



THE TOLL RECEPTOR FAMILY IN CHANNEL CATFISH

Sylvie M.A. Quiniou¹, Eva Bengtén²

¹ USDA-ARS, Catfish Genetics Research Unit, Stoneville, MS

² Dept of Microbiology, UMMC, Jackson, MS



Abstract

The commercial production of channel catfish, *Ictalurus punctatus*, is the largest enterprise in U.S. aquaculture. Similar to other aquaculture species, disease is a prevalent problem. Toll-like receptors (TLRs) are involved in recognition of microbes through pathogen-associated molecular patterns (PAMPs) and thus are important to innate immunity. We present here the identification of 10 channel catfish TLRs (1, 4, 7, 8, 9, 13 (21), 14 (18), 19, 20 and 22) in addition to the 3 previously described catfish TLRs (2, 3 and 5). The TLR sequences were isolated using degenerate primers, except for TLR 13 that was obtained by BAC end sequencing. Each TLR was fully sequenced following identification of specific BAC clones for each of them with the exception of TLR 9, which was sequenced by direct genomic sequencing. Southern blot analyses demonstrated that most catfish TLRs were single copy genes except for TLR4, 5 and 20, which exist as two copies. Phylogenetic analyses of the catfish TLR genes show that they group with their respective counterparts from other species. Expression of each of these genes was determined for 11 different tissues.

Methods

Degenerate primers were constructed based on multi species alignments for stretches of 7 conserved amino acids. Products were separated on agarose. Bands of predicted size were cloned and sequenced. Based on the sequences obtained, PCR primers were designed to screen the CCBL1 BAC library. The TLR genes were sequenced by walking the identified BAC clones with the exception of TLR 9 for which a BAC clone was not found. TLR 9 was sequenced by direct genomic sequencing using the Clontech GenomeWalker kit. Total RNA of 11 tissues was extracted from four 1 year old fish using TRI-reagent. 500 ng of total RNA was used for cDNA synthesis using the Bio-Rad iScriptcDNA synthesis kit. A total of 500 ng of cDNA was used in 35-cycle PCR for expression analyses. Sequence alignments and phylogenetic analysis were based on amino acid alignment of the TIR domains using CLUSTALW. Neighbor-joining (NJ) trees with pair wise gap deletions were drawn using MEGA v3.1 with 1, 000 bootstrap replications.

Results

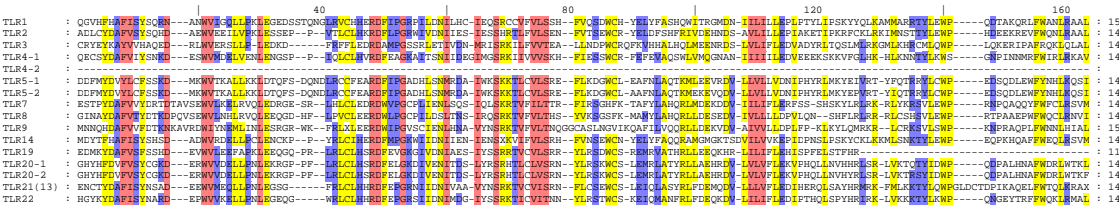


Figure 1: Amino acid alignment of the TIR domain of catfish TLRs. Residues that are conserved in 95% of the sequences are shaded red, 75% of the sequences in yellow and 55% of the sequences in purple.

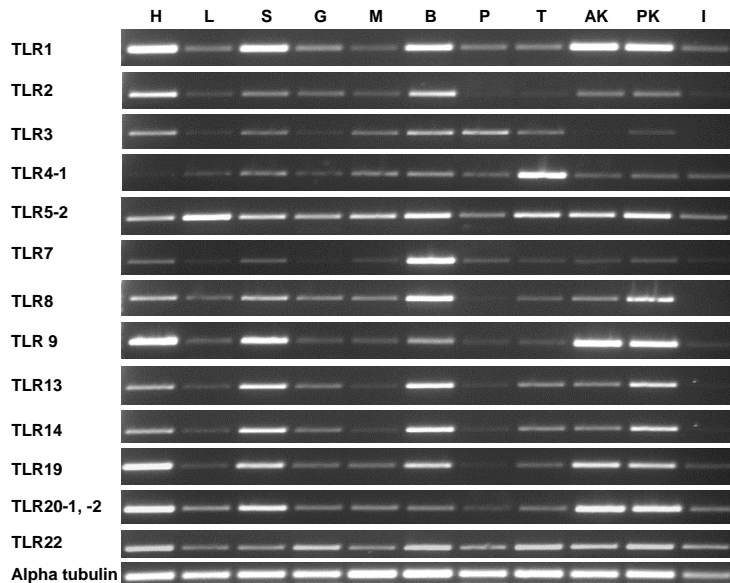


Figure 2: Expression analysis of catfish TLRs in 1 year old fish. Alpha tubulin was used as a positive control for constitutive expression in H: Heart; L: Liver; S: Spleen; G: Gill; M: Muscle; B: Brain; P: Pituitary Gland; T: Testis; AK: Anterior Kidney; PK: Posterior Kidney; I: Intestine.

Conclusion

In the future, expression of all Toll receptors will be studied by real-time PCR to determine their role, if any, in bacterial and viral diseases of catfish.

Acknowledgments

The authors wish to thank Thishya Perera for her technical help.

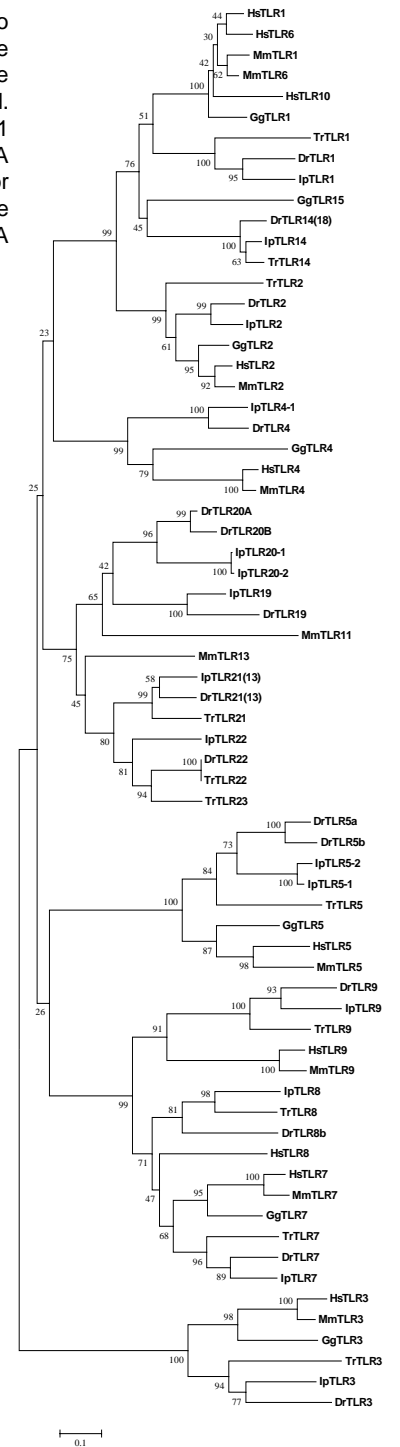


Figure 3: Un-rooted phylogenetic tree of TLRs in vertebrates. Bootstrap values are indicated. Branch lengths reflect amino acid substitutions and a scale bar is shown below the tree. Hs: Human; Mn: Mouse; Gg: Chicken; Dr: Zebrafish; Tr: Fugu; Ip: Catfish