Dutrudi Panprommin 2008: Analysis of Expressed Sequence Tags in Liver and Muscle Tissues of Günther's Walking Catfish, *Clarias macrocephalus*. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Supawadee Poompuang, Ph.D. 158 pages.

Expressed sequence tags have been generated from cDNA libraries constructed from liver and muscle tissues of adult female walking catfish. Two thousand and twenty-nine randomly picked cDNA clones, 991 from the liver library and 1,038 from the muscle library were sequenced. A total of 1,334 EST clones showed significant sequence similarity to known genes in the databases, representing 303 genes from the liver library and 234 genes from the muscle library. Fifty-one full-length genes were identified in both libraries. Eighty-nine EST clones contained 95 microsatellite repeat sequences. The majority of EST sequences from both libraries matched sequences identified from other Siluriformes particularly channel catfish (513 EST clones, 38.5%) and zebrafish (284 EST clones, 21.3%). A number of identified genes appeared to be expressed in specific tissues. Genes associated with primary functions of liver, metabolism, defense and homeostasis, and signaling and communication, were well represented in liver cDNA library. Vitellogenin genes were highly expressed in the liver of female walking catfish. Further, genes responsible for innate immune function, in particular, acute phase proteins were found only in the liver. In contrast, genes encoding structural proteins were restricted to the muscle library. ESTs represented structure and motility (actin, myosin, troponin, and parvalbumin) were relatively highly expressed in muscle cDNA library. Analysis of the cDNA libraries indicates that EST approaches can provide effective way for characterizing expressed genes in walking catfish.

Walking catfish displayed different isotype characteristics of contractile proteins, parvalbumin and troponin, in skeletal muscle, gill and skin. Expression of these proteins in skin and gill indicated their involvement in ionic and osmotic regulation of fish body in addition to their primary role of muscle contraction and relaxation. However, the mechanism of these proteins in osmoregulation was unknown. Phylogenetic analysis of these proteins revealed close evolutionary relationship between walking catfish and channel catfish. Amino acid sequence similarities among parvalbumin, troponin C, and myosin light chain III were observed in walking catfish, suggesting that these three types of protein might share a common ancestor.

The complete cDNA sequence for walking catfish vitellogenin, (VTG) (4,192 bp) contained 51 bases of 5'-untranslated region, the open reading frame of 4,050 bp coding for a 1,350 amino acids, 60 bases of 3'-untranslated region and a poly (A) tail of 31 nucleotides. The deduced amino acid sequence of the walking catfish VTG shared 58.9%, 56.9%, 41.7%, 32.6% and 8.1% identity with VTGs of carp, zebrafish, rainbow trout, clawed frog and chicken, respectively. To establish the relationship between GSI and the level of hepatic VTG transcript, monthly change in GSI of females was monitored over a 1-year period from June 2006 to May 2007. The relative copy number of VTG mRNA was determined by quantitative real-time PCR. Significant variations of mean GSI values and VTG mRNA levels ($P<0.05$) were observed among months. From June to August, mean GSI values ranged from 8.60± 0.35% to 13.07±0.59%. Highest mean GSI value (18.27 ± 2.51%) and maximum VTG transcript levels were observed in September. From October through March, ovarian weight steadily declined with the lowest mean GSI value (0.77 ± 0.38%) in January. The relative levels of VTG transcript correlated well with GSI. The expression profile of VTG gene reflected the annual changes in reproductive cycle of female walking catfish, showing high levels during breeding period and being lowest during resting periods.

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24 / 3 / 2008