COMPARISON OF *Oreochromis sp* GROWTH HORMONE PRODUCTION BY *Aspergillus niger* AND *Pichia pastoris*

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Introduction. Growth hormone (GH) is a polypeptide hormone of approximately 22 kDa, it regulates mainly the somatic growth on vertebrates (1). Several studies have showed the biologic effects of recombinant GH on fish when it is administered by injection, immersion baths, oral incubation and dietary delivery (1, 2, 3).

The interest in the growth hormone to fish production has increased due to the acuiculture has become the fastest growing alimentary industry in the world, being *Oreochromis sp* the second fish with most commercial interest (4).

Aspergillus niger and Pichia pastoris have been widely used for the industrial production of homologous and heterologous proteins with successful results (1, 5).

The aim of this work is evaluate the production potential of both expression systems for *Oreochromis sp* growth hormone (OGH) recombinant production.

Methods. *Oreochromis sp* growth hormone gene was designed from the original nucleotide sequence optimizing the codons usage to *A. niger* and *P. pastoris* preferences and synthetized. For *A. niger* expression pIGF#18 fusion plasmid under the control of *Gla*A promoter and pAB4.1 pyrG gene carrying plasmid for transformant selection were cotransformed in AB4.1 *A. niger* strain that is auxotrophic to pyrG.

For Pichia pastoris pPICZαA expression plasmid under the control of *AOX* promoter was used and the construction was used to transform GS115 *P. pastoris* strain and transformants were selected by resistance to Zeocin.

Culture was performed in Geshlaghi media for *A. niger* using modified starch as carbon source to induce the production of the OGH, and for *Pichia* YEPD with Methanol as inductor was used.

The presence of OGH polypeptide in culture supernatant will be analyzed by SDS PAGE and later it will be purified by affinity chromatography using the QIAExpress 6X HIS kit from Qiagen as a 6X His tail included in the carboxy terminal of the designed gene

Results.

Table 1. Similarity percent between OGH gene and other fish species.

Organism	aminoacid similarity	Nucleotide similarity
O. mossambicus	100 %	84 %
O. niloticus	99 %	84 %
Amphiprion ocellaris	91 %	77 %
O. urolepis	99 %	74 %
Micropterus salmoides	91 %	71 %

Table 2. Production of OGH by *A. niger* and *P. pastoris.*

Organism	OGH mg/L	Production time
A. niger	5.8	60
P. pastoris	11.2	72

Even when OGH production is quite low for both expression systems in each case a protein with the expected molecular weight of OGH was obtained when the right inductor was included in the culture media. *P. pastoris* produced more than the double OGH than *A. niger*. In both cases the production curve followed the growth curve and time of maximal production was very close.

Conclusions. *P. pastoris* is better OGH producer than *A. niger* in a very close time production. This makes *Pichia* is a better option if the commmercial production of this proteic hormone is to be attained as it can be easily used in high volume processes.

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