論文要約（4 （2）関係）

博士論文要約（Summary）

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連合農学研究科
United Graduate School of Agricultural Sciences
専攻
Course: Biological Science and Technology
氏名
Name: TRIEU THANH TUAN

タイトル **Title**	Cytochrome P450 family 1, acetylcholinesterase, and antioxidant enzymes in Javanese medaka *Oryzias javanicus* as biomarkers potential against environmental pollutions (ジャワメダカ *Oryzias javanicus* のシトクロム P450 ファミリー1，アセチルコリンエステラーゼ，および抗酸化酵素群の環境指標としての有用性に関する研究)

キーワード（　　）（　　）（　　）
Key word: CYP1, biomarker, acetylcholinesterase, antioxidant enzymes, *Oryzias javanicus*

「序論及び目的」Introduction / Purpose

Expression of detoxification and antioxidant enzymes at the genetic and protein levels can reveal the potential cellular and physiological effects of pollutants and can further increase the sensitivity of environmental assessments. This study was carried out to provide better understanding of marine medaka Oryzias javanicus cytochrome P450 1 (CYP1) family, AChE, and antioxidant enzymes with respect to environmental conditions such as heavy fuel oil-contaminated food, water-accommodated fraction of heavy fuel oil, bioremediation of heavy oil-polluted, chlorpyrifos insecticide, salinity stress and starvation.

「材料及び方法」Materials / Method

1. Study 1: Cloning, sequencing and Characterization of CYP1 Genes

Medaka fish was exposed to 500 ppb β-NF for 24 hours. Liver was dissected for total RNA extraction, then cloning and sequencing.

2. Study 2: Heavy Oil-Contaminated Food Induced CYP1 Genes Expression in the Fish Tissues

Javanese medaka was fed an oil-contaminated food at levels of 0% (control) or 1%. Liver, gills, muscle and intestine were collected from the fish after 24 hours for CYP1 genes expression analysis.

3. Study 3: Effects of Water Accommodated Fraction of Heavy Oil on CYP1 Genes Expression

The fish were exposed to the control (seawater only) or 100% WAF of heavy oil for 24 hours. Intestine, liver and gills were collected for CYP1 genes expression analysis.

4. Study 4: Expression of CYP1 Genes in Embryos at 10-day Post-Fertilization through Bioremediation of Heavy Oil-Polluted Seawater

The experiment consists of three treatments. In the control treatment (C), we added 1.5 L sand-filtered seawater and 0.51 g/L slow-release fertilizer into the pipe. In the second treatment (P), we added 1.5 L sand-filtered seawater and 30 g heavy fuel oil into the pipe. In the third treatment (PF), we added 1.5 L sand-filtered seawater, 0.51 g/L slow-release fertilizer and 30 g of heavy fuel oil. Thirty Javanese medaka embryos 10 days post-fertilization were introduced to three petri dishes (10 embryos/dish) containing 10 mL of water obtained from each treatment. Embryos were sampled before and 48 hours after exposure for CYP1 genes expression analysis.

5. Study 5: Effects of Chlorpyrifos Insecticide on CYP1 Genes Expression and AChE Activity in the Fish

The medaka fish was exposed to various concentration of chlorpyrifos insecticide (0 mg/L; 0.02 mg/L; 0.1 mg/L; 0.5 mg/L) for 5 days. Liver, gills and intestine were collected after 1, 3 and 5 days of exposure for CYP1 genes expression analysis.

The fish remaining after 5 days exposure to the chemical was moved to the new experimental system and keeping in clean water for 5 or 15 days. Brain, muscle and liver were sampled after 1, 3 and 5 days of exposure and after 5 or 15 days keeping in clean water for determining AChE activity.

6. Study 6: Chlorpyrifos Insecticide Induced Antioxidant Genes Expression in the Fish Tissues

The experimental design, collecting samples and analysis as described in the Study 5. Liver, gills and intestine were collected after 1-, 3- and 5-day exposure & after holding the fish in clean water for 5 or 15 days. Antioxidant genes expression in the fish tissues were evaluated at each time point of sampling.

7. Study 7: Salinity Shock Induced CYP1 Genes Expression in the Fish Tissues

The medaka fish was directly transferred from seawater to freshwater. Liver, gills, muscle, and intestine were collected from the fish after 24 hours for CYP1 genes expression analysis.

8. Study 8: CYP1 Genes Expression in Starved Medaka Fish Tissues

The fish were either starved or fed (control group) for 1 week. Liver, gills, muscle and intestine were collected from the starved fish for CYP1 genes expression analysis.

「結果」Results

1. In this study, the full-length cDNA of three cytochrome P450 1 (CYP1) family from the medaka fish were firstly identified and analyzed to evaluate their potential as an index for monitoring of environmental pollutions. The full-length sequences are around 2 kb (CYP1A: 2439 bp; CYP1B1: 1984 bp; CYP1C1: 2601 bp) with deduced protein lengths approximately 500 amino acid residues (CYP1A: 530; CYP1B1: 517; CYP1C1: 525). The presence of several signature motifs in the deduced amino acid sequences confirmed their identity as cytochrome P450 enzymes. The obtained cDNA sequences of Javanese medaka CYP1A, CYP1B1, and CYP1C1 has been deposited in the GenBank/EMBL database with an accession numbers of KJ689303, KJ689304, and KJ689305, respectively.

2. Effect of salinity shock was studied additionally by transferring the fish from seawater to freshwater and keeping them for 24 hours. Salinity stress caused CYP1A, -1B1, and -1C1 inductions highest in gills, suggesting physiological function of CYP1s in acclimation to salinity changes.

3. When the fish was starved for 1 week, CYP1A, -1B1, and -1C1 expressions in the checked tissues tended to be down-regulated.

4. Feeding the fish with heavy fuel oil-contaminated food for 24 hours induced the CYP1 genes, showing highest levels of the transcripts in intestine and lowest levels in liver.

5. When the fish were exposed to water-accommodated fraction of heavy fuel oil for 24 hours, highest levels of expression of CYP1A were found in gills and lowest levels in intestine. For CYP1B1, the highest expression was found in gills, while the expressions in liver and intestine were similarly lower than in gills. CYP1C1 showed an induction pattern different from those of other CYP1s i.e. with highest expression in liver and down-regulation in gills and intestine.

6. Expression of the CYP1 genes in embryos (10-day post-fertilization) kept in heavy oil-polluted seawater for 48 hours indicated that CYP1A was expressed higher in bioremediation treatment than in the control, while CYP1B1 and -1C1 expressions were down-regulated in all the treatments.

7. The fish exposed to various concentrations of chlorpyrifos (0.01-0.5 mg/L) for 5 days showed induction of CYP1 genes with various expression patterns among the organs examined, liver, gills, and intestine, and highest induction observed was more than 40-fold. Chlorpyrifos depressed AChE activity in the tissues with significant dependency on concentrations of the insecticide and exposure time, resulting in long-term inhibition at higher concentrations of the insecticide. Recovery was occurred after holding the fish in clean water for 5 days, but the activity in all exposure groups was still significantly lower than that in the control group. Full recovery was only found in liver AChE activity after keeping the fish in clean water for 15 days at the lowest concentration of chlorpyrifos.

8. The genes of antioxidant enzymes, CAT, G6PD, GPx, GR, GST, SOD, and UB, showed relatively short-term inductions in liver, gills, and intestine of the fish exposed to various concentrations of chlorpyrifos (0.01-0.5 mg/L) for 1 to 5 days. After holding chlorpyrifos-exposed fish in clean water for 5 days or 15 days, the expression of these genes were back to the control level in all the tissues examined at all levels of chlorpyrifos.

「結論及び考察」Conclusion/ Consideration

These findings suggest usefulness of the induction of the genes studied in environmental assessment and provide sensitive multi-biomarkers to characterize toxicological impacts in monitoring aquatic environments. In addition, the results are important for understanding the molecular basis of biotransformation and detoxification in this specie, and also have evolutionary significance for understanding the diversity and history of the CYP superfamily. It also provides a good tool for evaluation the health status of the organism itself or the environments in a specific area.