Angkanalak Janganan 2010: Molecular Characterization of Complementary DNAs (cDNAs) and Expression Analyses of Caspase-3 and Granzyme Genes in Nile Tilapia (*Oreochromis niloticus* Linn.). Master of Science (Aquaculture), Major Field: Aquaculture, Department of Aquaculture. Thesis Advisor: Mr. Prapansak Srisapoome, Ph.D. 178 pages.

Two full lengths of complementary DNAs (cDNAs) involved in program cell death (apoptosis) (caspase-3 and granzyme genes) of Nile tilapia immune system were discovered by searching in a Nile tilapia cDNA library and 5' Rapid Amplification cDNA Ends techniques. The full-length of a cDNA encoded for Nile tilapia caspase-3 (Casp3-TL) was 2,612 bp containing 5' and 3' untranslated region of 79 and 1,684 bp respectively. Open reading frame (ORF) of this cDNA was identified to be 846 bp or 282 amino acid residues. Structural analysis of Casp-TL revealed that this protein contained prodomian, large subunit and small subunit without a putative hydrophobic leader sequence. Important motifs indicating the caspase-3 characteristics which were found in caspase-3 genes of other organisms were well conserved. Besides, the full length of Nile tilapia granzyme cDNA (Granz-TL) was also cloned and characterized. The Granz-TL consisted of 1,412 bp which donated to 125 and 519 bp of 5' and 3' UTR. The ORF of Granz-TL was 765 bp long and equal to 255 amino acids. Mature protein of Granz-TL was identified to possess 3 different active sites or catalytic traids (His_{67}-Asp_{110}-Ser_{208}) of serine protease signature motifs. Phylogenetic and multiple sequence analyses indicated that Granz-TL was placed at the same group as granzyme A/K of fish and granzyme A and granzyme K of higher vertebrates. Expression analysis by RT-PCR exhibited a very low expression of Casp3-TL transcripts in every determined tissue of a normally experimental fish. On the other words, the highest expression level of Granz-TL was observed in peripheral blood leukocytes (PBLs), while mild expression levels were shown in head kidney, spleen and trunk kidney. No changes in expression levels of Casp3-TL mRNAs were determined in experimental fish injected with viable *Streptococcus agalactiae*. However, highly up-regulated transcripts of Granz-TL mRNA were clearly found in brain, head kidney, spleen and trunk kidney of *S. agalactiae* stimulated fish. Additionally, expression analysis by quantitative real-time RT-PCR also indicated that fish intraperitoneally injected with *S. agalactiae* $1x10^7$ and $1x10^9$ CFU/ml were not significantly changed in Casp3-TL mRNAs, but Granz-TL transcriptional levels were obviously up-regulated with 11- and 3- fold changes at hour 24 compared to control *S. agalactiae* uninjected fish ($P<0.05$). *In vitro* experiment was also carried out, PBLs of Nile tilapia were separately incubated with two different stimuli, *Aeromonas hydrophila* and concanavalin A. Suppressed expression of Casp3-TL was recorded at hour 48 in PBLs exposed to viable *A. hydrophila* and down-regulated was also determined in concanavalin A exposed PBLs at 48 and 72 hours after incubating. Unexpectedly, control PBLs, PBLs together incubated with *A. hydrophila* and concanavalin A were simultaneously exhibited high up-regulated expression of Granz-TL at 48 and 72 hours of experimental period and no significant differences of fold changes were evaluated at every time of quantitative real-time PCR detection ($P>0.05$).

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