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Cloning of Qiantang River Triangular Bream (*Megalobrama terminalis*) IGF-I Gene and Expression of the Recombinant pre-IGF-I in *Escherichia coli*

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Abstract The insulin-like growth factor (IGF-I) gene (GenBank accession no. AY247412) of Qiantang River triangular bream (*Megalobrama terminalis*) was cloned for the first time from the liver by reverse transcriptase polymerase chain reaction. The gene was inserted into pMD 18-T vector to construct the recombinant plasmid pMD 18-T/IGF-I. Sequence analysis indicated that the IGF-I cDNA consisted of 486 nucleotides encoding 161 amino acids, which spanned the complete signal peptide, mature peptide (including B, C, A, and D domains), and E-domain. Analysis of the E domain indicated that triangular bream IGF-I gene belonged to the IGF-I Ea-2 subtype. To construct the expression plasmid, the IGF-I gene was subcloned into prokaryotic expressing vector pGEX-4T-1. The recombinant plasmid pGEX-4T-1/IGF-I was transformed into *Escherichia coli* BL21 (DE3), and the transgene expression was observed after being induced with isopropylthiogalactoside. The results of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting indicated that the recombinant fusion protein had immune activity, and the molecular weight was about 47 kDa. The results of SDS-PAGE and thin-layer scanning showed that the yield of fusion protein had been enlarged with prolonging time. When the time of induced expression was 1, 2, 3, 4, 5, and 6 h, the expression amount was approximately 1.4, 4.3, 8.1, 11.3, 16.3, and 18.8% of total bacterial protein, respectively.

Keywords Qiantang River triangular bream (*Megalobrama terminalis*) · Insulin-like growth factor-I (IGF-I) · Cloning · Prokaryotic expression · Antigenicity

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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, osmotic pressure regulation, etc., plays an important role in growth and generation of fishes [1, 2]. Since salmon (*Oncorhynchus tshawytscha*) IGF-I cDNA was cloned in 1989 for the first time [3], IGF-I cDNAs of other fishes, such as rainbow trout (*Oncorhynchus mykiss*) [4], catfish (*Clarias macrocephalus*) [5], shark (*Squalus acanthias*) [6], black sea bream (*Sparus auratus*) [7], sea scorpion (*Cottus scorpius*) [8], goldfish (Gf) (*Carassius auratus*) [9], flounder (*Paralichthys olivaceus*) [10], tilapia (*Oreochromis mossambicus*) [11], bluntnose bream (Bb) (*Megalobrama amblycephat*) [12], grass carp (Gc) (*Ctenopharyngodon idellus*) [13], zebrafish (*Denio rerio*) [14], common carp (Cc) (*Cyprinus carpio*) [15], etc., have also been cloned or expressed in *Escherichia coli* in recent years.

In taxonomy, Qiantang River triangular bream (*Megalobrama terminalis*) (QRTb) belongs to the Megalobrama genus, Cyprinid family. As an ideal commercial fish, the artificial culture and breeding of QRTb have been well studied. However, few reports can be found on the germplasm characteristics of QRTb [16]. To study of the germplasm characteristics of QRTb, QRTb IGF-I gene was cloned (GenBank accession no. AY247412) from the liver by reverse transcriptase polymerase chain reaction (RT-PCR), and the recombinant pre-IGF-I was expressed in *E. coli* BL21 (DE3). A preliminary investigation of the expression condition and antigenicity of the recombinant fusion protein are also reported in this paper. This study provides basic data for research into QRTb's genetics, germplasm identification and protection, and production of the recombinant pre-IGF-I.

Materials and Methods

Experimental Animal

QRTb was supplied by the Fisheries Science Research Institute of Academy of Agriculture Sciences of Hangzhou Municipality.

Bacterium and Reagents

The *E. coli* TG1 and BL21 strains were saved by the Biochemical Laboratory of the College of Animal Science, Zhejiang University. The pMD 18-T Vector Systems, *Bam*HI, *Eco*RI, *Not*I, DNA Marker DL3000, and DNA Marker DL6000 were purchased from TakaRa (Shiga, Japan); expression vertor pGEX-4T-1 from Pharmacia Company (New York, NY, USA); the protein molecular weight marker from MBI Fermentas (Burlington, Canada); the polyvinylidene fluoride (PVDF) Western blotting membrane from Roche (Basel, Switzerland); and Coomassie Brilliant Blue R250 (Fluka), agarose, low melting point agarose, calcium chloride, chloroform, and other chemical reagents from Shanghai Sangon Biological Engineering Technology and Services (Shanghai, China). The Western blotting test kit for IGF-I was purchased from BOSTER (Wuhan, China). Its primary antibody is rabbit IgG anti-IGF-I and binds only to the homologous sequence (53 Asp–Leu–Arg–Arg–Leu–Glu–Met–Tyr–Cys–Ala–Pro–Leu–Lys–Pro–Ala–Lys–Ser–Ala 70) of IGF-I when it occurred in the particular three-dimensional conformation that was characteristic of the native form of IGF-I without reacting with insulin or IGF-II or any other proteins. Its secondary antibody is rat anti-rabbit IgG conjugated a peroxidase.

Primer Designation and Synthesis

According to IGF-I gene sequence of Bb (GenBank no. AF332865), a pair of primers was designed and synthesized by Shanghai Sangon Biological Engineering Technology and Services. The 3′-primer (P1) was 5′ CGCGGATCCTTACTAAATGCGATAGTTTC 3′; the 5′-primer (P2) was 5′ CGGAATTCATGTCTAGCGGACATTTC 3′. To facilitate cloning of PCR products, a *Bam*HI restriction site was inserted into 5′-terminal of the P_1 and an *Eco*RI restriction site was inserted into 5′-terminal of the P_2.

Total RNA Extraction from Liver of QRTb

QRTbs were killed by cutting off their heads. Liver tissues were rapidly separated and ground in liquid nitrogen. Total RNA was extracted from 50 mg tissue with an RNA Extraction Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. Agarose gel electrophoresis of formaldehyde-denatured RNA showed that the quality of extracted RNA met the requirements for RT-PCR.

IGF-I cDNA Cloning and Sequencing

According to the instruction of THERMSCRIPT RT-PCR System (Promega), the first strand of cDNA was synthesized from 10 μg of total RNA with 3′-primer using Moloney murine leukemia virus RT at 42°C for 1 h. The PCR amplification program was as follows: denaturing at 94°C for 2 min, 10 cycles with denaturing at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 68°C for 1 min; 25 cycles with denaturing at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 68°C for 1 min, followed by a final extension at 72°C for 10 min then storage at 4°C. The RT-PCR products were electrophored through a 1.0% agarose gel and reclaimed with a 1.0% low-melting-point agarose gel. After separation and purification, the RT-PCR products were ligated into pMD 18-T vectors to obtain recombinant plasmid pMD 18-T/IGF-I. *Escherichia coli* TG1 strain was transformed with the ligation product according to Hanahan [17]. Luria–Bertani (LB) agar plates (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0), containing 50 μg/ml ampicillin, 40 μl *X*-gal (20 mg/ml) and 4 μl isopropylthiogalactoside (IPTG) (200 mg/ml), were used to screen the transformants. Plasmids from three positive clones were verified primarily by PCR and sequenced by Shanghai Sangon Biological Engineering Technology and Services.

Construction and Transformation of Expression Plasmid pGEX-4T-1/IGF-I

The expression plasmid pGEX-4T-1 and recombinant plasmid pMD 18-T/IGF-I were digested with both *Eco*RI and *Not*I. The IGF-I gene fragment was purified, reclaimed, and ligated with the linearized plasmid pGEX-4T-1 by T_4 DNA ligase (overnight at 4°C). The constructed plasmid pGEX-4T-1/IGF-I was transformed into *E. coli* TG1 strain. LB agar plates, containing 50 μg/ml ampicillin, were used to screen transformants. Then, the *E. coli* BL21 was transformed with the recombinant plasmid from positive clone TG1 by the $CaCl_2$ method. The obtained expression plasmid pGEX-4T-1/IGF-I was verified by both *Eco*RI and *Sal*I. The inserted fragment was oriented by PCR with the 3′-primer and the 5′-primer.

Fusion Expression of QRTb Pre-IGF-I in *E. coli* BL21

A positive colony was picked and incubated in 5 ml of LB medium with 50 μg/ml ampicillin overnight at 37°C with shaking (160 rpm). The overnight culture 50 μl was

added into 5 ml fresh LB medium with 50 μg/ml ampicillin and incubated at 37°C with vigorous shaking (210 rpm) until an OD_{600} of 0.3–0.5 was achieved. Then, the IPTG was added to a final concentration of 0.5 mM. After induction for 1, 2, 3, 4, 5, and 6 h, 1-ml samples of the culture were taken. Bacteria were harvested by centrifugation at 12,000×*g* for 5 min at 4°C, and the pellets were frozen at −20°C.

Frozen bacterial pellets were thawed and resuspended in 45 μl of lysis buffer (50 mM Tris-Cl, pH 8.0; 1 mM EDTA; 100 mM NaCl). Then, 5 μl lysozyme (10 U/μl) was added until the pellet became a single-cell suspension, and the suspension was incubated at 25°C for 30 min. After that, three freeze–thaw cycles were performed using −20°C for freezing and 25°C water bath for thawing. Subsequently, the 50-μl loading buffer was added into samples and boiled for 5 min and then centrifuged for 5 min at 12,000 rpm. The supernatant was an expression of QRTb pre-IGF-I in *E. coli* BL21 and was analyzed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

SDS-PAGE and Western Blotting Analysis

To analyze the recombinant protein, SDS-PAGE was performed in the Mini-Protein system (Bio-Rad, Hercules, CA, USA). After electrophoresis, one of the gels was stained with Coomassie Brilliant Blue R250 to visualize the protein bands, and the protein profile was analyzed by a dual-wavelength TLC Scanner. The proteins of the other gel were transferred onto PVDF membrane according to the manufacture's directions on a 0.65-mA/cm^2 membrane for the Western blotting analysis. The PVDF membrane was blocked with 5% nonfat dried milk powder in PBS with 0.05% Tween 20 (blocking solution). The membrane was probed with the primary antibody in blocking solution (1:20). A peroxidase-conjugated rat anti-rabbit IgG was used as the secondary antibody (1:400), and the signal was detected with H_2O_2 and diaminobenzidine as a chromogenic substrate.

Results

Cloning of IGF-I Gene from QRTb by RT-PCR

Total RNA from QRTb liver was expanded by RT-PCR, and 1% agarose gel electrophoresis showed that the RT-PCR product was a specific fragment of about 500 bp (Fig. 1A). RT-PCR products were obtained in low-melting-temperature agarose gels and ligated with pMD 18-T vectors. *Escherichia coli* TG1 was transformed with the recombinant plasmid pMD 18-T/IGF-I by the $CaCl_2$ method to obtain transformants. Three positive clones were screened out primarily by PCR, and 1% agarose gel electrophoresis showed that the PCR product was a specific fragment of about 500 bp (Fig. 1B).

cDNA Sequence of QRTb IGF-I Gene

The postive clone was sequenced by Shanghai Sangon Biological Engineering Technology and Services. The results showed that QRTb IGF-I gene consisted of a complete open reading frame (ORF) of 486 bp (submitted to GenBank, accession no. AY247412). QRTb IGF-I preprotein contained 161 amino acids, which spanned the complete signal peptide, mature IGF-I, and E-domain. Signal peptide contained 132 nucleotides encoding 44 amino acids. Mature IGF-I contained 210 nucleotides encoding 70 amino acids, which included B domain (1 Gly–Thr 29), C domain (30 Gly–Arg 41), A domain (42 Gly–Ala 62), and D

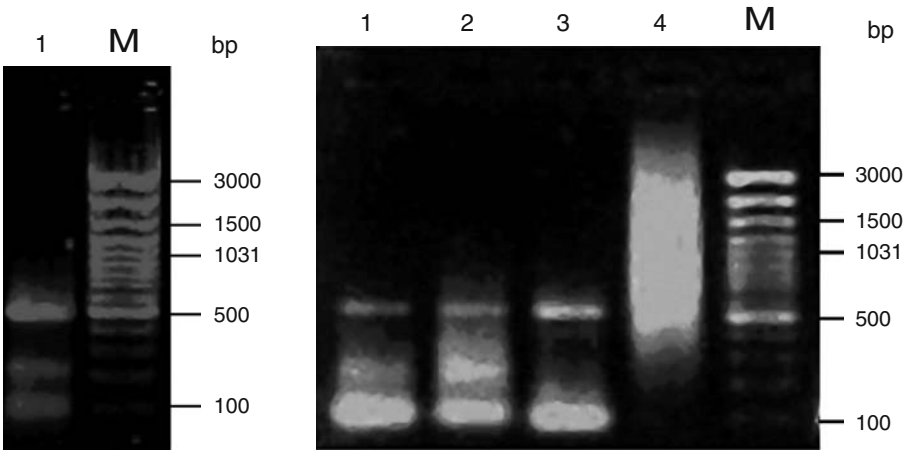

Fig. 1 **A** RT-PCR products. *Lane 1*: PCR product. *M*: DNA marker. **B** Construction of clone plasmid. *Lanes 1*, *2*, and *3*: Positive clones. *Lane 4*: Negative control. *M*: DNA marker

domain (63 Pro–Pro 70). E-domain consisted of 144 nucleotides encoding 47 amino acids. Analysis of the E domain indicated that cloned QRTb IGF-I gene belonged to the IGF-I Ea-2 subtype (Fig. 2).

Recombinant Expression Plasmid pGEX-4T-1/IGF-I

The positive clones *E. coli* BL21 were screened out primarily by PCR (Fig. 3A). The recombinant expression plasmid pGEX-4T-1/IGF-I from positive clone *E. coli* BL21 was

Signal sequence

ATG TCT AG C GGA CAT TTC TTC CAG GGG CAT TGG TGT GAT GTC TTT AAG TGT ACC ATG CGC	60
Met Ser Ser Gly His Phe Phe Gln Gly Hid Trp Cys Asp Val Phe Lys Cys Thr Met Arg	20
TGT CTC TCG TGC ACC CAC ACC CTC TCA CTG GTG CTG TGC GTC CTC GCG TTG ACT CCC GCG	120
Cys Leu Sex Cys Thr His Thr Leu Ser Leu Val Leu Cyc Val Leu Aln Leu Thr Pro Ala	40
ACA CTG GAG GCG	132
Thr Leu Glu Ala	44

Mature peptide

GGT CCG GAG ACGCTG TGC GGG GCG GAG CTT GTA GAC ACG CTG CAG TTT GTG TGT GGA GAC	60
Gly Pro Glu Thr Leu Cyc Gly Ala Glu Leu Val Asp thr Leu Gin Phe Val Cyc Gly Asp	20
AGG GCG TTT TAT TTC AGC AAA CCA ACA GGA TAT GGG CCT AGT TCG AGA CGG TCA CAC AAC	120
Arg Gly Phe Tyr Phe Sex Lys Pro Thr Gly Tyr Gly Pro Ser Ser Arg Arg Ser His Asn	40
CGC GGC ATT GTG GAC GAA TGC TGC TTT CAA AGC TGC GAA CTG CGG CGC CTC GAG ATG TAC	180
Arg Gly Ile Val Asp Glu Cyc Cys Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr	60
TGT GCA CCT GTG AAA ACC GGC AAA ACT CCA	210
Cys Ala Pro Val Lys Thr Gly Lys Thr Pro	70

E domein

CGA TCC CTA CGA GCG CAA CGG CAC ACA GAT ATC ACCAGG ACA GCA AAG AAA CCT ATA TCT	60
Arg Ser leu Arg Ala Gln Arg His Thr Asp Ile Thr Arg Thr Ala Lys Lys Pro Ile Ser	20
GGA CAT AGC CAC TCT TCC TGT AAG GAG GTT CAT CAGAAGAAC TCA AGC CGA GGAAAC ACA	120
Gly His Ser His Ser Ser Cys Lys Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr	40
GGG GGC AGAAAC TCT CGC ATT TAG	144
Gly Gly Arg Asn Tyr Arg Ile	47

Fig. 2 Nucleotide sequences and extrapolated amino acid sequences of the QRTb IGF-IcDNA

double-digested with restriction endonuclease *Eco*RI/*Sal*I, and 1% agarose gel electrophoresis showed that the double digestion product included a specific fragment of about 500 bp (Fig. 3B).

SDS-PAGE and Western Blotting Analysis of Fusion Protein Glutathione-*S*-Transferase-IGF-I

SDS-PAGE analysis showed that the induced expression products involved a specific band with MW about 47 kDa (Fig. 4). This was consistent with the expected molecular mass of the fusion protein of pGEX-4T-1/IGF-I, which consisted of the glutathione-*S*-transferase (GST) (29 kDa) and pre-IGF-I (17.8 kDa).

SDS-PAGE gel, used for analyzing expression products, was measured by a CS-930 Dual-wavelength TLC Scanner (Shimadzu, Kyoto, Japan). Measuring wavelength was at 590 nm. The results indicated that expression amount of the fusion protein increased with prolonging time. When induced time was 1, 2, 3, 4, 5, and 6 h, the expression products were approximately 1.4, 4.3, 8.1, 11.3, 16.3, and 18.8% of total bacterial protein, respectively.

The antigenicity of the recombinant fusion protein was demonstrated by Western blotting analysis using the Western blotting test Kit for IGF-I. A protein was recognized, which had the same size as the recombinant fusion protein as shown in the Western blotting (Fig. 5).

Discussion

QRTb, Bb, mud carp (Mc), Gc, Gf, and Cc all belong to cyprinid family. QRTb and Bb belong to cyprinoid, cultrinae. Mc belongs to cyprinoid, labeoninae. Gc belongs to

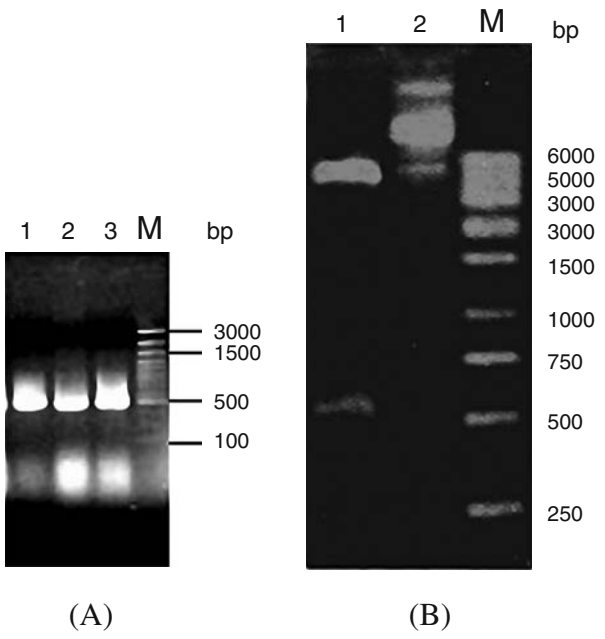

Fig. 3 **A** Electrophoresis of PCR product of recombinant expression plasmid. *Lanes 1*, *2*, and *3*: PCR products. *M*: DNA Marker. **B** Recombinant plasmid pGEX-4T-1/IGF-I was digested by *Eco*RI/*Sal*Irestriction enzymes. *Lane 1*: Double digestion product. *Lane 2*: Expressing plasmid. *M*: DNA marker

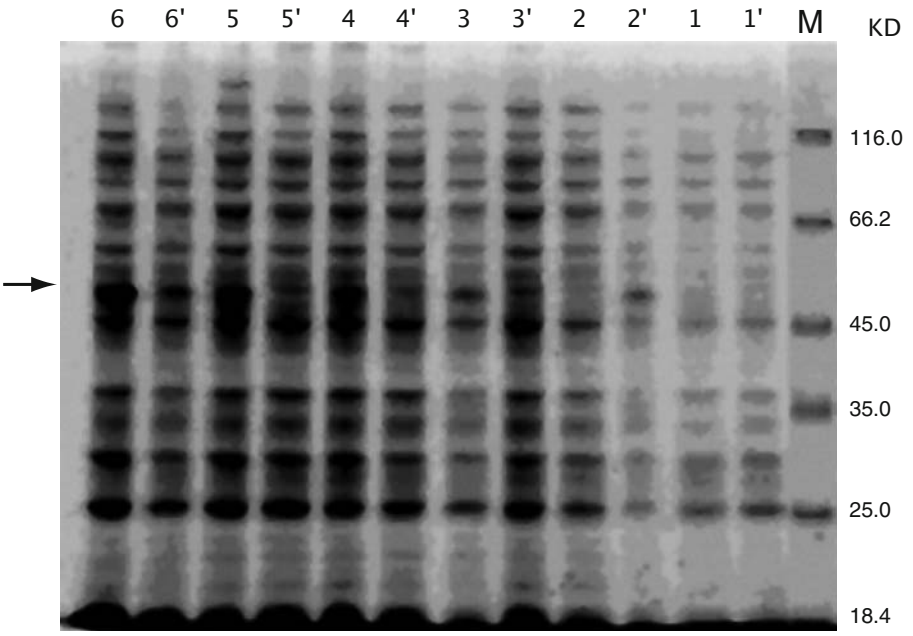

Fig. 4 The expression of pGEX-4T-1/IGF-I in *E. coli* BL21 was analyzed with SDS-PAGE. The recombinant plasmid transformed into *E. coli* was cultured in LB medium and induced with IPTG. *Lanes 1*, *2*, *3*, *4*, *5*, and *6*: pGEX-4T-1/IGF-I with IPTG induction. *Lane 1′*, *2′*, *3′*, *4′*, *5′*, and *6′*: pGEX-4T-1/IGF-I without IPTG induction. *M*: Protein marker

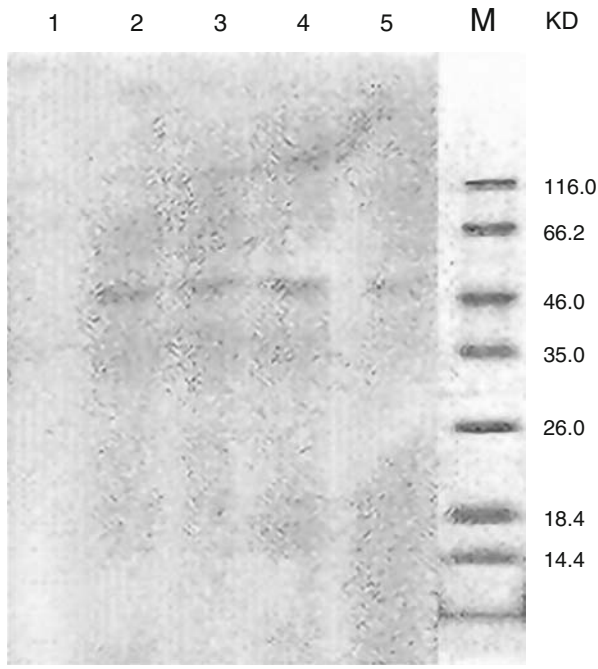

Fig. 5 Western blotting analysis of pGEX-4T-1/IGF-I expression product from the *E. coli* BL21. *Lane 1*: pGEX-4T-1/IGF-I without IPTG induction. *Lanes 2*, *3*, *4*, and *5*: pGEX-4T-1/IGF-I with IPTG induction. *M*: Protein marker

Table 1 The identity of pre-IGF-I cDNA in *Cyprinus carpio*.

	Nucleotide sequences of signal peptide (bp/identity)	Nucleotide sequences of mature IGF-I (bp/identity)	Nucleotide sequences of E peptide (bp/identity)	Nucleotide sequences of pre-IGF-I (bp/identity)
QRTb	132/100%	210/100%	144/100%	486/100%
Bb	132/99.2%	210/100%	144/100%	486/99.8%
Mc	132/98.5%	210/94.8%	144/93.1%	486/96.7%
Gc	183/70.5%	210/97.1%	144/100%	537/88.8%
Gf	132/97.0%	210/92.9%	144/95.1%	486/94.7%
Cc	183/71.6%	210/94.3%	144/91.0%	537/85.8%

cyprinoid, leuciscinae. Gf and Cc belong to cypinoid, cyprininae. The cDNA sequence of QRTb IGF-I gene consists of 486 nucleotides, which was very similar to that of Bb. QRTb ORF nucleotides share 99.8% sequence identity with that of Bb, including 100% sequence identity in mature IGF-I and E-domain and 99.2% sequence identity in signal peptide with difference in the 98th nucleotide. The IGF-I cDNA sequence of Mc (GenBank no. AY069945) contains 486 nucleotides, including the signal peptide, mature peptide, and E domain. The three domain regions share 98.5, 94.8, and 93.1% identity with that of QRTb, respectively. The IGF-I cDNA sequence of Gc contains 537 nucleotides [13], the signal peptide nucleotide sequence of which has considerable difference from that of QRTb, only 70.5% sequence identity. Whereas the nucleotide sequence of mature peptide and E domain share 97.1% and 100% identity, respectively. The IGF-I cDNA sequence of Gf (GenBank no. AF001006) contains 486 nucleotides, in which the nucleotide sequences of the signal peptide, mature peptide, and E domain share 97.0, 92.9, and 95.1% identity with that of QRTb, respectively. The IGF-I cDNA sequence of Cc (GenBank no. AF465830) contains 537 nucleotides, which has considerable difference from that of QRTb, with the signal peptide, the mature peptide, and E domain sharing 71.6, 94.3, and 91.0% identity, respectively (Table 1). It shows the high homology between cultrinae and labeoninae and considerable difference between leuciscinae and cyprininae. However, leuciscinae and cyprininae had high homology.

QRTb and Bb both belong to magalobrama genus. The pre-IGF-I shares 99.4% sequence identity between them and only one amino acid is different out of the 161 amino acids. In the 161 amino acids, the mature peptide and E-domain shares 100% sequence identity, signal peptide 97.7% identity (Table 2). Bb contains a Phe^{33} instead of Cys^{33} in QRTb signal peptide, which has more scientific significance to investigate further when and where QRTb and Bb parted.

Table 2 The identity of the pre-IGF-I amino acid sequence in *Cyprinus carpio*.

	Signal peptide (AA/identity)	Mature IGF-I (AA/identity)	E peptide (AA/identity)	Pre-IGF-I (AA/identity)
QRTb	44/100%	70/100%	47/100%	161/100%
Bb	44/97.7%	70/100%	47/100%	161/99.4%
Mc	44/97.7%	70/98.6%	47/87.2%	161/95.0%
Gc	61/68.9%	70/98.6%	47/100%	178/88.8%
Gf	44/100%	70/98.6%	47/91.5%	161/96.9%
Cc	61/70.5%	70/98.6%	47/85.1%	178/85.4%

QRTb, Mc, Gc, Gf, and Cc belong to cyprinid family but different subfamilies. The differences in pre-IGF-I amino acid sequence between them are more evident than differences between QRTb and the same genus Bb. The pre-IGF-I amino acid sequence of Mc and Gf consists of 161 amino acids, in which the signal peptide, mature peptide, and E peptide share 97.7 and 100%, 98.6 and 98.6%, and 87.2 and 91.5% identity with that of QRTb, respectively. The pre-IGF-I amino acid sequence of Gc and Cc consists of 178 amino acids, in which the signal peptide contains 61 amino acids and shares 68.9 and 70.5% identity with that of QRTb. Among the 70 amino acids of mature peptide of QRTb, the difference appears in the 69th amino acid compared to that of Gc and in the 66th amino acid compared to that of Cc, the identity being both 98.6%. In E domain, QRTb has the same amino acid sequence with Gc but shows difference at 3rd, 11th, 12th, 19th, 20th, 22nd, and 27th amino acids compared with that of Cc [15], with 85.1% identity.

The GST gene fusion system is an integrated system for the expression, purification, and detection of fusion proteins produced in bacterial, yeast, mammalian, and insect cells [18]. The sequence encoding the GST protein is incorporated into an expression vector, generally upstream of the multicloning site. The QRTb IGF-I gene was subcloned into the multicloning site of this vector. Induction of the vector resulted in expression of a fusion protein—GST-pre-IGF-I (GPI). The MW of GST and pre-IGF-I of QRTb is, respectively, about 29 and 17.8 kDa, so the MW of GPI is about 47 kDa, which is consistent with the results of SDS-PAGE and Western blotting. Purification of the GPI was facilitated by the affinity of the GST protein for glutathione residues. Glutathione residues were coupled to a resin and the expressed protein product was brought into contact with the resin. The GPI bound to the glutathione-resin complex and all other nonspecific proteins could be washed off. Then, the GPI was released from the resin using a mild elution buffer, which was of low pH. A thrombin restriction site (Leu Val Pro Arg ↓ Gly Ser) was inserted between GST and pre-IGF-I; it was possible to remove the GST from the GPI by using a thrombin, which would be adequate for purification of the QRTb pre-IGF-I polypeptide.

Western blotting was used to identify specific antigens with polyclonal or monoclonal antibodies. The primary antibody was monoclonal and could recognize the homologous sequence (53 Asp–Leu–Arg–Arg–Leu–Glu–Met–Tyr–Cys–Ala–Pro–Leu–Lys–Pro–Ala–Lys–Ser–Ala 70) of IGF-I when it occurred in the particular three-dimensional conformation that was characteristic of the native form of IGF-I. The homologous sequence shared 66.7% homology with the QRTb IGF-I mature peptide A12-D8 (53 Glu–Leu–Arg–Arg–Leu–Glu–Met–Tyr–Cys–Ala–Val–Lys–Thr–Gly–Lys–Thr–Pro 70). In two sequences above, the common sequence 54 Leu–Arg–Arg–Leu–Glu–Met–Tyr–Cys–Ala 62 may possibly play a role in antigenic determinant.

In this investigation, the QRTb IGF-I gene was cloned and expressed in *E. coli*. The productivity and the antigenicity of the recombinant fusion protein were tested. The GST gene fusion system (pGEX-4T-1 vector) for expression of QRTb IGF-I gene may be a potential useful approach in production of IGF-I polypeptide.

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