

Non-Mammalian Pattern Recognition Receptor from Rock Bream (*Oplegnathus fasciatus*): Genomic Characterization and Transcriptional Profile upon Bacterial and Viral Inductions

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Abstract : Toll like receptors (TLRs) are a phylogenetically conserved family of pattern recognition receptors, which participates in the host immune responses against various pathogens and pathogen derived mitogen. TLR21, a non-mammalian type, is almost restricted to the fish species even though those can be identified rarely in avians and amphibians. Herein, this study was carried out to identify and characterize TLR21 from rock bream (*Oplegnathus fasciatus*) designated as RbTLR21, at transcriptional and genomic level. In this study, the full length cDNA and genomic sequence of RbTLR21 was identified using previously constructed cDNA sequence database and BAC library, respectively. Identified RbTLR21 sequence was characterized using several bioinformatics tools. The quantitative real time PCR (qPCR) experiment was conducted to determine tissue specific expressional distribution of RbTLR21. Further, transcriptional modulation of RbTLR21 upon the stimulation with *Streptococcus iniae* (*S. iniae*), rock bream iridovirus (RBIV) and *Edwardsiella tarda* (*E. tarda*) was analyzed in spleen tissues. The complete coding sequence of RbTLR21 was 2919 bp in length which can encode a protein consisting of 973 amino acid residues with molecular mass of 112 kDa and theoretical isoelectric point of 8.6. The anticipated protein sequence resembled a typical TLR domain architecture including C-terminal ectodomain with 16 leucine rich repeats, a transmembrane domain, cytoplasmic TIR domain and signal peptide with 23 amino acid residues. Moreover, protein folding pattern prediction of RbTLR21 exhibited well-structured and folded ectodomain, transmembrane domain and cytoplasmic TIR domain. According to the pair wise sequence analysis data, RbTLR21 showed closest homology with orange-spotted grouper (*Epinephelus coioides*) TLR21 with 76.9% amino acid identity. Furthermore, our phylogenetic analysis revealed that RbTLR21 shows a close evolutionary relationship with its ortholog from *Danio rerio*. Genomic structure of RbTLR21 consisted of single exon similar to its ortholog of zebra fish. Several putative transcription factor binding sites were also identified in 5' flanking region of RbTLR21. The RbTLR 21 was ubiquitously expressed in all the tissues we tested. Relatively, high expression levels were found in spleen, liver and blood tissues. Upon induction with rock bream iridovirus, RbTLR21 expression was upregulated at the early phase of post induction period even though RbTLR21 expression level was fluctuated at the latter phase of post induction period. Post *Edwardsiella tarda* injection, RbTLR transcripts were upregulated throughout the experiment. Similarly, *Streptococcus iniae* induction exhibited significant upregulations of RbTLR21 mRNA expression in the spleen tissues. Collectively, our findings suggest that RbTLR21 is indeed a homolog of TLR21 family members and RbTLR21 may be involved in host immune responses against bacterial and DNA viral infections.

Keywords : rock bream, toll like receptor 21 (TLR21), pattern recognition receptor, genomic characterization

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