Molecular cloning of Insulin-like Growth Factor-I from triangular bream (Megalobrama terminalis)

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Abstract
Triangular bream (Megalobrama terminalis) IGF-I DNA and gene were cloned from triangular bream liver for the first time by RT-PCR. Sequence analysis indicated that the IGF-I cDNA consisted of 486 nucleotides encoding 117 amino acids which spanned the complete signal peptide, B, C, A, D and E domains. Analysis of the E domain indicated that triangular bream IGF-I belongs to the IGF-I Ea-2 subtype. Compared to bluntnose bream (Megalobrama amblycephala), another member of the same Megalobrama genus, triangular bream IGF-I shared 99.8% identity in cDNA sequence and 99.4% in predicted amino acid sequence. However, considerable differences were found in comparison to with common carp (Cyprinus carpio) and grass carp (Ctenopharyngodon idellus), which are members of the same family but of a different genus.

Introduction
The insulin-like growth factor-I (IGF-I) of fish, which consists of 70 amino acids and serves as a mediator for cell division, differentiation, embryonic development, growth regulation, cell death restraint and osmotic pressure regulation, etc., plays an important role in the growth and generation of fishes (Duan et al. 1997; Reinecke et al. 1998). Since salmon (Oncorhynchus tsawytscha) IGF-I DNA was cloned in 1989 for the first time (Cao et al. 1989), IGF-I cDNA of other fishes, such as rainbow trout (Oncorhynchus mykiss) (Shamblott et al. 1993), common carp (Cyprinus carpio) (Liang et al. 1996), tilapia (Oreochromis mossambicus) (Reinecke et al. 1997; Schmid et al. 1999), catfish (Clarias macrocephalus) (Mcrophy et al. 1994), black sea bream (Sparus auratus) (Duguay et al. 1996), sea scorpion (Cottus scorpius) (Loeffling et al. 1998), bluntnose bream (Megalobrama amblycephata) (Bai et al. 2001), grass carp (Ctenopharyngodon idellus) (Hua et al. 2001), goldfish (Carassius auratus) (Kermouni et al. 1998) etc. have also been cloned and investigated in recent years.

In taxonomy, triangular bream (Megalobrama terminalis) belongs to the Megalobrama genus, Cyprinid family. As an ideal commercial fish, the artificial culture and breeding of triangular bream have been well studied. However, few reports can be found on the germplasm characteristics of triangular bream (Li et al. 2002). In the experiment reported here, triangular bream IGF-I cDNA and gene were cloned (GenBank Accession No. AY247412) from triangular bream liver by RT-PCR to provide basic data for research into its genetics, breeding, germplasm identification and protection and finally to promote the development and utilization of biological feed additives.

Materials and methods
Total RNA extraction
Triangular bream were supplied by the Fisheries Science Research Institute of Hangzhou Municipality. Liver tissues of Triangular bream were rapidly separated and ground in liquid nitrogen. Total RNA was extracted from 50 mg tissue with an RNA Extraction Kit.
Formaldehyde denatured electrophoresis of total RNA (Promega) following the manufacturer’s instructions. Total RNA quality was examined by formaldehyde denatured electrophoresis.

**Primer design and RT-PCR**

After analyzing the IGF-I of representative fish species (Liang et al. 1996; Hua et al. 2001; Bai et al. 2001; Duan et al. 1997; Reinecke et al. 1998), a pair of primers were designed and synthesized based on the conserved region of blunt-nose bream IGF-I ORF (Opening reading frame), which is closely related to Triangular bream: 3′-primer (5′-CGCGGATCCCTTTACTAAATGCGATAGTTTC-3′) and 5′-primer (5′-CGGAATTCATGTCTAGGAGCATTTC-3′). The 5′-primer was expected to extend from the first amino acid code ATG of IGF-I signal peptide, while the 3′-primer was expected to extend in reverse from the stop code TAG. A BamHI restriction site and an EcoRI restriction site were inserted into 3′-primer and 5′-primer, respectively, to facilitate cloning of PCR products. The first strand of cDNA was synthesized from 10 µg of total RNA with 3′-primer using MMLV RT (Promega) at 42 °C for 1 h. The PCR amplification program was as follows: 10 cycles with denaturing at 94 °C for 2 min, denaturing at 94 °C for 45 sec, annealing at 50 °C for 45 sec and extension at 68 °C for 1 min; 25 cycles with denaturing at 94 °C for 45 sec, annealing at 55 °C for 45 sec and extension at 68 °C for 1 min followed by a final extension of 10 min at 72 °C then storage at 4 °C. RT-PCR products were analyzed by electrophoresis on 1.0% agarose gel.

**cDNA cloning and sequencing**

After separation and purification, RT-PCR products were ligated with pMD 18-T vectors (Takara). E. coli TG1 was transformed with the recombinant DNA by the CaCl2 method to obtain transformants. Three positive clones were screened out primarily by gel electrophoresis, and analyzed by digesting with BamHI and EcoRI, then sequenced by the Beijing Genomics Institute (Hangzhou), Genomics and Bioinformatics Center, Chinese Academy of Science.

**Results**

**Total RNA quality of triangular bream liver**

Formaldehyde denatured electrophoresis on agarose gel showed that the total RNA quality met the requirements for RT-PCR (Figure 1).

**Results of RT-PCR**

Total RNA of Triangular Bream liver was expanded by RT-PCR. Gel electrophoresis showed that the RT-PCR product was a specific fragment of about 500 bp (Figure 2).

RT-PCR products were obtained in low melting temperature agarose gels and ligated with vectors. E. coli TG1 was transformed with the recombinant DNA by the CaCl2 method to obtain transformants.