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Transcriptional Evidence for the Involvement of MyD88 in Flagellin Recognition: Genomic Identification of Rock Bream MyD88 and Comparative Analysis

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Abstract : The MyD88 is an evolutionarily conserved host-expressed adaptor protein that is essential for proper TLR/ IL1R immune-response signaling. A previously identified complete cDNA (1626 bp) of OfMyD88 comprised an ORF of 867 bp encoding a protein of 288 amino acids (32.9 kDa). The gDNA (3761 bp) of OfMyD88 revealed a quinquepartite genome organization composed of 5 exons (with the sizes of 310, 132, 178, 92 and 155 bp) separated by 4 introns. All the introns displayed splice signals consistent with the consensus GT/AG rule. A bipartite domain structure with two domains namely death domain (24-103) coded by 1st exon, and TIR domain (151-288) coded by last 3 exons were identified through in silico analysis. Moreover, homology modeling of these two domains revealed a similar quaternary folding nature between human and rock bream homologs. A comprehensive comparison of vertebrate MyD88 genes showed that they possess a 5-exonic structure. In this structure, the last three exons were strongly conserved, and this suggests that a rigid structure has been maintained during vertebrate evolution. A cluster of TATA box-like sequences were found 0.25 kb upstream of cDNA starting position. In addition, putative 5'-flanking region of OfMyD88 was predicted to have TFBS implicated with TLR signaling, including copies of NF□B1, APRF/ STAT3, Sp1, IRF1 and 2 and Stat1/2. Using qPCR technique, a ubiquitous mRNA expression was detected in liver and blood. Furthermore, a significantly up-regulated transcriptional expression of OfMyD88 was detected in head kidney (12-24 h; >2-fold), spleen (6 h; 1.5-fold), liver (3 h; 1.9-fold) and intestine (24 h; ~2-fold) post-Fla challenge. These data suggest a crucial role for MyD88 in antibacterial immunity of teleosts.

Keywords : MyD88, innate immunity, flagellin, genomic analysis
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