Molecular cloning and expression analysis of a C-type lectin in the rock bream Oplegnathus fasciatus

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C-type lectins are crucial for pathogen recognition, innate immunity, and cell-cell interactions. In this study, a C-type lectin gene was cloned from the rock bream. The full-length RbCTL cDNA was 729 bp with a 429 bp ORF encoding a 164-residue protein. The deduced amino acid sequence of RbCTL had all of the conserved features crucial for its fundamental structure, including the four cysteine residues involved in sulfide bridge formation and potential Ca2+/carbohydrate-binding sites. RbCTL contains a signal peptide one single carbohydrate recognition domain. It showed 29.4% similarity to the C-type lectin of rainbow trout. RbCTL mRNA was predominately expressed in gill and head-kidney tissue and expressed less in peripheral blood leukocytes, trunk-kidney, spleen, liver, intestine and muscle. Expression of RbCTL was differentially upregulated in rock bream stimulated with LPS, Con A/PMA and poly I:C.

Key words: Rock bream, C-type lectin, PBLs, LPS, Con A/PMA, poly I:C

In animals, innate constitutes the first defense against microbial invasion based on pattern recognition. The ability of host humoral - or cell-associated lectins to recognize exposed carbohydrates on the cell surfaces of potential pathogens considered a primary role for pattern recognition molecules in innate immunity (McGreal et al., 2004). Calcium-dependent (C-type) lectins are a major group of pattern-recognition receptors (PRRs) with one or more characteristic carbohydrate recognition domains (CRDs), that possibly mediate pathogen recognition and are important vertebrate and invertebrate innate immunity due to their ability to bind specific carbohydrates on microbe surfaces in a Ca2+-dependent manner (Homeskov et al., 2003; Vasta et al., 2004; Fujita et al., 2004).

Lectins are multivalent carbohydrate-binding proteins that function as important PRRs. According to their distinct structures and functions, lectins are classified as calnexin C-, L-, P-, I-, R- and S- types (Janeway and Medzhitov, 2002). The C-type lectin family has been well-studied in vertebrates and invertebrates. All C-type lectins share the same structural features including CRD sequences, disulfide-bond positions, and calcium binding sites (Drickamer, 1999).

In fish, C-type lectins have been identified in the carp (Cyprinus carpio) (Fujiki et al., 2001), rainbow trout (Oncorhynchus mykiss) (Zhang et al., 2000) and
eel (*Anguilla japonica*) (Tasumi *et al*., 2002). Lectins interacting with yeast and fish bacterial pathogens have also been functionally characterized in the conger eel (*Conger myriaster*) (Tsutsui *et al*., 2007) and coho salmon (*Oncorhynchus kisutch*) (Yousif *et al*., 1994). Lectins that bind to specific sugars such as mannose (Ottiner *et al*., 1999; Konstantina and Ioannis, 2006; Ourth *et al*., 2007), fucose (Honda *et al*., 2000), rhamnose (Okamoto *et al*., 2005) and galactose (Vitved *et al*., 2000) have also been isolated and characterized in fish.

The rock bream, *Oplegnathus fasciatus*, is one of the most economically important fisheries resources in Korea. It habits the Pacific Ocean, including southern parts of the Korean Peninsula, Japan, Taiwan, and Hawaii. It’s infrequent compared with other commercially important fishes in Korea. Red sea bream iridovirus (RSIV) disease is the major cause of rock bream mass mortality in Korea (Oh *et al*., 2007).

In this study, we identified a novel C-type lectin from *O. fasciatus*. C-type lectin was cloned from a constructed cDNA library of rock bream. The cloning and sequencing of the RbCTL, multiple alignment and phylogenetic analysis of the deduced amino acids, tissue distribution and mRNA expression pattern of the RbCTL was investigated.

Materials and methods

1. Cloning and sequencing rock bream RbCTL cDNA

The RbCTL cDNA was identified in the analysis of expressed sequence tags (ESTs) of rock bream liver that were stimulated with the LPS cDNA library (Kim *et al*., 2010). The similarity analyses of the nucleotide and protein sequences were conducted using the program BLAST at the US National Center for Biotechnology Information (NCBI http://blast.ncbi.nlm.nih.gov/Blast.cgi). The determined nucleotide and deduced amino acid sequences and multiple sequence alignments were analysed with GENETYX ver. 8.0 (SDC Software Development Co., Ltd., Tokyo, Japan). The signal peptide was predicted using the online SignalP 3.0 programme (http://www.cbs.dtu.dk/services/SignalP/) and domain identification was analysed with the PROSITE and SMART programmes (http://smart.embl-heidelberg.de/).

2. Fish, tissues sampling, and expression analysis

Rock bream (approximately 200 g) were purchased from TongYeong fish market in Korea. The fish were maintained in a laboratory recirculating seawater system at 20°C for domestication. They were anesthetized using benzocaine (Sigma-Aldrich, Co, USA). For PBL sampling, a heparin-treated syringe was used to collect blood from a tail unit vein. The peripheral blood leukocytes (PBLs) were prepared as described previously (Park *et al*., 2003). The head-kidney, trunk-kidney, spleen, liver, intestine, gill and muscle tissue samples were dissected from the fish.

Total RNA was extracted from rock bream PBLs, head-kidney, trunk-kidney, spleen, liver, intestine, gill and muscle tissue using the TRIzol reagent (Invitrogen, USA). Total RNA was treated with Recombinant DNase I - RNase free (TaKaRa, Japan) to remove DNA. To synthesize cDNA by reverse transcription, 2 μg total RNA, oligo dT primer and dNTP mixture, RNase free dH2O were reacted for 5 min at 65°C and immediately