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Characterization and expression pattern of myostatin in *Paralichthys olivaceus*

Sang Beum Lee and Hyung-Joo Jin
Faculty of Marine Bioscience and Technology, Kangnung National University

Introduction

Muscle tissues express many muscle-specific genes, including myostatin (also known as GDF8) that is a member of the transforming growth factor-β superfamily. Myostatin is expressed in developing and adult muscle tissues and functions to negatively regulate muscle growth in mammals (McPherron et al., 1997). Myostatin has recently been isolated from many fish species, including zebrafish (McPherron et al., 1997), salmonids (Ostbye et al., 2001; Rescan et al., 2001; Roberts and Goetz, 2001), tilapia, white bass (Rodgers and Weber, 2001; Rodgers et al., 2001), catfish (Kocabas et al., 2002), and sea bream (Maccatrozzo et al., 2001b). Also, myostatin mRNA was found to be expressed in several tissues that are muscle, gill filaments, eyes, spleen, ovaries, gut, brain, and testes (Maccatrozzo et al., 2001a,b; Rodgers et al., 2001). In this study, we described the sequence and expression pattern of the myostatin gene in *P. olivaceus*.

Materials and Methods

1. Full-length cDNA isolation

Total RNA was extracted from *P. olivaceus* muscle tissue using RNA extraction Kit (Trizol, Invitrogen). One micrograms of total RNA from adult muscle tissue was reverse transcribed using a reverse transcriptase (Superscript II, Invitrogen) and oligo(dT) primers to obtain first-strand cDNA. Primers for 5′ end region of myostatin were designed on the basis of published sequences of teleost myostatin. The 5′-end region of myostatin was amplified by using DNA Walking SpeedUp Premix Kit (Seegene, Korea). A full-length cDNA of the myostatin was cloned with a Capfishing™ Full-length cDNA Premix Kit (Seegene, Korea) according to the manufacturer's protocol using gene specific primers based on the myostatin fragments known by DNA Walking SpeedUp Premix Kit (Seegene, Korea).

2. Tissue distribution of myostatin by RT-PCR

Total RNA was extracted as described above from brain, gill, heart, intestine, kidney, liver, muscle of and spleen of *P. olivaceus*. The initial cDNA synthesis and two-step PCR

cycling program were performed by incubating samples, followed by PCR with MSTN1 (forward) and MSTN2 (reverse) primers chosen on both sides of open reading frame of sequenced myostatin.

Results and summary

The full-length myostatin gene (GeneBank: DQ412048) in *P. olivaceus* was 1599 bp with open reading frame of 1134 bp, encoding for a 377 amino acids that showed 63-92% protein simility with other vertebrate myostatins, containing a conserved proteolytic cleavage site (RXXR) and conserved cysteine residues in the C-terminus. Based on RT-PCR, the myostatin gene was predominantly expressed in the brain and muscle, with limited expression in other tissues.

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