

Full Length Research Paper

Physiological responses and expression of Mn-superoxide dismutase mRNA in *Phascolosoma esculenta* exposed to benzo(a)pyrene (BaP)

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cDNA encoding Mn-superoxide dismutase (MnSOD) and 18S rRNA of *Phascolosoma esculenta* were cloned in this study. The partial MnSOD cDNA obtained consisted of 411 bp, encoded a protein of 137 amino acids and contained a MnSOD signature (DVWEHAYY). Physiological activity responses and expression of MnSOD mRNA in *P. Esculenta* exposed to benzo(a)pyrene (BaP) for 4, 24, 48, 96, 168 and 336 h were also studied, and results indicate that *P. Esculenta* showed a strong ability to resist BaP stress. The expression levels of MnSOD mRNA were highest at 4 h of exposure, while the physiological activity levels of MnSOD were highest at 96 h, but then returned to baseline levels. The different response rates indicated that there was a very intricate regulating mechanism that prevents the damaging effects of the oxidative stress imposed by BaP. This result suggests that the expression of MnSOD mRNA in *P. Esculenta* in a short time can be used as a sensitive biomarker to alarm an early acute BaP contamination.

Key words: *Phascolosoma esculenta*, benzo(a)pyrene (BaP), Mn-superoxide dismutase (MnSOD), gene expression, physiological activity.

INTRODUCTION

Phascolosoma esculenta also known as Chinese caterpillar fungus, is an organism on the Chinese coast that is an important source of sea food and materials for medicines. *P. esculenta*, belonging to the phylum Sipuncula, which has a special phylogenesis (José and Harlan, 2009), is a small phylum of coelomate marine worms closely related to polychaete annelids. It has a body divided into a retractable portion and a trunk, with the anus located towards the anterior end of the body, and has an open circulatory system. Sipunculans have been used as model systems for studying invertebrate immunity and the results obtained regarding some of their immunities are comparable to the results obtained from

vertebrates (Giuseppina, 2009). *P. esculenta* usually lives in the intertidal mud habitat of coastal areas. However, the increasing pollution of coastal areas in China has given rise to public concern about the food safety of marine products including *P. esculenta* produced from such areas. The oxidative stress caused by persistent environmental contaminant was considered as one of the main factors that affects the healthy *P. esculenta* in these areas. For this reason, the ecotoxicological monitoring of *P. esculenta* in coastal producing areas is important in Chinese sea aquaculture.

Benzo(a)pyrene (BaP) is a five-ring polycyclic aromatic hydrocarbon (PAH) widely distributed in nature. BaP may trigger mutagenesis and subsequent pathological processes (Hannah, 1982), including carcinogenesis (Neal and Rigdon 1967; Lee and Shim, 2007). The increase in industrialization, agriculture and the subsequent deposition effluents on the southeast coast of China has made

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great contribution to BaP pollution in these areas. One recent study indicated that the total concentrations of 15 PAHs in the soils ranged from 28.2 to 1432.3 ng·g⁻¹ and 4 to 6 rings PAHs were the dominant compounds by analyzing 33 soil samples (0 to 10 cm layer) collected from various functional zones including industrial areas, residential areas, scenic areas and agricultural fields in Quanzhou city southeast of China (Lin et al., 2011). High levels of PAHs were usually found in the estuary and coastal areas in China; for example the total concentration of 16 PAHs varied from 105.3 to 5118.3 ng·g⁻¹ dry weight in surface sediments of Xiamen Western Harbor southeast of China, and 4 to 6 rings PAHs were the dominant compounds (Tian et al., 2004). The total concentrations of PAHs of other different site sediment samples of Xiamen Western Harbor could range from 70 to 33000 ng·g⁻¹ dry weight, and the BaP concentration of some site was 30800 ng·g⁻¹; occupying the highest level among the PAHs in these coastal sediments southeast of China (Zhang et al., 1996). In Pearl River Delta and Estuary southeast of China, the total concentration of PAHs ranged from 408 to 10811 ng·g⁻¹ (Mai et al., 2000). All these results suggest that there are ecological risks of the PAHs in some estuary and coastal areas southeast of China, and BaP has attracted more attention because of its wide distribution and high level among the PAHs.

BaP introduction into the environment could bring about severe damage to human health through the food chains (Lioy, 1988). The BaP sediments are the food and the habitat for *P. esculenta*, and the bodywall of *P. esculenta* is the main parts of the food for people. Parts of BaP and its metabolites could be distributed on the bodywall by the process of biotransport and biotransformation of BaP from the sediments. BaP metabolites for example 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (anti-BPDE), 3-hydroxybenzo[a]pyrene (3-OH-BP) and 9-hydroxybenzo[a]pyrene (9-OH-BP) etc., can oxidatively generate DNA damage via regulation on the oxidative stress response and the expression of CYP1 metabolism enzymes (An et al., 2011) and BaP-DNA adducts could be formed (Park and Schatz, 1999), thereby inducing base substitution (Gao et al., 1996; Eisenstadt et al., 1982) that could increase cancer risk. *P. esculenta* is at risk from BaP pollution and also increases the human health risk as a popular seafood of southeast coastal areas of China due to increasing anthropogenic pollution entering its habitat environment.

Superoxide dismutases (SODs) involved in the defense system against reactive oxygen species (ROS), are a class of important antioxidant enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide in nearly all cells exposed to oxygen (Igor et al., 2002). Several common forms of SOD exist: they are proteins cofactored with copper and zinc, or manganese, iron, or nickel. SOD plays a key antioxidant role. Mn-superoxide dismutase (MnSOD) is expressed in many cell types and tissues at relatively high levels. It is also

highly regulated by a variety of intracellular and environmental cues, and it plays an important role in cancer generation and development (Igor et al., 2002). MnSOD has been suggested to have tumor suppressor function and is located in the mitochondria where the majority of MnSOD is generated during respiration (Hu, 2005). The increase in MnSOD expression in ovarian cancer is a cellular response to intrinsic ROS stress and the scavenging of superoxide by SOD may alleviate the ROS stress, and thus reduce the simulating effect of ROS on cell growth (Hu, 2005). Since SOD is as an important part of the immune system that is responsible for the defense of the host, any fluctuation in the immune response could potentially affect resistance of *P. esculenta* to diseases or pollution. Measurements of the immune status may predict whether the health of populations of *P. esculenta* health is at risk and could also indicate the impact of BaP on *P. esculenta* farming.

Although *P. esculenta* served as a delicious sea food in local restaurants, ecotoxicological researches related to pollutants have been poorly reported, and the transcriptional regulation of *P. esculenta* SOD at mRNA level with respect to environmental pollutants has not been studied. The present study therefore aimed to: (1) clone cDNA encoding MnSOD and 18S rRNA of *P. esculenta*; (2) investigate the dose- and time-related effects of BaP on SOD mRNA using quantitative polymerase chain reaction; and (3) compare the physiological activities and gene expressions of MnSOD from *P. esculenta* so as to further understand the host defense and their contribution to the alarming BaP pollution in coastal estuary wetland habitation.

MATERIALS AND METHODS

P. esculenta used in the experiments were obtained from Luoyang mangrove intertidal mud sediment of Quanzhou Bay, southeast of China. The average weight of *P. esculenta* was 4.8 g and the average length was 8 cm. Some sediment habituated by these individuals was also brought back to laboratory to be prepared for the experiment.

BaP was purchased from Sigma (HPLC grade, USA) and was dissolved in cyclohexane. All other chemicals were of reagent grade and obtained from local commercial sources.

Experiment design

To examine the differential expression of SOD transcripts during the BaP exposures, four exposure experiments were performed. Healthy *P. esculenta* were incubated in tanks containing local sediment at room temperature (28 ± 1 °C) for five days to acclimate the worm to laboratory conditions prior to BaP exposures. The experiments were designed as follows: the control group without BaP (CK₀); the solvent control (CK₁); and the four toxicant concentrations, with three replicates of each concentration. BaP concentrations were designed based on the pollution concentrations in the coastal sediment southeast of China (Zhang et al., 1996; Tian et al., 2004). BaP was first mixed with sediment to give final concentrations of 100, 500, 2500 and 10000 ng BaP/g dry

sediment, which simply were regarded as A, B, C and D treatment groups. Portions of the sediments (350 g) from each of the treatment groups were placed in tanks. The experiment in each group was replicated three times. The tanks were covered with materials to prevent light penetration but allow air penetration. Samples were collected after 4, 24, 48, 96, 168 and 336 h exposure to BaP. Three *P. esculenta* from each group were taken at the end of each exposure period. Samples of body wall from each individual *P. esculenta* were surgically removed and immediately frozen in liquid nitrogen and stored at -80°C for further use.

cDNA cloning and nucleotide sequence analysis

P. esculenta MnSOD cDNA sequence (GenBank accession no. EF062359) was first amplified from bodywall RNA with reverse transcription polymerase chain reaction (RT-PCR). Total RNA of *P. esculenta* bodywall was isolated using the TRIzol method (Invitrogen). Degenerate primers for cDNA cloning of MnSOD were designed with reference to recognize the conserved region of various invertebrate species of the MnSOD reported in NCBI. MnSOD forward primer (5'-CTG(T) CAT(C) CAC C(A)A(G) C(A/G) AAG CAC CA-3') and MnSOD reversed primer (5'-AG(A)G(A) TAG(A) TAA(G/C) GCA(G) TGC(T) TCC CA -3') was used for cloning MnSOD. Gene-specific primers were designed from the previously determined DNA sequence to confirm the *P. esculenta* MnSOD partial sequences. The amplified cDNA fragments were cloned into the pGEM-T Easy vector following the instructions provided (Promega Corporation, Madison, WI, USA). Recombinant bacteria were identified by blue/white screening and confirmed by PCR. Plasmids containing the inserted MnSOD were used as a template for DNA sequencing. Nucleotide sequence was determined by the automatic sequencer ABI Prism 310 Genetic Analyzer (Applied Biosystems). The MnSOD cDNA sequence and its translated amino acid sequence were analyzed and compared using the BLASTX and BLASP search program (<http://ncbi.nlm.nih.gov/BLAST>) with a GenBank database search. Multiple alignments of the amino acid sequences were finished on <http://www.ebi.ac.uk/Tools/es/cgi-bin/clustalw2>.

In addition, *P. esculenta* 18S rRNA cDNA sequence (GenBank accession no. HM591303) was first amplified from bodywall RNA with RT-PCR. The specific primers were chosen using highly conserved regions according to those species of the Phylum Sipuncula reported in NCBI. *P. esculenta* 18S rRNA cDNA fragment was used as the internal control for quantitative real-time PCR.

Quantification of SOD mRNA level by real-time PCR

Total RNA of *P. esculenta* was isolated from pooled bodywall tissues using the TRIzol method (Invitrogen). Total RNA was treated with RNase-free DNaseI (Roche) to remove possible contamination by DNA molecules and reverse-transcribed into cDNA using the One-Step TaKaRa Primescript™ RT Reagent Kit (Perfect Real-time, DRR037S, TaKaRa). In order to examine the MnSOD mRNA expression changes, oligonucleotide primers were redesigned according to the released MnSOD cDNA sequence on GenBank (EF062359) and 18S rRNA cDNA sequence on GenBank (HM591303) as follows: MnSOD 2F (5'-GAACCTACAGGGG-ACTTGCTT-3'), MnSOD 2R (5'-CAAACATCAATGCCAAACAGT-3') 18S rRNA 2F (5'-TGCTTTCGAGTTGTTCTGTCT-3'), 18S rRNA 2R (5'-CCTCGGTTCTATTTTGTGGTTTTTC-3'). The gene-specific primers were designed with the Primer Express(r) Software v3.0 (Applied Biosystem). Expected PCR products of target gene (MnSOD) and housekeeping control gene (18S rRNA) were 220 and 127 bp, respectively.

Real-time PCR assays were performed using the methodology of ABI Power SYBR Green PCR Master Mix and an ABI 7500 System

(Applied Biosystems), according to the manufacturer's instructions. QPCR was performed as follows: denaturation at 95°C for 3 min; 40 cycles of denaturation at 95°C for 20 s, annealing at 56°C for 30 s and a final extension at 72°C for 40 s. As an internal control, experiments were duplicated with 18S rRNA, and all data were expressed as change with respect to corresponding 18S rRNA calculated threshold cycle (C_T) levels. Triplicate assays per cDNA sample were carried out to determine the average.

Determination of MnSOD activities

MnSOD activities were determined according to the method of Marklund and Marklund (1974), where CuZnSOD activities inhibited by 1.5 mM KCN and MnSOD activities were measured. One unit MnSOD activity was described as the amount of total protein that cause 50% inhibition of pyrogallol autoxidation.

Statistical analysis

Results were presented as mean \pm standard deviation (SD). Each data was analyzed from three *P. esculenta* for each exposure period. The levels of statistical significance were determined by one-way ANOVA and results were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

RT-PCR and cDNA cloning

The degenerate primer pair, was used to amplify the 411 bp fragment of the partial MnSOD. The cDNA sequence was predicted to encode a protein of 137 amino acids (GenBank accession no. EF062359, 2007), containing a MnSOD signature (DVWEHAYY) (Cheng et al., 2006; Lin et al., 2010) (Figure 1). The fragment showed significant similarity to MnSOD of other aquatic life-forms and also consistency to MnSOD cloned afterward by Wang et al. (2009) (GenBank accession no. GQ487656) in GenBank database. The deduced amino acid sequence of *P. esculenta* MnSOD was compared to those from other marine species by BLASTX in NCBI. The deduced amino acid sequence of *P. esculenta* MnSOD showed similarity of 71% with *Macrobrachium rosenbergii* MnSOD (GenBank accession no. AAZ81617.1) and *Fenneropenaeus chinensis* MnSOD (GenBank accession no. ABB05539.1), 70% with *Xenopus laevis* MnSOD (GenBank accession no. NP_001083968.1), 69% with both *Charybdis feriatus* MnSOD (GenBank accession no. O96347.1) and *Rachycentron canadum* MnSOD (GenBank accession no. ABC71306.2), and 68% with *Anguilla anguilla* MnSOD (GenBank accession no. ABF50548.1). Multiple alignments of these amino acid sequences on <http://www.ebi.ac.uk/Tools/es/cgi-bin/clustalw2> are shown in Figure 1. The sequences showed relatively high identity, which suggested that MnSOD gene should be well conserved in many marine organisms.

The 545 bp fragment of the partial 18S rRNA of *P.*

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Macrobrachium      MLSFSRLFASR---GLVAAG-----VRSCQKHTLPDLPYDYNALEPVICAEIMQLHHS 50
Fenneropenaeus    MLAATRLFSAR---GLAALG-----VRSQQKHTLPDLAYDYNALEPVICAEIMQLHHS 50
Charybdis         -MLLARAFARR---SLRAG-----LWCRQKHTLPDLPYDYGALEPTISAEIMQLHHS 48
Phascolosoma      -----
Rachycentron      -MLCRVQIIRR----CAASLTQSIHQVAAARQKHTLPDLTYDYGALEPHINAEIMQLHHS 55
Anguilla          -MLCRFNHIPR---CAISLS---CVVSSKQKHTLPDLTYDYGALEPHISAEITQLHHS 51
Xenopus           -MLCRLSVCGRGRMRCVPALA----YSFCKEKHTLPDLPYDYGALQPHISAEIMQLHHS 54

Macrobrachium      KHHQTYVNNLNVAAEKLAEAHAKGDVSTVIVSLAPALKFNGGGHINHTIFWQNLSPDGG-E 109
Fenneropenaeus    KHHQTYVNNLNVAAEKLAEAAKGDVNTVIVSLAPALKFNGGGHINHTIFWQNLSPDGG-E 109
Charybdis         KHHQTYVNNLNVAAEKLAEAKEKGDVSTIIVSLAPALRFNGGGHINHSIFWQNLSDAGG-E 107
Phascolosoma      KHHQTYVNNLNVAAEQIAEATANEDVTRLVALQAQAFKFNNGGGHINHSIFWQNLSPDGG-E 59
Rachycentron      KHHATYVNNLNVTEEKYQEAALAKGDVTAQVALQPALKFNGGGHINHTIFWTNLSPNGGGE 115
Anguilla          KHHATYVNNLNVTEEKYKALAKGDVTAQVALQPALKFNGGGHVNHTIFWTNLSPNGGGE 111
Xenopus           KHHATYVNNLNVTEEKYAEALAKGDVTTQVSLQAALKFNNGGGHINHTIFWTNLSPNGGGE 114
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Macrobrachium      PSGELLAAINRDFGSLDAMKSQLSAATIAVQSGSGWGLGYNKQKQALQIATCPSQDPLQA 169
Fenneropenaeus    PAGDLLAAINKDFGSDAMKAQLSAATVAVQSGSGWGLGFNKQKQSLQIATCANQDPLEA 169
Charybdis         PEGELLAAINRDFGSENMKNQLSAQTAVVQSGSGWGLGY-IAEGALQIATCPNQDPLEA 166
Phascolosoma      PTGDLAAIIRDFDLENLKSTMSAAAVGVQSGSVAWLGYCKNTDSLQVATCANQDPLQG 119
Rachycentron      PQGELMEAIKRDFGSEKMKESMAATVAVQSGSGWGLGYDKESGTLRIAACANQDPLQG 175
Anguilla          PQGELMEAIKRDFGSEKLRKMSMASVAVQSGSGWGLGYDKESGRLRVAACSNQDPLQG 171
Xenopus           PQGELLDAIKRDFGSEKFKELNNTVSVGVQSGSGWGLGYNKDSNRLQLAACANQDPLQG 174
* *:: ** :*.*.: :: :. :::*****.***: . *:::*.****:.

Macrobrachium      STGLVPLFGIDVWEHAYYLQYKNVR-----PDYVNAIWNVANWNDISARFAAAK---- 218
Fenneropenaeus    TTGLVPLFGIDVWEHAYYLQYKNVR-----PDYVNAIWKVANWKDISARFKAASK-- 220
Charybdis         TTGLVPLFGIDVWEHAYYLQYKNVRRIMLKPISLIGRISLQSGMQSKNATSCSGA 224
Phascolosoma      TTGLVPLFGIDVWEHAYY----- 137
Rachycentron      TTGLIPLLIGIDVWEHAYYLQYKNVR-----PDYVKAIWNVISWENVAERLQTAKK--- 225
Anguilla          TTGLIPLLIGIDVWEHAYYLQYKNVR-----PDYVKAIWNVNVNENITERFQSAKK--- 221
Xenopus           TTGLIPLLIGIDVWEHAYYLQYKNVR-----PDYLKAIWNVINWENVTERYQASKK--- 224
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Figure 1. Multiple alignments of *Phascolosoma esculenta* MnSOD (ABN13920.1) with *Macrobrachium rosenbergii* MnSOD (AAZ81617.1), *Fenneropenaeus chinensis* (ABB05539.1), *Xenopus laevis* MnSOD (NP001083968.1) *Charybdis feriatius* MnSOD (O96347.1), *Rachycentron canadum* MnSOD (ABC71306.2) and *Anguilla anguilla* MnSOD (ABF50548.1).

esculenta was amplified by using RT-PCR (GenBank accession no. HM591303). Result indicate that the partial 18S rRNA cDNA sequence was highly similar to those species from Sipuncula, and showed similarity of 100% with *Antillesoma antillarum* (GenBank accession no. DQ299951.1), 99% with *A. antillarum* (GenBank accession no. AF519259.1), *Siphonosoma vastum* (GenBank accession no. DQ300003.1), *Cloeosiphon aspergillus* (GenBank accession no. DQ299968.1) and *Phascolosoma capitatum* (GenBank accession no. DQ299986.1).

MnSOD physiological activities of *P. esculenta* exposed to BaP

The individual amount of *P. esculenta* have no obvious loss in the experiments of the 100 ~ 10000 ng BaP/g dry sediment exposure, thus *P. esculenta* showed a strong ability to resist environment BaP stress. Few individuals with the effect of BaP treatments on MnSOD activities are given in Figure 2. The results indicate that the obvious effects of the 100 ~ 10000 ng BaP/g dry sediment exposure on the physiological activity of MnSOD in *P.*

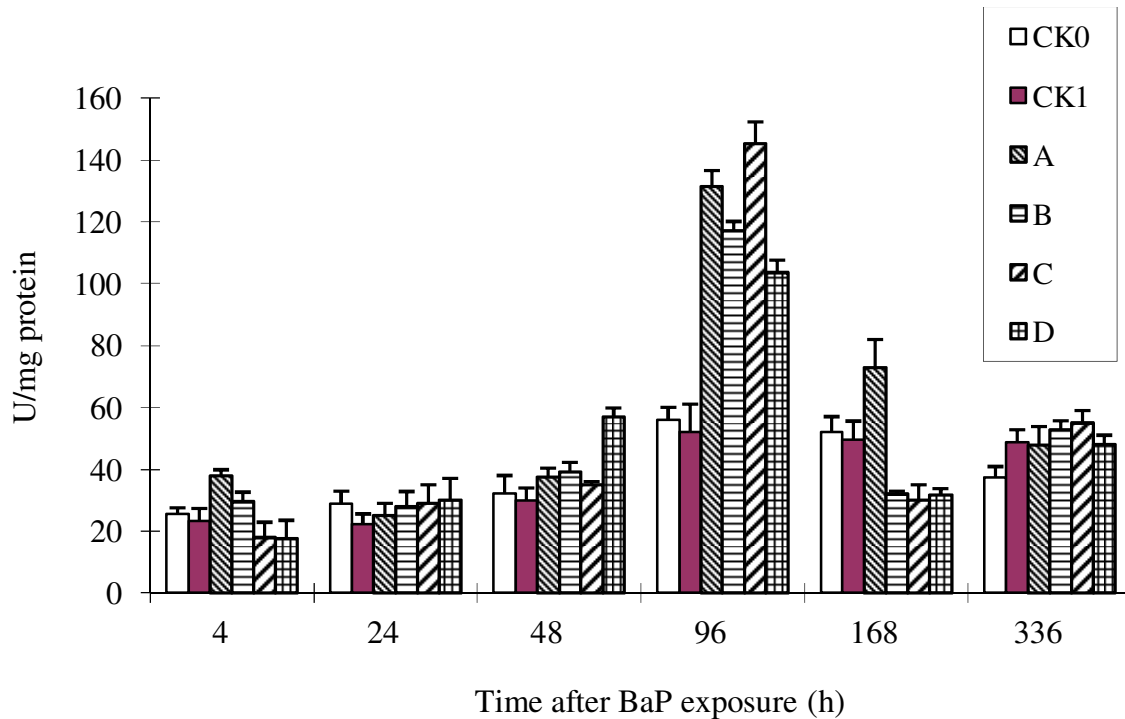


Figure 2. MnSOD activities of *Phascolosoma esculenta* bodywall after BaP exposure. CK₀ is the control group without BaP; CK₁ is the solvent control; A, B, C and D treatment groups are the BaP treatment groups with 100, 500, 2500 and 10000 ng BaP/g dry sediment concentrations respectively.

esculenta was different either with exposure time or with exposure concentration. It is difficult to find the change rule between the activity and the exposure concentration. However, the trend of the activity change could be easily found in each BaP treatment group with the exposure time. The physiological activity levels of MnSOD increased till 96 h and then decreased nearly to the normal level. This showed that the oxidative damage brought by BaP was counteracted by MnSOD. At 96 h post exposure, the MnSOD activities in the highest dose level (group D) increased by 491.9% over that of 4 h post exposure, and increased by 99.21% over that of CK₁ at 96 h exposure period. The MnSOD activities in the lowest dose level (group A) also increased by 247.6% over that of 4 h post exposure, and increased by 153.1% over that of CK₁ at 96 h exposure period. All these changes were significant ($p < 0.05$), thus suggesting that the exposure period is an important factor affecting the MnSOD activities.

Marine organisms protect themselves from the oxidative stress by involving various antioxidant enzymes. In this study, different antioxidant enzymes had different response characteristic to BaP contamination. The effect of different BaP concentrations on the activities of SOD, glutathione peroxidase (GPx) and catalase (CAT) in *Boleophthalmus pectinirostris* liver was also studied under experimental condition (Feng et al., 2001). The results obtained showed that the SOD activities and GPx

activities increased significantly with BaP exposure in higher concentration. SOD activity was induced significantly in 72 h exposure, while GPx activity was induced significantly at 168 h, after which they both decreased to some extent with the prolonged exposure. The activity change patterns of our results were also similar.

QPCR for MnSOD mRNA expression

The level of MnSOD mRNA was measured by QPCR. MnSOD transcripts in *P. esculenta* bodywall increased significantly 4 h after exposed to BaP (Figure 3). However, the transcript of MnSOD declined after 24 h and returned to the original value after 48 h. With the prolonged exposure, the expression levels of MnSOD mRNA at different concentrations decreased to some extent. At 4 h BaP exposure period, the expression levels of MnSOD mRNA was dramatically upregulated, and the expression levels of A, B, C, D BaP treatment group increased by 5.2, 5.1, 4.88, 4.27 times over that of CK₁, respectively. The MnSOD transcripts in *P. esculenta* bodywall were highest in 4 h after BaP exposure, indicating an induction of the MnSOD transcript system in a short time. Compared with the physiological activity of MnSOD (Figure 2), we found that the responses between the MnSOD activity and the gene expression to the BaP exposure were at different rate. The response rate of the

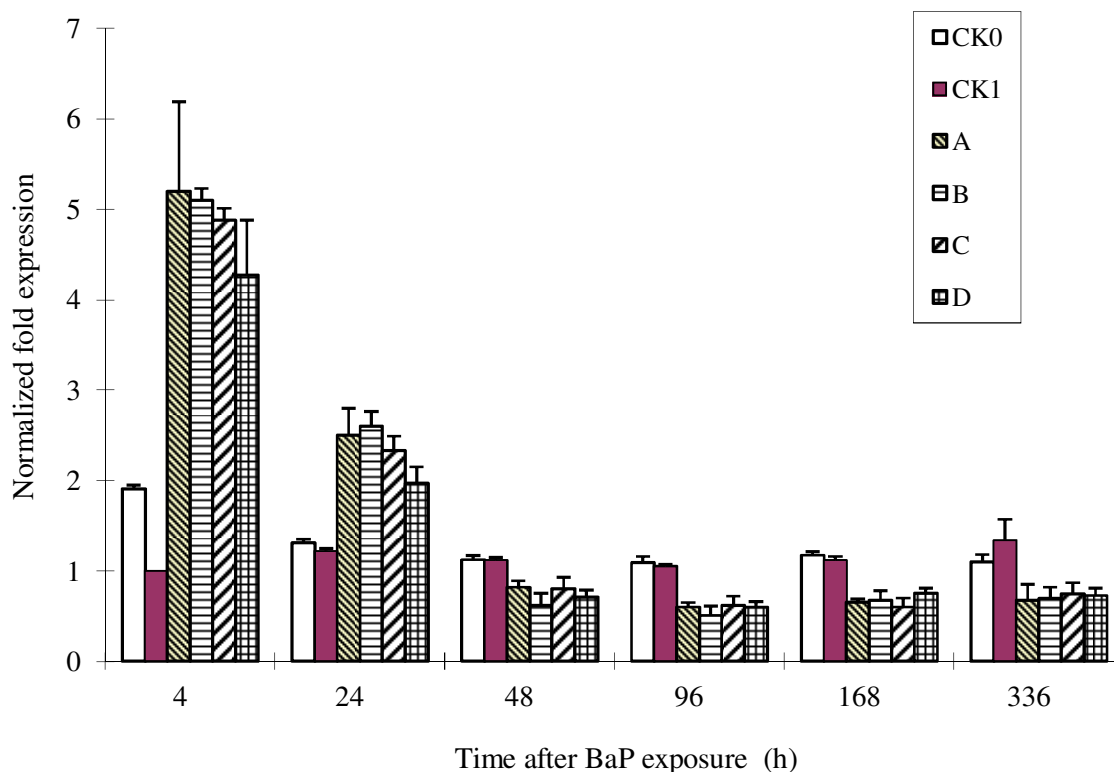


Figure 3. Quantitative real-time RT-PCR analyses of MnSOD expression in bodywall of *Phascolosoma esculenta* after 4, 24, 48, 96, 168 and 336 h BaP exposure. CK₀ is the control group without BaP; CK₁ is the solvent control; A, B, C and D treatment groups are the BaP treatment groups with 100, 500, 2500, 10000 ng BaP / g dry sediment concentrations respectively.

activity was slower than that of gene expression. Similarly, protein expression of GPx increased significantly in diabetic animals compared to the controls, though the activities remained constant (Gökhan, 2009). The results show a very intricate regulating mechanism to prevent the damaging effects of oxidative stress induced by BaP. The results also suggest that the expression of MnSOD mRNA in *P. esculenta* response can be used as a sensitive biomarker to control the earlier acute BaP contamination.

Conclusion

Both MnSOD and 18S rRNA of *P. esculenta* bodywall were first cloned, and the partial MnSOD cDNA obtained consisted of 411 bp and encoded a protein of 137 amino acids. It also contained a MnSOD signature (DVWEHAYY). The 18S rRNA cDNA sequence cloned and used as a housekeeping control gene was consistent with those species from Sipuncula. Our results indicate that *P. esculenta* has a good tolerance to BaP stress and showed a strong ability to resist BaP stress. The responses between the MnSOD activity and the gene expression to the BaP exposure are at different rates, and the response rate of the activity was slower than that

of gene expression. The physiological activity levels of MnSOD increased with the prolonged exposure time and were highest at 96 h, while the expression levels of MnSOD mRNA were highest at 4 h. There was a very intricate regulating mechanism that prevents the damaging effects of oxidative stress imposed by BaP. The responses of the activity and the mRNA expression levels of MnSOD, particularly their return to baseline levels with the prolonged BaP exposure time, suggest that MnSOD played a key role in counteracting the oxidative damage induced by marine BaP contamination. This result indicates that the expression of MnSOD mRNA in *P. esculenta* in a short time can be used as a sensitive biomarker to control the earlier acute BaP contamination.

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