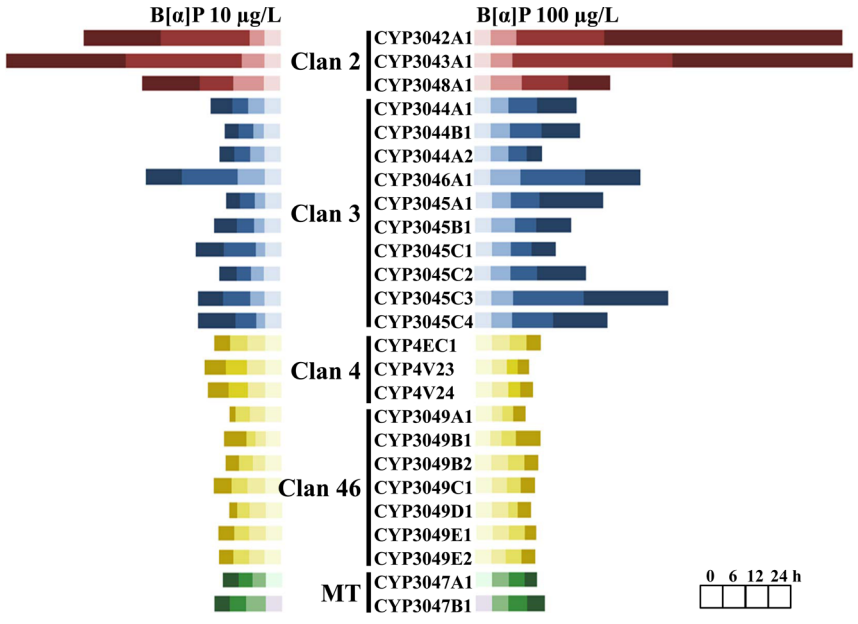


**Figure 2.** Transcript profiles of *B. koreanus* whole *CYPs* in response to different concentrations (10 and 100 μg/L) of B[*a*]P exposure for 24 h. Values were distributed by fold change in different time courses (0, 6, 12, and 24 h). Fold changes were represented by cutoff of valid $p$ values as analyzed in Table S2 of the Supporting Information.

clan 2, were highly induced by B[*a*]P exposure over different concentrations and time courses.

**Analysis of Transcriptional Responses and Enzyme Activities of Phase II Detoxification- and Antioxidant Mechanism-Related Genes.** To understand B[*a*]P effect on *CYP*s-triggered metabolism of *B. koreanus*, we analyzed transcriptional responses of several genes that are involved in the phase II detoxification and antioxidant defense system at different concentrations of B[*a*]P exposure for 24 h (Figure 3).

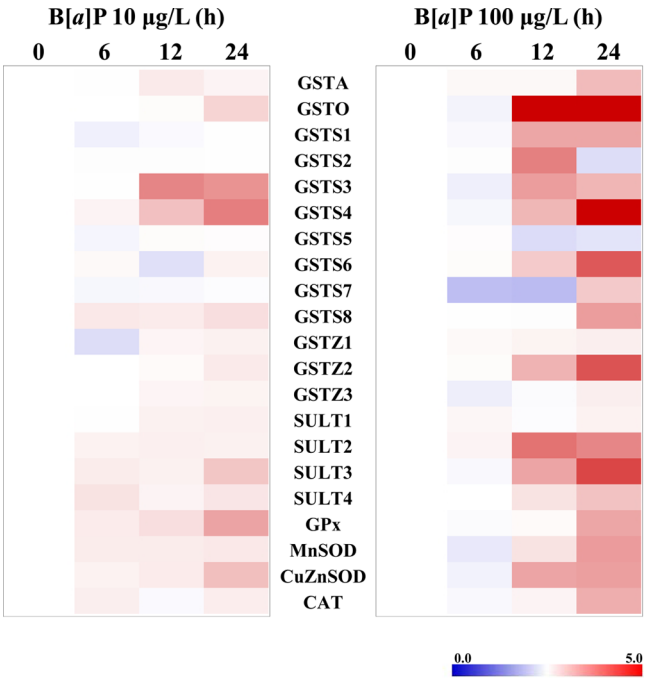


**Figure 3.** Transcript profiles of *B. koreanus* phase II detoxification- and antioxidant system-related genes in response to different concentrations (10 and 100 μg/L) of B[*a*]P exposure for 24 h. Expression profiles were represented by a heat map.

Different *GST* isotypes were characterized by phylogenetic analysis with multiple alignments (Figure S8 and S9, Supporting Information). Their inducibilities against oxidative stress inducers were confirmed in our preliminary study (Figure S10 and Table S5, Supporting Information). In the case of phase II biotransformation enzymes, most *GST* isotypes and *SULT* transcripts were significantly upregulated in the 100 μg/L of B[*a*]P treatment groups with ≥2.0 fold increase. Regarding the transcriptional modulation of antioxidant enzymes, antioxidant genes such as *GPx*, *MnSOD*, *CuZnSOD*, and catalase (*CAT*) were also significantly induced in the 100 μg/L of B[*a*]P treatment groups at 24 h.

Regarding enzymes involved in the phase II detoxification system, *B. koreanus* GST and SULT enzyme activities were significantly induced upon B[*a*]P exposure (10 and 100 μg/L) for 24 h (Figure 4), indicating that detoxification activity can be induced by B[*a*]P exposure as a measure against hazardous components of B[*a*]P. GSH concentrations and antioxidant enzyme activities of GPx, GR, and SOD were significantly elevated by B[*a*]P exposure (10 and 100 μg/L) for 24 h (Figure 4), suggesting that B[*a*]P exposure induced an oxidative stress in *B. koreanus*.

**Transcript Profiling of Heat Shock Protein (*hsp*) Genes in Response to B[*a*]P Exposure.** To analyze the effect of B[*a*]P on transcript modulation of *hsp* genes, we exposed rotifers to different concentrations of B[*a*]P (1, 10, 100 μg/L) and measured the transcript level at different time courses: 12 h (Figure 5A) and 24 h (Figure 5B). As a result, transcripts of high molecular weight *hsps* (*hsp70, hsc70, hsp90α*, and *hsp90β*) were induced by B[*a*]P for 12 h in a dose-dependent manner, while transcripts of *hsp21*, *hsp30*, *hsp40*, and *hsp60* were decreased at 10 and 100 μg/L of B[*a*]P exposure for 12 h with a $p$ value cutoff ($P < 0.05$). After exposure of B[*a*]P over 24 h, overall transcript profiles reached the background level.

## DISCUSSION

In *B. koreanus*, sublethal effects became apparent by a slight retardation of population growth rate over 10 days, even though acute toxic effects of B[*a*]P were not observed for up to 24 h. On the basis of this result, we hypothesized that in vivo physiological activity such as growth rate and reproductive events can be affected directly or indirectly by B[*a*]P exposure, as rotifer might consume metabolic energy for the increased endogenous metabolism such as the defensome response within the limited total energy budget. B[*a*]P is known to have xenoestrogenic activity and is a potential endocrine disruptor.[36] Although there are no studies as yet on the effect of B[*a*]P treatment for population growth of aquatic invertebrates, 8 and 20 μg/L of B[*a*]P treatment impaired swimming activity and embryogenesis in the amphipod *Gammarus duebeni* and *Chaetogammarus marinus*, respectively,[37] and several reproductive parameters such as survival and growth rate were affected by B[*a*]P treatment in the Pacific oyster *Crassostrea gigas*.[38] In mammals, the potential influence of PAHs including B[*a*]P on physiological aspects is well reported. However, in aquatic invertebrates, the effect of B[*a*]P on intracellular detoxification mechanisms including *CYP*s-triggered innate metabolism or its dysregulation has not yet been investigated.

The *CYP*s constitute one of the largest gene families in the molecular detoxification mechanism of phase I oxidative metabolic system.[3,4] In vertebrates, xenobiotic inducible *CYP1−4* families are considered as the most important chemical defense system, and the *CYP1* family (*CYP1A*, *CYP1B*, *CYP1C*, and *CYP1D*) has been extensively studied and found to be a reliable biomarker for diverse environmental pollutants.[3,21,25,39] However, transcription factor-mediated regulation of the *CYP1* family is not investigated in invertebrates as yet due to the absences of both *CYP1* family and an appropriate characterization systems, while xenobiotic sensing receptors such as aryl hydrocarbon receptor (*AhR*) have been characterized in response to PAHs including B[*a*]P in vertebrates.[40] With increasing genome information by NGS technologies, *CYP1*-like genes or other relevant *CYP*s as potential biomarkers upon PAH pollution have focused on cloning in invertebrates[41,42] but a conserved biotransformation mechanism has not yet been investigated. In *B. koreanus*, phylogenetic topology analysis revealed that two genes (*CYP3043A1* and *CYP3048A1*), belonging to the clan 2 member, made a sister branch with a cluster of the vertebrates *CYP1* and the invertebrate *CYP307* family. Therefore, we can expect a potential role of two rotifer *CYP*s as invertebrate *CYP1*-like genes that would be regulated via a xenobiotic metabolism in this species.

In this study, two rotifer *CYP* genes in clan 2 (*Bk-CYP3042A1* and *Bk-CYP3043A1*) were significantly induced by different concentrations of B[*a*]P exposure over time courses. Also, several *CYP3* families showed the upregulated

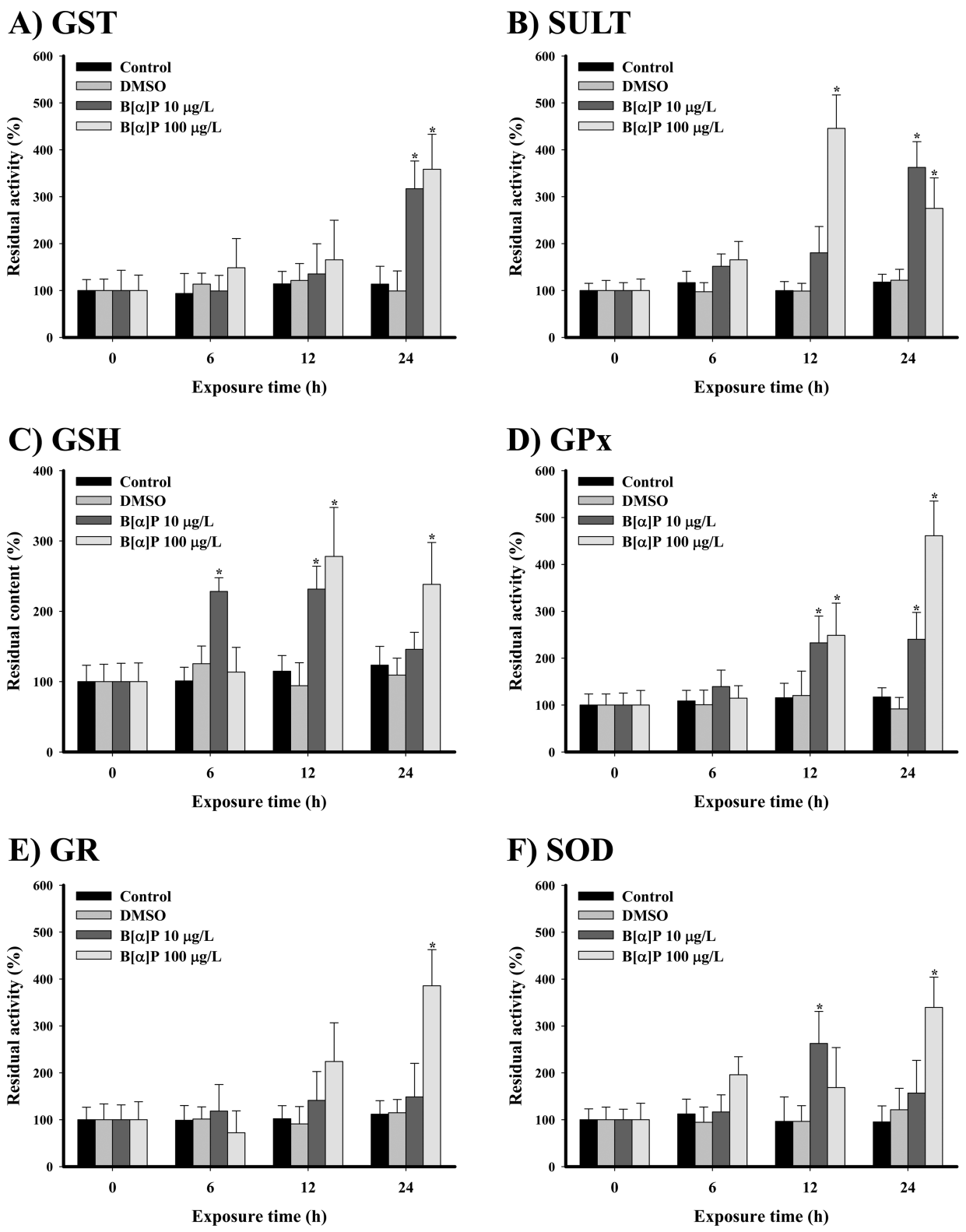


**Figure 4.** Enzyme activities of phase II detoxification-related proteins, (A) GST and (B) SULT, and oxidative stress-related protein, (C) GSH, (D) GPx, (E) GR, and (F) SOD by different concentrations of B[*a*]P exposure for 24 h *B. koreanus*. The remaining activities were recorded as percentage relative to control. Significant difference over control values are indicated by different symbols on the data bar ($P < 0.05$) analyzed by multiple comparison ANOVA. Data are means ± SD of three replicates of exposed embryos. The symbol (∗) indicates $P < 0.05$.

transcriptional rates at a relatively high concentration of B[*a*]P (100 μg/L) exposure. Thus, we suggest that these *CYP* families (*CYP 2/3*) would play a compensatory role for major biotransformation activity against B[*a*]P in this species, as rotifers lack the *CYP1* family as shown in vertebrates. Regarding transcript profiles of the *CYP* family in this species, we found that the *CYP2/3* families were involved in an acutely upregulated pattern of expression suggesting them to be

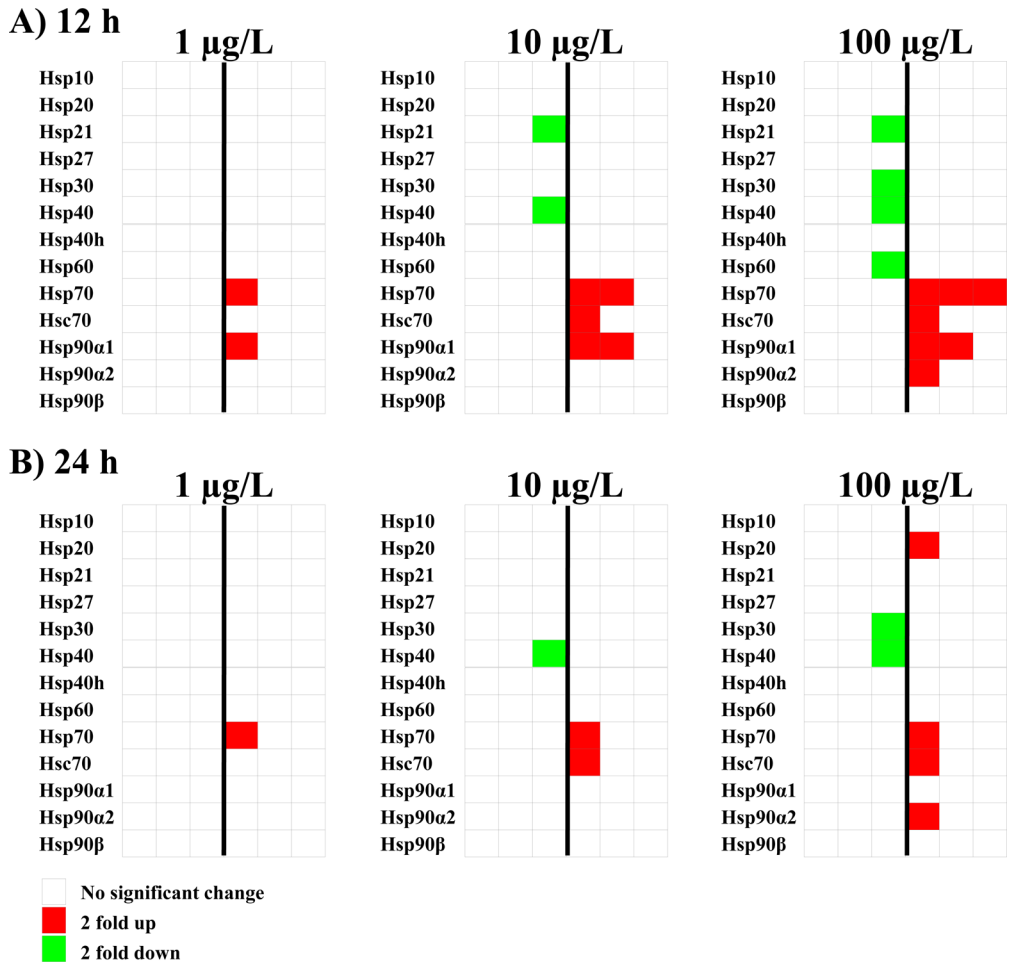


**Figure 5.** Transcript profiles of *B. koreanus Hsps* in response to different concentrations (1, 10, and 100 μg/L) of B[*a*]P exposure for (A) 12 and (B) 24 h with *p* value cutoff ($P < 0.05$).

important defensome components and recognizing their crucial roles for eliminating diverse xenobiotics including PAHs in aquatic invertebrates.[26,41−46] Therefore, we concluded that B[*a*]P could induce several *CYP* genes including *CYP2/3* families to metabolize B[*a*]P to detoxify or biotransform B[*a*]P intermediates in *B. koreanus*. Regardless of the defensome induction, transcriptional levels of several *CYP*s involved in clans 4, 46, and MT were decreased in the 100 μg/L treatment of B[*a*]P-exposed *B. koreanus*. In fact, this family has been known to be involved in the detoxification, lipid metabolism, and hormonal function of mammals as well as insects and waterfleas.[27,47] Therefore, transcriptional regulation in these *CYP* clans provides an interesting topic for our further study. Subsequently, we analyzed the transcriptional responses and activities of several metabolizing enzymes to investigate whether rotifers have conserved detoxification mechanisms like vertebrates or whether an alternative metabolic system was possibly employed for survival.

Phase II biotransformation is generally considered as a detoxification pathway producing more polar metabolic products that are more easily excreted.[48] In the rotifer *B. koreanus*, transcriptional levels and enzyme activities of GST and SULT were significantly induced by B[*a*]P exposure, indicating that phase II detoxification metabolism was modulated by B[*a*]P for conjugating GSH with oxidized functional groups of B[*a*]P metabolites.[49] Also, the relative activities of *B. koreanus* phase II enzymes represented the relative presence of detoxified B[*a*]P metabolites, as B[*a*]P and its metabolites are substrates for GSTs and SULT.[50,51] Although contradictory results on GST activity have been reported in vertebrates as an epoxide is not formed during pyrene biotransformation, it can be explained that oxidative effects of B[*a*]P treatment triggered GST induction for catalyzing thiolization to GSH conjugates. Taken together, our results suggest that a series of detoxification mechanisms from *CYP*s involved in biotransfromation were induced for metabolic activation of B[*a*]P with potentially conserved function for detoxication in *B. koreanus*.

In vertebrates, many antioxidant enzymes with phase II biotransformation enzymes are targets for reactive electrophilic metabolites of B[*a*]P that result in oxidative stress.[52,53] To expand our knowledge on the effects of B[*a*]P, the antioxidant defense system was further examined. On the basis of the initial increase and subsequent decrease of GSH level for 24 h, we suggest that conjugation of GSH with B[*a*]P occurred within a short time period in *B. koreanus*, resulting in the depletion of the GSH at 24 h. Through significantly increased transcriptional levels of antioxidant enzymes and their activities, we suggest that B[*a*]P or its intermediates can be a crucial modulator in both detoxification metabolism and antioxidant defense systems in *B. koreanus*. In addition, this kind of coinduction of antioxidant defense system with phase II detoxification systems suggests a synergistic role in mediating the eliminating the effects of B[*a*]P in *B. koreanus*.

*Hsps* is an important member of the chemical defensome in aquatic invertebrates, as they have significant response mechanism such as chaperoning activity and damaged protein degradation against various environmental contaminants.[54] In *B. koreanus*, we found that there were two different transcriptional regulations of the cellular stress protein *Hsps* after B[*a*]P exposure. Thus, genes for low molecular size hsp (*hsp21, hsp30, hsp40, hsp60*) were down-regulated but genes for high molecular size hsp (hsp70, hsc70, hsp90) showed upregulation. Regarding the transcriptional induction of *Hsp* genes of *B. koreanus*, we suggest that intracellular stress was induced by B[*a*]P exposure based on a strong involvement of genes coding for high molecular size *Hsp*. In case of the decreased transcriptional pattern of *Hsp* genes, we assume that specific or unexpected combined effects of B[*a*]P with its metabolites would be involved in the inhibitory potential of expression of genes coding for low molecular size *hsps*. However, further studies would be required to analyze the potential role of each *Hsp* at a cellular level, as different metabolites of B[*a*]P may have different modulatory effects. Taken together, transcripts of several *Hsp* genes were modulated by directly regulating transcriptional level of *Hsp* genes upon B[*a*]P or its metabolites in *B. koreanus,* although the regulatory mechanism of B[*a*]P is yet to be clarified.

*B. koreanus* defensome shows likely nonspecific molecular responses on transcriptional or biochemical effects against B[*a*]P treatment due to the evolutionary gap of detoxification capacity between invertebrates and vertebrates. We, therefore, focus here on an understanding of molecular mechanisms of invertebrates as to whether invertebrates have conserved molecular and biochemical response capacities for adaptations to environmental changes. Goldstone et al. reported that the chemical defensome of aquatic invertebrates could be conserved. [55] This is characterized by environmental sensing and responsive genes in genomes of the sea urchin *Strongylocentrotus purpuratus* and the sea anemone *Nematostella vectensis*.[2,55] Colbourne et al. suggested that the water flea *Daphnia pulex* has an ecoresponsive genome as it has conserved responsive genes to environmental challenges.[56] Moreover, to date numerous studies suggested conserved or specific molecular responsive systems in aquatic invertebrates, although invertebrate damage response mechanisms are only partially understood as yet. Subsequently, several aquatic invertebrates have been used as model animals for environmental monitoring based on their conserved responsive mechanisms.

In rotifers including *B. koreanus*, there is increasing evidence of a conserved molecular response mechanism. Rotifers have an effective antioxidant protection system and conserved progesterone hormone functions.[57,58] Genomic structure analysis of several genes that are involved in DNA replication and repair mechanisms revealed that rotifer have a compact and conserved genomic organization, and their transcriptional responses against radiation damage demonstrate similar response mechanisms as shown in vertebrates.[33,59] Also in rotifer, a primitive neural system and/or neurological response mechanism were suggested that include conserved acetylcholinesterase (*AChE*) functions and AChE receptors against neurotoxic compounds .[60−63] In addition, common responses to environmental stressors such as reorganization of cellular organelles and proteins, induction of P-glycoprotein activity or antioxidant defense system, and *hsps* modulation were identified.[64−68] Therefore, we suppose that similar stress responses including the induction of the defensome may have evolved in rotifer and other invertebrates that were retained in vertebrates, even though a considerable evolutionary gap exists at lower taxon level in eukaryotes on detoxification and metabolism of B[*a*]P.

In conclusion, our results show that the application of whole *CYP* profiling with comprehensive analysis of the detoxification system is a valuable testing method for a better understanding on the mode of action of PAHs including B[*a*]P as a novel approach using aquatic invertebrates. To date, the knowledge on *CYP* genes and their regulation and specific function are extremely limited in aquatic invertebrates. In *CYP1A*-deficient invertebrates, several reports have been published to highlight the potential role of other *CYP* family in PAH metabolism.[44,45,69,70] Despite this limitation, whole spectrum profiling of *CYP*s could be a useful method for investigation of novel function of invertebrate *CYP* genes compared to vertebrate the *CYP1* family that has been extensively employed for environmental monitoring. Taken together, our observations highlight the potential effect of B[*a*]P and/or its metabolites in the modulation of physiological parameters along with the induction of a defensome in rotifers, even though the mode of action of B[*a*]P-triggered physiological changes are not fully understood as yet in rotifers, and the in-depth molecular response mechanism should be analyzed and clarified in invertebrates.

## ■ ASSOCIATED CONTENT

### Supporting Information

Gene annotation, phylogenetic analysis, general methods, and raw value of transcript profiles were incorporated in Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


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### Notes

The authors declare no competing financial interest.

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