ANALYSIS OF GROWTH HORMONE IN TENCH (*TINCA TINCA*)

ANALÝZA RŮSTOVÉHO HORMONU LÍNA OBECNÉHO (*TINCA TINCA*)

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ABSTRACT

The aim of this work is to prepare proceeding protocols for students of practical course of "Fish Genetics" at MUAF. We have designed sets of primers and sequenced a part of tench (Tinca tinca) growth hormone gene. We also designed other sets of primers to sequence the rest of the gene. The RNA isolation was performed from the muscle of the tench and cDNA was synthesized from the total RNA with reverse transcriptase. Sets of primers for housekeeping genes were designed and will be used for quantitative analysis of growth hormone in tench by using real-time RT-PCR.

Key words: growth hormone, tench

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INTRODUCTION

Tench (*Tinca tinca*) is a traditional fish bred in ponds in Central and Eastern Europe. According to FAO statistics, tench production reaches in average 3 200 tons in European countries from the year 2000. In the Czech Republic pond production of tench was 244 tons in the year 2005 (Mandelíková and Ženíšková, 2006).

The increased requirements of European market cannot be satisfied by tench production in ponds and thus, possibilities for non-traditional breeding approaches to rearing the early and juvenile stages under controlled conditions of intensive aquaculture are searched for. Using a proper feeding strategy, the rearing of tench larval stages can be carried out successfully in recirculation facilities (Wolnicki and Gorny, 1995; Wolnicki *et al*., 2003). If compared to juvenile stages of other cyprinids, the growth of tench upon commercial feeds is extremely slow, even in case of addition of natural food (Quirós and Alvariño, 1998; Wolnicki *et al*., 2003, Wolnicki *et al*., 2006).

Growth hormone (GH) is one of the polypeptide hormones secreted by somatotrophs in the anterior portions of the pituitary glands of vertebrates. It is involved in the regulation of somatic growth and maintenance of protein, lipid, carbohydrate, and mineral metabolisms (Ganong, 1983).

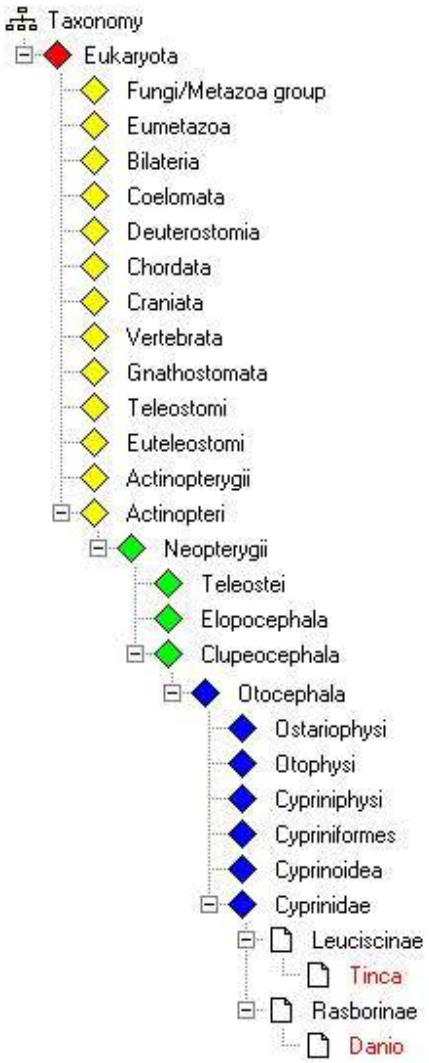
Growth hormone is a member of the protein family that also includes prolactin, somatolactin and placental lactogen. The functions of fish growth hormones are similar to those of mammalian growth hormones, both *in vivo* and *in vitro*. These include stimulation of the expression of rainbow trout IGF-1 (Moriyama, 1995), a downstream gene on the growth hormone stimulation signal transduction pathway (Hammerman, 1989) and stimulation of chum salmon peripheral blood leukocyte proliferation *in vitro* (Sakai *et al*., 1996).

Growth manipulation of fish is an important task in aquatic biotechnology (Acosta *et al.*, 2007), the use of aquatic biotechnology in fish breeding is just the latest technological response, but also the most controversial, but it can contribute to productivity increases in aquaculture and lower customer price for fish (Aerni, 2004).

The aim of this work is to prepare proceeding protocols for students of practical course of “Fish Genetics” at MUAF.

MATERIAL AND METHODS

Fig.1 Taxonomy tree



DNA Extraction: samples of tench (*Tinca tinca*) muscles for DNA analysis were obtained at Department of Fishery and Hydrobiology at MUAF. The DNA was isolated by JetQuick Kit (Genomed). Sequence of *Tinca tinca* growth hormone (*GH*) no. DQ980027 was used as a pattern for primer setting. Then the primers were designed in OLIGO v4.0 programme.

PCR: volume 25 µl contained: 12 µl HotStar Mix (QIAGEN), 0.5 µl dNTP (Roche), 0.5 µl primer LGH A, 0.5 µl primer LGH B (see Tab. 1), 10 µl PCR ultra H_2O water (Top-Bio). A temperature gradient PCR was used at cycler MJ Research PTC-200 (MJ Research) and the best temperature was chosen for other reactions. The used conditions for other PCR were: 95 ºC 15 min, 35 cycles of 94 ºC for 30 s, 57 ºC for 30 s and 72 ºC for 30 s, and final elongation 72 ºC for 10 min and cool down to 4 ºC.

The gained PRC product was sequenced by ABI PRISM 3100 Avant Genetic Analyzer (Applied Biosystems), and compared with DQ980027 in ClustalW. The sequence was also confirmed by cloning with the use of pDrive Cloning Vector and QIAGEN EZ Competent Cells (QIAGEN). The plasmid DNA was isolated and sequenced using an ABI PRISM 3100. The sequence was compared with genomic DNA and DQ980027 in ClustalW. Then other sets of primers (see Tab. 1) were designed according to the sequence DQ980027 to do the seguencing of the rest of the gene.

RNA extraction: fresh muscle of tench was put to RNA*later* (QIAGEN) to stop RNase activity and then the sample was stored in the freezer (-20 ºC). For RNA extraction the FastRNA Pro Green Kit (Q-BIOgene) was used. We gained 60 µl of RNA which was purified by Dnase (Top-Bio). cDNA was synthesized from the total RNA with Omniscript Reverse Transcriptase (QIAGEN) and oligo(dT)20 primers (Invitrogen).

After that we designed sets of primers for housekeeping genes: *GAPDH*, *HPRT*, *EF1* and *TUBA1* (see Tab1). For the pattern sequence we used AY818346 (*GAPDH*), NM_212986 (*HPRT*), ENSDART00000023156 (*EF1*) and NM_194388 (*TUBA1*) of Danio rerio (Tang *et al.*, 2007), which is phylogeneticly very closed to the tench (see Fig. 1) (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=7955; http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=27717).

Tab. 1 Information on used primers for PCR and real-time RT-PCR

Abbreviation	Primers 5´-3´	GenBank accession number or reference
LGH 1A	GGT GGT GCT GGT TAG TTT GTT	DQ980027
LGH 1B	TGT GTT TCA TCT TTC CCA GTG	
LGH 2A	CAA CAA TGC AGT CAT CCG TG	
LGH 2B	GGC AGG GAG TCG TTA TCA TC	
LGH 3A	TGA TAA CGA CTC CCT GCC G	
LGH 3B	TTT CCA CCT TGT GCA TGT CC	
LGH 4A	GCT TTC GTC TTC TGG CTT GC	
LGH 4B	ACA GGG TGC AGT TGG AAT CC	
F_GAPDH 1A	CGC TGG CAT CTC CCT CAA	AY818346 (Tang et al., 2007)
F_GAPDH 1B	TCA GCA ACA CGA TGG CTG TAG	
F_GAPDH 2A	TCA ACG CAC TCA TTT CCT AGC	AY818346
F_GAPDH 2B	ACA CCC AAA ATG GCA ACA AC	
F_HPRT1 1A	ATC AGC GAA ACA GGA AAG GAG	NM_212986 (Tang et al., 2007)
F_HPRT1 1B	CTG CGG TGA GCT GCA CTA CT	
F_HPRT1 2A	TGC ACA TAG AAT TGG TTT GCT C	NM_212986
F_HPRT1 2B	ATG TGA TTG GAT GAT CAG GTT GT	
F_EF1α 1A	CTG GAG GCC AGC TCA AAC AT	ENSDART00000023156 (Tang et al., 2007)
F_EF1α 1B	ATC AAG AAG AGT AGT ACC GCT AGC ATT AC	
F_EF1α 2A	CAG GCT GAC TGT GCT GTG C	ENSDART00000023156
F_EF1α 2B	TCA CTC CCA GGG TGA AAG C	
F_TUBA1 1A	TGG AGC CCA CTG TCA TTG ATG	NM_194388 (Tang et al., 2007)
F_TUBA1 1B	CAG ACA GTT TGC GAA CCC TAT CT	
F_TUBA1 2A	GCT TCA CCT CTC TGC TGA	NM_194388
F_TUBA1 2B	AGC GGT GGA GAC CTG AGG	

RESULTS AND DISCUSSION

Concentration of isolated DNA was 0.114 µg/µl from muscle with purity 112 % and 0.591 µg/µl from fin with purity 100 %. The DNA was used for PCR, where we amplified products with length around 400 bp and 800 bp for LGH 1A1B and around 1200 bp for LGH 2A2B (Fig. 2). The two products of LGH 1A1B were cut from gel, isolated, purified and sequenced on ABI PRISM 3100. The product of LGH 2A2B was purified and sequenced too. The sequences of these products were compared in ClustalW and the homology was 100%. We combined primer LGH 2A and LGH 1B and we gained a product with length around 600 bp.

The products of LGH 2A1B and LGH 2A2B were also cloned with the use of pDrive Cloning Vector and Competent Cells. The isolated plasmid DNA was cut by restriction enzyme EcoRI (see Fig. 3), the length of the plasmid was 3000 bp and the length of inserts were around 600 bp (LGH2A1B) and around 1200 (LGH 2A2B). This confirmed that we had cloned the right insert. Then the plasmid DNA was purified and sequenced and compared with the previous sequences in ClustalW. We gained the real length of the product (LGH 2A2B) – 1253 bp, where we determined exons and putative introns. The knowledge of

sequence helps us to determine the length of products after designing of new primers and with other work with cDNA.

We have designed two other sets of primers (LGH 3A3B and LGH 4A4B) for sequencing of the rest of the genes to know the all sequence of growth hormone gene.

Furthermore, on the base of homology of Danio rerio with tench we have designed sets of primers for housekeeping genes *GAPDH, HPRT, EF1* and *TUBA1*. These genes will be used for quantitative analysis of growth hormone in tench by using Real Time PCR.

Fig 2. PCR with primers LGH 1AB and LGH 2AB, marker M100

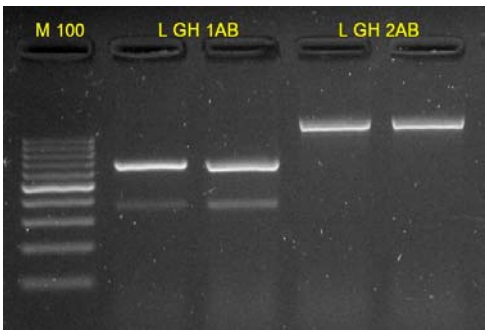
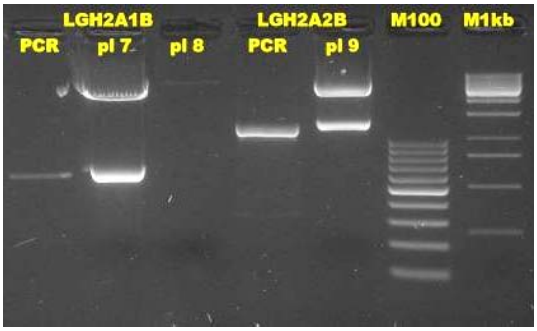


Fig. 3 PCR product LGH 2A1B (600 bp), plasmid with insert (pl 7), plasmid without insert (pl 8), PCR product LGH 2AB (1200 bp), plasmid with insert (pl 9), Marker M100 and M1kb



CONCLUSION

We have isolated DNA and RNA from the tench. Sets of primers were designed PCR reactions. The PCR products were sequenced and also cloned for confirmation of the sequence. We confirmed exons with DQ980027 sequence and determined putative introns. The sets of primers of housekeeping genes were designed and will be used for quantitative analysis of growth hormone in tench by using real-time RT-PCR.

The results will be used in practical course of “Fish Genetics” at MUAF. This work was necessary for preparation of this course.

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