Executive summary

Walking catfish, *Clarias macrocephalus* Günther is one of the commercially important freshwater cultured fish species in Thailand. Even though, hybrid catfish becomes the main cultured catfish species in the present, culture of *C. macrocephalus* remains importance for providing female broodstock used to generate the hybrid catfish. However, production of *C. macrocephalus* has been restricted due to disease outbreak especially caused by extoparasites and bacteria. Knowledge of fish immunity is therefore necessary to establish strategies for prophylaxis and control of disease in the culture.

In order to understand the walking catfish immunity at the molecular level, the cDNA encoding for the third complement component (C3) and the gamma subunit of the eighth complement component (C8γ), which previously obtained from EST study, were cloned and characterized. Moreover, their gene expression during larval development and in different tissues of mature walking catfish was also examined by RT-PCR. *In situ* hybridization was used to localize the expression of the complement components in the larval tissue. In addition, the expression of these two complement components in fingerlings supplemented with β-glucan was determined by semi-quantitative RT-PCR.

The cDNA encoding the partial (4140 bp) *C. macrocephalus* C3 was obtained by 5' RACE PCR amplification. The deduced amino acid sequence of the obtained C3 showed high sequence similarity to known teleost C3 proteins and was clearly grouped as a cluster with teleost C3 upon phylogenetic analysis. Alignment of amino acid sequences between of the obtained partial *C. macrocephalus* C3 and the corresponding part of other known C3 indicated that the obtained *C. macrocephalus* C3 contained many functionally important sites such as thiolester site, cleavage sits for C3 convertase and factor I as well as properdin binding site, which are present in mammalian C3. In a healthy adult fish, the mRNA of C3 was mainly expressed in the liver. *In situ* hybridization also indicated that liver hepatocytes were the main source of C3 synthesis in *C. macrocephalus.* Developmental expression study of C3 in different stage of *C. macrocephalus* larvae showed that C3 transcripts were immediately detected in *C. macrocephalus* after fish hatching and gradually increased as development progressed. *C. macrocephalus* C3 mRNA was not detected in eggs prior to fertilization. In addition, the expression level of C3 mRNA in liver increased significantly

induced after fish fed with β-glucan. The highest expression of C3 gene was observed at day 7 of stimulation.

The full-length cDNA sequence of *C. macrocephalus* C8γ was 886 bp in size encoding 211 amino acid sequences. The deduced amino acid sequence showed the highest similarity to C8γs from rainbow trout and zebra fish. *C. macrocephalus* C8γ also showed high similarity to mammalian C8γs and slightly lower similarity to other lipocalin proteins α-1-microglobulin and prostaglandin D-synthase. It seems to contain the lipocalin domain and all cysteine residues found in human C8γ. Phylogenetic tree also showed that *C. macrocephalus* C8γs was more closely related to C8γ of fishes and mammals than to other lipocalins. Tissue distribution analysis revealed constitutive expression of C8γ in walking catfish liver. Moreover, C8γ mRNA was already present in *C. macrocephalus* after fish hatching and slightly increased over larval development. There were any C8γ transcripts observed in unfertilized eggs of *C. macrocephalus*. In addition, we found that β-glucan did not affect the expression level of *C. macrocephalus* C8γ mRNA.

In conclusion, two complement components from walking catfish, *Clarias macrocephalus* were cloned and characterized. Expression analyses in different tissues of these two genes indicate that liver is the major organ for complement protein synthesis. Their expression levels were immediately detected in *C. macrocephalus* after hatching and slightly increased over larval development, suggesting that C3 and C8γ play an important role in the early immune response of fish larvae. Oral β-glucan administration enhanced the expression of C3, indicating an important role of C3 in immune system. However, the exact function of C8γ remains to be clarified.