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CHANIKUL CHUTRAKUL: OPTIMIZATION OF HETEROLOGOUS EXPRESSION OF GIANT CATFISH *PANGASIANODON GIGAS* GROWTH HORMONE IN *SACCHAROMYCES CEREVISIAE*. THESIS ADVISORS: SAKOL PANYIM, Ph.D., LILY EURWILAICHITR, Ph.D. 194 P. ISBN 974-662-109-2

Yeast *Saccharomyces cerevisiae* was used as a host to express and secrete giant catfish growth hormone (gcGH) cDNA by using yeast MFα1 secretory signal.

In order to achieve a high level of gcGH protein secretion into the culture medium, several approaches were studied. The study focused on changing gene dosage, altering nucleotide from "C" to "A" at minus three region preceding AUG start codon and changing the culture medium.

Altering nucleotide from "C" to "A" did not improve the gcGH protein production. Increasing the gene dosage increased the level of secreted gcGH protein. Both multicopy plasmid, YEp352 (*TEF1* promoter) and pYES2 (*GAL1* promoter), containing *2μ-ORI* sequence gave approximately 3-7 times higher amount of gcGH protein than single copy plasmid, pCEC122. The highest intracellular gcGH was 1.62 mg/l from transformants containing multicopy plasmid with *GAL1* promoter.

The level of secreted gcGH from yeast transformants grown in selective MM-URA was compared to the level from those grown in modified BMGY medium. Transformants containing each plasmid grown in BMGY rich medium gave 3-5 times higher amount of gcGH protein than those grown in MM-URA medium. The highest amount of gcGH in the medium was approximately 59.02 μg/l from transformants containing multicopy plasmid with *GAL1* promoter. The amount of secreted gcGH was approximately 4% of total gcGH produced. However, the multicopy plasmid with *TEF1* constitutive promoter is suggested for used in large-scale production because it can produce gcGH product during the growth and the use of inducer is not necessary.

It has been observed that the size of gcGH protein extracted from intracellular fraction was different from the size of gcGH protein extracted from secreted fraction when detected by Western blotting analysis.