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PHUWADOL BANGRAK: EXPRESSION AND SECRETION OF GIANT
CATFISH GROWTH HORMONE IN METHYLOTROPHIC YEAST *PICCHIA*
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The cDNA encoding mature giant catfish *Pangasianodon gigas* growth hormone (*gcGH*) was intracellularly and extracellularly expressed in the methylotrophic yeast, *Pichia pastoris*, under the control of methanol inducible promoter (*AOX1*). A cassette for intracellular expression of *gcGH* containing the ACCATG (Kozak sequence) as a start codon was constructed. The level of intracellular expression of the recombinant *gcGH* (22 kDa) was approximately 80 mg/l of induction medium harvested after 6 days of induction period. For secreted expression, *gcGH* was fused to the N-terminal signal sequence of *Saccharomyces cerevisiae* α -mating factor. The accumulated *gcGH* in the induction medium was approximately 200 mg/l. N-terminal amino acid sequencing of the secreted product indicated the presence of three additional residues. The first two amino acids, glutamic acid and alanine, resulted from incomplete processing of the MF α -1 secretion signal, and glutamic acid was obtained from in-frame fusion of *gcGH* cDNA with *Pichia* expression vector. Two potential N-glycosylation sites (Asn-X-Ser/Thr) were observed in the *gcGH*. Thus, the glycosylation of the secreted *gcGH* was examined. The result from Endoglycosidase II treatment and glycoprotein staining revealed that, similar to the growth hormone from other species, recombinant *gcGH* produced from *P. pastoris* is not glycosylated.