

Thesis Title A High Level Expression of Biologically Active
Fish Growth Hormone in *Eschericia coli*
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ABSTRACT

Fish is an economically important animal of Thailand. The success of commercial aquaculture depends on many factors such as fish strains, food, disease control and management. Furthermore, the rate at which fish grow from egg state to market size state is an important component of the economics of commercial aquaculture. Use of fish growth hormone represents a potential solution to this problem.

Giant catfish (*Pangasianodon gigas*) is the biggest freshwater catfish in the world and its growth rate is considerably fast. This may be involved in the regulation of various factors, and its growth hormone has been believed to be an efficient agent to raise the fish growth rate. Since the hormone concentration in fish pituitary is very low (about 1-4 mg/g of pituitary gland), an efficient method for large-scale preparation has been sought. Recombinant DNA technique is considered the most effective one.

Giant catfish growth hormone cDNA (*gcGH* cDNA) encoding mature growth hormone had been amplified by polymerase chain reaction (PCR) with designed primers derived from *gcGH* cDNA previously cloned in a Bluescribe vector. The expression plasmid, pUC12-*gcGH*, was constructed

and transformed to *E. coli* JM107 to produce gcGH in a high level. The 20.5 kDa protein which corresponds to the putative mature gcGH was efficiently expressed in *E. coli* carrying the plasmid in which the gcGH cDNA was under control of LacZ promoter. This protein comprised about 20 % of total cellular proteins. Recombinant gcGH produced in *E. coli* was in a form of inclusion bodies with density higher than those of other cytosolic proteins which allowed isolation by centrifugation. The amount of gcGH obtained from this method was up to 40-60 mg/l of culture. The purity of partially purified gcGH is approximately 94 %. The test of biological activity of this hormone showed that the growth of goldfish (*Carassius auratus*) was accelerated when the partially purified gcGH was administered by 4 intramuscular injections, at a dose of 0.1 and 1.0 ug/g body weight/week, and by 8 intramuscular injections only a dose of 0.1 ug/g body weight/week. But it did not raise the significant growth of catfish (*Pangasius sutchi*) when the hormone was administered by 4 intramuscular injections at a dose of 0.1 and 1.0 ug/g body weight/week. In addition, the growth of goldfish which received 4 intramuscular injections of very high dose (10 and 100 ug/g body weight/week) was not different from control group. This suggests that the recombinant gcGH can be used to increase growth rate of other fish species if it was administered with a suitable dose. However, a number of parameters must be addressed before its use, such as the route, the dose and the frequency of delivering hormone to the fish, including the kind of fish and its age.