

(学位第 3 号様式)

学 位 論 文 要 旨	
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題 目	Cytochrome P450 family 1, acetylcholinesterase, and antioxidant enzymes in Javanese medaka <i>Oryzias javanicus</i> as biomarkers potential against environmental pollutions (ジャワメダカ <i>Oryzias javanicus</i> 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)
<p>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 <i>Oryzias javanicus</i> cytochrome P450 1 (CYP1) family, AChE, and antioxidant enzymes with respect to environmental conditions. CYP1A, -1B1, and -1C1 cDNAs obtained were 2439 bp encoding 530 amino acids, 1984 bp encoding 517 amino acids, and 2601 bp encoding 525 amino acids, respectively. The determined sequences of the cDNAs were deposited in the GenBank/EMBL database with accession numbers of KJ689303, KJ689304, and KJ689305, respectively. Specific PCR primers for each of the CYP1 genes were successfully designed from the sequences. 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. 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>i.e.</i> with highest expression in liver and down-regulation in gills and intestine. 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. The fish exposed to various concentrations of chlorpyrifos (0.01-0.5 mg/L) 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. 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). 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. When the fish was starved for 1 week, CYP1A, -1B1, and -1C1 expressions in the checked tissues tended to be down-regulated. 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.</p>	