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Molecular Cloning and mRNA Expression of Cytochrome P450 (CYP450)-related Protein in the Pacific Oyster, *Crassostrea gigas*: A Water Temperature and Time Study

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Abstract: We cloned the complete complementary DNA (cDNA) of a Pacific oyster (*Crassostrea gigas*) cytochrome P450 (CYP450)-related protein using rapid amplification of cDNA ends (RACE). The cDNA included a 1470 bp open reading frame that began with the first ATG codon at position 103 bp and ended with a TAG stop codon at position 1573 bp (GenBank accession EF451959). The sequence had all major functional domains and characteristics of previously characterized CYP450 molecules, including the heme-binding region (FGVGRRRCVG) and putative arginine codon (R) integral to enzymatic function. An NCBI/GenBank database comparison to other CYP450 genes revealed that the deduced *C. gigas* CYP450 amino acid sequence is similar to that of mouse (*Mus musculus*) CYP450 2D9 (29%, accession AK078880), rabbit (*Oryctolagus cuniculus*) CYP450 2D/II (28%, AB008785), and white-tufted-ear marmoset (*Callithrix jacchus*) CYP450 2D (28%, AY082602). Thus, although the *C. gigas* CYP450 we cloned appears to belong to the 2D type of the CYP450 group, it has low similarity to this type. CYP450 mRNA expression increased over 6 h in *C. gigas* gills at 30°C and 10°C, and then decreased, indicating that CYP450 plays an important role in *C. gigas* exposed to water temperature changes. This finding can be used as a physiological index for Pacific oysters exposed to changing water temperatures.

Key words: cloning, *crassostrea gigas*, CYP450, expression, water temperature

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INTRODUCTION

Water temperature affects physiological functions of aquatic organisms, such as growth, propagation, metabolism, and osmoregulation, and thus exerts significant impact on the organisms. As a species inhabiting shallow waters and estuaries, the Pacific oyster, *Crassostrea gigas* is highly affected by water temperature. The optimal water temperature for Pacific oysters is 15-20°C. Outside this range, water temperature acts as a physical stress on Pacific oysters, adversely affecting their physiological state, including defense mechanisms (Zhang et al., 2006).

The heat shock protein family and protein 65-kDa, which is related to warm temperature acclimation, are used as biological markers for stress factors, such as water temperature change (Choi et al., 2008). Recently, the cytochrome P450s (CYP450s) have been suggested as biological markers that react sensitively to water temperature stress (Arukwe and Goksùyr, 2000). CYP450s are enzymes that contain heme (reduced hematin), which colors hemoglobin. Several factors, such as developmental stage, sex, diet, and seasonal changes in steroid levels and temperature, affect the basal level of CYP isozymes (Arukwe and Goksùyr, 2000). CYP450s are heme-dependent oxidases that catalyze a wide variety of reactions, including hydroxylation, epoxidation, N-demethylation, Odealkylation, deamination, sulfoxidation, and oxidative dehalogenation, by using NADPH and/or NADH as electron donors. They comprise a superfamily of heme-thiolate enzymes responsible for the oxidative, peroxidative, and reductive metabolism of many structurally diverse compounds (Pua et al., 2003).

Diverse varieties of CYP450 are found in plants, animals, bacteria, and other organisms, and CYP450s are the subject of active research. The enzyme families forming this gigantic

superfamily have different substrates and metabolic products. Isozymes that belong to different subfamilies characteristically form derivatives and have nuclear receptors specific to each group (Yoon et al., 2003). The very diverse CYP450 types are divided into families, subfamilies, and polypeptides and include CYP19A1, CYP11B1, CYP7a1, CYP1A, CYP2d2, CYP4A, CYP739A, CYP513E1, and many others. CYP450s in the 1A group have been cloned in a number of fish species, including rainbow trout (Berndtson and Chen, 1994), brook and lake trout (Rees and Li, 2004), red sea bream (Mizukami et al., 1994), and sea bass (Stien et al., 1998). Temperature compensation in relation to the CYP450 enzyme system during acclimation by fishes to environmental changes (e.g., water temperature changes) have been reported for killifish, salmonids, roach, and bluegill (Andersson and Koivusaari, 1985; Karr et al., 1985; George et al., 1990). However, among shellfish, changes in CYP450 enzyme activity and mRNA expression in relation to water temperature change have been studied only in the zebra mussel (*Dreissena polymorpha*) and mussel (*Mytilus galloprovincialis*; Ricciardi et al., 2006; Bebianno et al., 2007).

Thus, we cloned the complementary DNA (cDNA) of a CYP450-related protein and investigated its mRNA expression in the gills of Pacific oysters transferred from 20°C to 30°C and 10°C.

MATERIALS AND METHODS

Experimental oysters and water-temperature treatment

We used 1-yr-old Pacific oyster (n=100, average shell length: 112±10.7 mm; height: 31.1±5.4 mm; weight: 20.3±3.9 g) obtained from the oyster hatchery on Daebu Island in Goseong (Gyeongnam, Korea). These were acclimated in two circulating filter tanks (40 L, 50 oysters per a tank) for a week prior to experiment in the laboratory. Water temperature and photoperiod were maintained at 20°C and a 12L/12D cycle.

The oysters acclimated at 20°C (control group) directly transferred to 30°C and 10°C (experimental group), respectively. Gills of five oysters were randomly dissected in the following time period: 0, 1, 3, 6, 12 and 24 h. The tissues immediately were frozen in liquid nitrogen after collection and stored at −80°C until total RNA extraction.

Rapid amplification of cDNA 3' and 5' ends (RACE)

For RACE reactions, total RNA was extracted using a Trizol (Gibco/BRL, Grand Island, NY, USA) from the gill of Pacific oysters. Reverse transcription was performed using M-MLV reverse transcriptase (Bioneer, Daejeon, Korea). Using 3 μg of total RNA as the template, 5'-RACE-ready complementary DNA (cDNA) and 3'-RACE-ready cDNA were generated using the protocols and reagents provided in the CapFishing Full-length cDNA Premix kit (Seegene, Seoul, Korea). Gene-specific primers were selected from cgCYP450 partial cDNA (GenBank accession no. AF075692). For 3'-RACE, the 50 μL PCR reaction mixture contained 5 μL of 3'-RACE-ready cDNA, 1 μL of 10 μmol 3' target primer (5'-CTG TGA ATG CTG CGA CTA CGA T-3'), 1 μL of 10 μmol 3' RACE gene-specific primer (5'-CTG ACC GGT TCC TGG AGG AGG GAA AGT-3'), and 25 μL of SeeAmp Taq Plus Master Mix (Seegene). Polymerase chain reaction (PCR) was carried out for 40 cycles at 94°C for 45 sec for denaturation, 62°C for 45 sec for primer annealing, and 72°C for 90 sec for extension, followed by 5 min at 72°C for extension. The PCR product was amplified, cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA), and sequenced. For 5'-RACE, the 50 μL PCR reaction mixture contained 5'-RACE-ready cDNA, 5' gene-specific primer (5'-ACT TTC CTT CCT CCA GGA ACC GGT CAG-3'), 5' target primer (5'-GTC TAC CAG GCA TTC GCT TCA T-3'), and SeeAmp Taq Plus Master Mix, as for 3'-RACE, under the same PCR conditions. The PCR product was amplified, cloned into pGEM-T Easy Vector, and sequenced. The DNA and deduced amino acid sequences were analyzed using GENETYX-WIN (Software Development, Tokyo, Japan).

Reverse transcription polymerase chain reaction (RT-PCR)

2.5 μg of total RNA extracted from the gonad, digestive gland, mantle, gill and intestine of oysters under thermal stress was reverse transcripted with M-MLV reverse transcriptase (Bioneer) and oligo-d$(T)_{15}$ primer (Promaga). RT-PCR was conducted to determine the relative expression of cgCYP450 mRNA and 28S ribosomal RNA (cg28Sr) in various tissues of *C. gigas*. To optimize the number of cycles used for RT-PCR, the reactions (1 μL) from the gonad, digestive gland, mantle, gill and intestine of oysters exposed to water-temperature change were used as the template for RT-PCR amplification. cgCYP450- and cg28Sr-specific primers for RT-PCR were designed from published sequences: cgCYP450 forward primer, 5'-AGG CGA TAT GAC GAC GAG TT-3' and cgCYP450 reverse primer, 5'-ACG TGT TCA TCT GTG AGC CA-3'; cg28Sr forward primer, 5'-TGC TCT GGA CTG TCC TAG GA-3' and cg28Sr reverse primer, 5'-ACC GAT TCG CCA CTG ACC AT-3'. The cg28Sr was amplified in each PCR reaction as a loading control. The PCR products from 30 cycles of amplification were visualized on a UV-transilluminator after electrophoresis on 1% agarose gel containing ethidium bromide (0.5 μg/μL). The signal intensity was quantified using the Gel-Doc System and Gelpro 3.1 software (KBT, Incheon, Korea).

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   1 tgtctaccaggcattcgcttcatggggataggggatacttctttgtgttttctcacact
  61 cctaactgatggttaattgacaaaatgtcctaagaagcggaaatggtctctttaagcact
                                                M  V  S  L  S  T    6
 121 tttgaagtcatgacatcaaagagaaagaaaaagtccccgccgggaccatgtggcttaccc
      F  E  V  M  T  S  K  R  K  K  K  S  P  P  G  P  C  G  L  P   26
 181 ttccttggttctttttcgacatcgaccttaaaaatattcacctggattttctgaaatgg
      F  L  G  S  F  F  D  I  D  L  K  N  I  H  L  D  F  L  K  W   46
 241 aaggagcgatatggagatatcgtgtctttcaaaatgaacgggaagaattttcttgtactg
      K  E  R  Y  G  D  I  V  S  F  K  M  N  G  K  N  F  L  V  L   66
 301 aataatattgacattatcaggaaggcattcgagagcgacgaaatcggcgccttaatgagt
      N  N  I  D  I  I  R  K  A  F  E  S  D  E  I  G  A  L  M  S   86
 361 gatcgaccattaaatttcattggagaaaatatatttttggttacaaagacgttcttta
      D  R  P  L  N  F  I  G  E  N  I  F  F  G  Y  K  D  V  L  L  106
 421 aggcgatatgacgacgagtttatgaagatgaagaaactgatgatacgatcgatgaagctc
      R  R  Y  D  D  E  F  M  K  M  K  K  L  M  I  R  S  M  K  L  126
 481 cacgattataactcggacaagtttcaacagctgatgtcagaggagctttcacacatactg
      H  D  Y  N  S  D  K  F  Q  Q  L  M  S  E  E  L  S  H  I  L  146
 541 tctaaattccagaagacagaaggaaagccaacggagcctatggacattttggtgccgtcc
      S  K  F  Q  K  T  E  G  K  P  T  E  P  M  D  I  L  V  P  S  166
 601 ttctgtaacatcatcggaatgctgttcacagggcgccgatgccaggacgaggacaggctc
      F  C  N  I  I  G  M  L  F  T  G  R  R  C  Q  D  E  D  R  L  186
 661 cttaaggtcttggtggacttcgaccgagacggggacaccatgattcagccccaggttcac
      L  K  V  L  V  D  F  D  R  D  G  D  T  M  I  Q  P  Q  V  H  206
 721 gcagtgtacaaactattcccctggattcgtcattctcccggctattacggcggtctgtat
      A  V  Y  K  L  F  P  W  I  R  H  S  P  G  Y  Y  G  G  L  Y  226
 781 cgcaatgttattcgggggggaacagaactacacaatctggtccaagatatgaagagtaaa
      R  N  V  I  R  G  G  T  E  L  H  N  L  V  Q  D  M  K  S  K  246
 841 tatgacaaaaccgaggttcaaaacttcatccacgaacttctgggggagcatcaggacttt
      Y  D  K  T  E  V  Q  N  F  I  H  E  L  L  G  E  H  Q  D  F  266
 901 gctgaggacccggataaaggctggctcacagatgaacacgttctaggaatgattatggat
      A  E  D  P  D  K  G  W  L  T  D  E  H  V  L  G  M  I  M  D  286
 961 cttatcaacacgtccgtgttgacaaccaaagctgtgatggcaggtgccctctttctcctc
      L  I  N  T  S  V  L  T  T  K  A  V  M  A  G  A  L  F  L  L  306
1021 tctcactttccggagatacaggaaaagattcgtgaggagatcattaacattgtcggctct
      S  H  F  P  E  I  Q  E  K  I  R  E  E  I  I  N  I  V  G  S  326
1081 cgatctcccacgacagaggacatggcgtcaatgccgtacacggaggcctgtatgatggag
      R  S  P  T  T  E  D  M  A  S  M  P  Y  T  E  A  C  M  M  E  346
1141 atcttacgctatcagtcccatcttcccctcaccgcgccccacgcaaacctaagccaggaa
      I  L  R  Y  Q  S  H  L  P  L  T  A  P  H  A  N  L  S  Q  E  366
1201 gtagaactggagggctacacgatccccaaaggaacggtgatttcgggaacttgtttgcc
      V  E  L  E  G  Y  T  I  P  K  G  T  V  I  F  G  N  L  F  A  386
1261 tgccaccacgatgagaccgtgtaccctgacccctgggagttcaaacctgaccggttcctg
      C  H  H  D  E  T  V  Y  P  D  P  W  E  F  K  P  D  R  F  L  406
1321 gaggaaggaaagttagtcggagccgaccacccagcggtcagaaattttattggatttggc
      E  E  G  K  L  V  G  A  D  H  P  A  V  R  N  F  I  G  F  G  426
1381 gtcgggaggcggcggtgcgtgggtcagcaaatggctagaatcagaatgttcctgtacccc
      V  G  R  R  R  C  V  G  Q  Q  M  A  R  I  R  M  F  L  Y  P  446
1441 acgtgtctcttgcagaaatttaaaatcgaggtcccaaaagatacgtcacttccgtcacat
      T  C  L  L  Q  K  F  K  I  E  V  P  K  D  T  S  L  P  S  H  466
1501 gacccaagagcgttactttcggagtcccctgtgattttaccgccacctatgcagtattgt
      D  P  R  A  L  L  S  E  S  P  V  I  L  P  P  P  M  Q  Y  C  486
1561 tctgttgagtgttgaacgtgaatgctcgagatctcagtctacagagaccggttgtgaatg
      S  V  E  C  *                                                490
1621 aaagttacttgttatgtataatacatggctagtcttttgcttttatttattagagatatg
1681 atgttatttattgaaatccagaaaataaatgttagaaacatccaaaaaaaaaaaaaaaaa
1741 aaaa
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Fig. 1. cDNA and deduced amino acid residue sequence of cytochrome P450 (CYP450) in Pacific oyster. Nucleotide number is shown on the left and the amino acid residue number is shown on the right. The start codon (position 103 bp), putative arginine residue critical to enzymatic function (position 748 bp), stop codon (position 1573 bp) and putative poly-adenylation signals (1665, 1686 and 2004 bp) are underlined and boldfaced. Heme-binding region (position 1375 bp) is boxed and boldfaced. This sequence data is available from the NCBI/GenBank nucleotide sequence databases with the accession number EF451959.

Quantitative PCR (QPCR)

QPCR was conducted to determine the relative mRNA expression of cgCYP450 using total RNA extracted from gills of control and oysters treated water-temperature change. With 2.5 μg of total RNA as a template, cDNA were synthesized using M-MLV reverse transcriptase (Bioneer). First-strand cDNA synthesis was conducted using oligo-$d(T)_{15}$ primer (Promega). Primers for QPCR were designed with reference to known gene sequences of the Pacific oyster as follows: cgCYP450 forward primer (5'-GGT GAA TGT TAC CAA GGA AGG-3'), cgCYP450 reverse primer (5'-GTT ACG ATA CAG CAA GGA GAT G-3'), cg28Sr forward primer (5'-AAA CAC GGA CCA AGG AGT CT-3') and cg28Sr reverse primer (5'-AGG CTG CCT

TCA CTT TCA TT-3'). QPCR amplification was conducted using a Bio-Rad MiniOpticon™ System (Bio-Rad, CA, USA) and iQ™ SYBR Green Supermix (Bio-Rad), according to the manufacturer's instructions. QPCR was carried by denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 55°C for 15 sec and extension at 72°C for 20 sec. The values based on normalization of individual samples to cg28Sr and then comparison to control group. To ensure that the primers amplified a specific product, we performed a melt curve (data not shown), as well as analyzed the PCR product size using capillary electrophoresis (Agilent Technologies, Santa Clara, CA). All primers used were shown to amplify only one size of template, melting at only one temperature. PCR products were also confirmed by sequencing (data not shown). All analyses were based on the calculated threshold cycle time (CT) values of the PCR products. The CT was defined as the PCR cycle at which the fluorescence signal crossed a threshold line that was placed in the exponential phase of the amplification curve. After the PCR program, QPCR data from five replicate samples were analyzed with analysis software of the system (Bio-Rad) to estimate transcript copy numbers for each sample. mRNA expression levels stood for an *n*-fold difference relative to 28Sr as the internal control.

Statistical analysis

The existence of significant differences between the data obtained from each experiment was tested using one way analysis of variance (ANOVA Tukey's test or LSD test) with the SPSS statistical package (version 10.0) at a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Pacific oysters are bivalves belonging to the family Osteidae, order Tetrabranchia, class Pelycypoda. Classification of Pacific oyster CYP is difficult, and only two studies have been conducted: one on CYP356A1 (GenBank accession no. EF645271) and the present study on the CYP450-related protein we separated.

An NCBI/GenBank database comparison of CYP450 genes revealed that the deduced amino acid sequence of cgCYP450 is similar to mouse (*Mus musculus*) CYP450 2D9 (AK078880; 29%), rabbit (*Oryctolagus cuniculus*) CYP450 2D/II (AB008785; 28%), and white-tufted-ear marmoset (*Callithrix jacchus*) CYP450 2D (AY082602; 28%). We first cloned the Pacific oyster CYP450 and then compared its amino acid sequence to that of other species, families, and subfamilies. We classified Pacific oyster CYP450 as belonging to family 2, subfamily D.

We investigated CYP450 mRNA expression in Pacific oyster tissues exposed to 30°C (high temperature) and 10°C

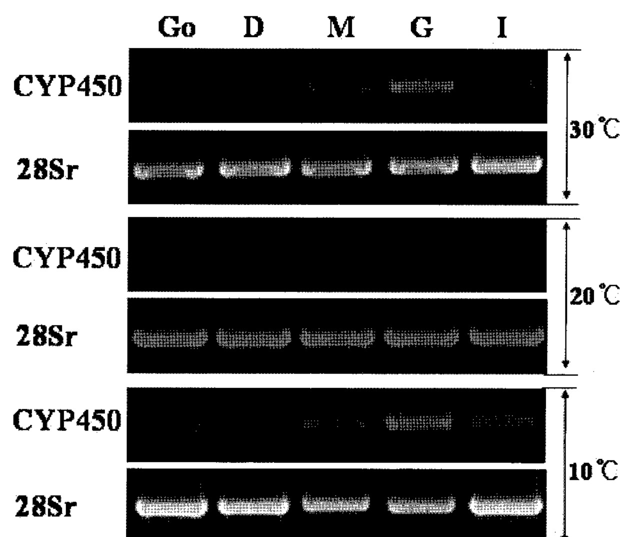

Fig. 2. Tissue-specific expression of cytochrome P450 (CYP450) mRNA in various tissues from the Pacific oyster, *C. gigas* transferred from 20°C (control) to 30 and 10°C for 6 h by RT-PCR. Amplification of 28S ribosomal RNA was used as an internal control. Go: gonad; D: digestive gland; M: mantle; G: gill; I: intestine.

(low temperature) water. Although the highest expression was observed in gill tissue, we also observed expression in gonad, mantle and intestine tissues (Fig. 2). As the primary interface in oysters between the hemolymph and cytoplasm and the outside environment, the gill directly absorbs, and has a large contact surface with, seawater. This direct impact triggers a greater expression of CYP450 mRNA in the gill than in other tissues. Thus, we used RNA extracted from gill tissue to conduct real-time PCR on changes in the CYP450 mRNA expression level of Pacific oysters after exposure to rapid water temperature changes (Fig. 3). We found no significant difference in CYP450 mRNA expression in the control group at 20°C. However, in the 30 and 10°C test groups, expression levels increased significantly over 6 h, and then decreased ($P<0.05$). Ricciardi et al. (2006) reported decreased CYP450 activity in zebra mussels acclimated to 20°C as water temperature was increased or decreased 1°C per day. Bebianno et al. (2007) reported that CYP450 activity in *Mytilus galloprovincialis* differed according to changes in environmental factors, such as water temperature and salinity. Yoon et al. (2003) also suggested that physical environmental factors, such as water temperature changes, affected the expression of CYP450 mRNA. We found that CYP450 mRNA was expressed in various tissues of Pacific oysters in relation to rapid changes in water temperature (Fig. 2). CYP450 mRNA expression significantly increased up to 6 h in all test groups in 30°C and 10°C water, and then decreased, stabilizing at the level of the control group. We assume that this increase in CYP450 mRNA expression resulted from unstable physiological metabolism due to stress from rapid

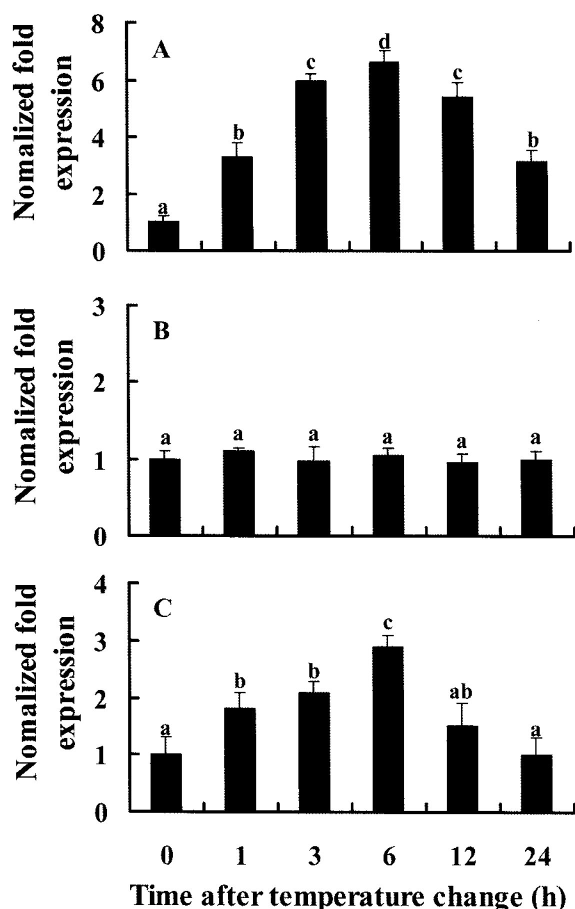

Fig. 3. Time-related effect on Cytochrome p450 (CYP450) mRNA levels in the gill of Pacific oyster during water-temperature change (A: 30°C, B: 20°C and C: 10°C) as determined by QPCR. Results are expressed as fold change with respect to levels of 28S ribosomal RNA for the same sample. Values with dissimilar letters are significantly different (P<0.05) from one another. Values are means±SD (n=5).

water temperature change. We further suggest that increase over 6 h in CYP450 mRNA expression and subsequent decrease and stabilization reflect the process of acclimation to a high or low water temperature environment. Thus, in Pacific oysters exposed to high or low water temperature, CYP450 mRNA expression stabilized after 6 h because of the water temperature compensation and metabolism control functions of CYP450. However, other studies have reported both a gradual increase in CYP450 activity in the hepatopancreas of fishes in relation to water temperature decrease (Andersson and Forlin, 1992) and decreased CYP450 activity in low water temperature in an estuarine fish (Stegeman and Hahn, 1994). Therefore, studies on the mechanism of CYP450 and water temperature change are needed.

In summary, we separated for the first time the entire 1,744-bp cDNA of CYP450 from Pacific oyster and compared the homogeny of this cDNA base sequence to base sequences of mouse, rabbit, and white-tufted-ear marmoset CYP450 in the NCBI database. We found low similarities of 29%, 28%, and 28%, respectively. We also used RT-PCR to observe CYP450 mRNA expression levels in various tissues of Pacific oysters and found that expression was highest in gill tissue. CYP450 mRNA expression in Pacific oyster gills exposed to rapid water temperature change increased significantly over 6 h and then decreased. We conclude that CYP450 mRNA expression occurred as Pacific oysters became acclimated to water temperature changes. This finding can be used as a physiological index for Pacific oysters exposed to water temperature change.

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REFERENCES

Andersson T and Förlin TL (1992) Regulation of the cytochrome P450 enzymes system in fish. *Aquat Toxicol* 24: 1-20.

Andersson T and koivusaari U (1985) Influence of environmental temperature on the induction of xenobiotic metabolism by β-naphthoflavone in rainbow trout, *Salmo gairdneri*. *Toxicol Appl Pharmacol* 80: 43-50.

Arukwe A and Goksùyr A (2000) Strain specific patterns in temperature adaptation of CYP isoenzyme levels in Atlantic salmon (*Salmo salar*). *Mar Environ Res* 50: 61-81.

Bebianno MJ, Lopes B, Guerra L, Hoarau P, and Ferreira AM (2007) Glutathione S-tranferases and cytochrome P450 activities in *Mytilus galloprovincialis* from the South coast of Portugal Effect of abiotic factors. *Environ Int* 33: 550-558.

Berndtson A and Chen T (1994) Two unique CYP1 genes are expressed in response to 3-methylcholanthrene treatment in rainbow-trout. *Arch Biochem Biophys* 310: 187-195.

Choi CY, An KW, Choi YK, Jo PG, and Min BH (2008) Expression of Warm Temperature Acclimation-Related Protein 65-kDa (Wap65) mRNA, and Physiological Changes with Increasing Water Temperature in Black Porgy, *Acanthopagrus schlegeli*. *J Exp Zool* 309A: 206-214.

George S, Young P, Leaver M, and Clarke D (1990) Activities of pollutant metabolising and detoxication systems in the liver of the plaice, *Pleuronectes platessa*: Sex and seasonal variations in non-induced fish. *Comp Biochem Physiol C* 96: 185-192.

Karr SW, Reinert RE, and Wade AE (1985) The effects of temperature on the cytochrome P-450 system of thermally acclimated bluegill. *Comp Biochem Physiol C* 80: 135-139.

Mizukami Y, Okauchi M, Arizono K, Ariyosh IT, and Kito H (1994) Isolation and sequence of cDNA-encoding a 3-methylcholanthrene-inducible cytochrome-P450 from wild red-sea bream, *Pagrus major*. *Mar Biol* 120: 343-349.

Pua EC and Lee YC (2003) Expression of a ripening-related

cytochrome P450 cDNA in Cavendish banana (*Musa acuminata* cv. Williams). *Gene* 305: 133-140.

Rees CB and Li W (2004) Development and application of a realtime quantitative PCR assay for determining CYP1A transcripts in three genera of salmonids. *Aquat Toxicol* 66: 357-368.

Ricciardi F, Binelli A, and Provini A (2006) Use of two biomarkers (CYP450 and acetylcholinesterase) in zebra mussel for the biomonitoring of Lake Maggiore (northern Italy). *Ecotoxicol Environ Saf* 63: 406-412.

Stengeman JJ and Hahn ME (1994) Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. (in) G. K. Ostrander and D. Malins (eds.), Aquatic toxicology: molecular, biochemical and cellular perspectives. Lexis Publishers, Boca Raton, pp. 87-206.

Stien X, Amichot M, Berge J, and Lafaurie M (1998) Molecular cloning of a CYP1A cDNA from the teleost fish *Dicentrarchus labrax*. *Comp Biochem Physiol C* 121: 241-248.

Yoon SJ, Kim IC, Yoon YD, and Lee JS (2003) Assessment of toxic effect in aquatic environment and the fish cytochrome P450 1A (CYP1A) gene. *Korean J Environ Biol* 21: 1-7.

Zhang Z, Li X, Vandepeer M, and Zhao W (2006) Effects of water temperature and air exposure on the lysosomal membrane stability of hemocytes in Pacific oyster, *Crassostrea gigas* (Thunberg). *Aquaculture* 256: 502-509.

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