

1. Title of the sub-project:

Toll-like receptors in phylogenetically divergent fish species-their contribution in modulating the innate immunity

2. Sub-project code: NAIP/Comp4/C30018

3. Names of the lead consortium leader and partners:

Consortium leader: Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, Orissa

Consortium partners:

1. Central Inland Fisheries Research Institute (CIFRI), Barrackpore, Kolkata, W. B
2. Madras Veterinary College, TANUVAS, Chennai

Consortium PI: Dr.M.Samanta, Senior Scientist, CIFA, Bhubaneswar

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Consortium Co-PI: Mr. P. Maurye, Scientist, CIFRI, Barrackpur

Dr. G. Dhinakar Raj, Professor, TANUVAS, Chennai

Consortium website:

4. Objectives:

- To study variation in TLR gene expression induced by three types of pathogens / ligands in phylogenetically divergent fishes *viz.* carp, catfish and shark.
- Assessing the divergence of key TLR genes playing a role in the innate immunity of fishes.
- To understand the contribution of TLR signaling pathway(s) in the innate immunity of fishes.
- Strategic delivery of TLR - ligands and testing their efficacy to modulate innate immunity *in-vivo*.

5. Duration: 3 years 3 months (2008-09 to 2011-2012)

6. Total estimated cost: Rs. 358.58 lakh

7. The rationale:

To meet the ever increasing demand, fish culture is now based on intensive-culture systems, where multiple species like rohu (*L. rohita*), catla (*Catla catla*), mrigal (*C. mrigala*) (called together as Indian major carps, IMC) are cultured in high stocking density. In intensive-aquaculture, diseases like: epizootic ulcerative syndromes (EUS), aeromoniasis, bacterial gill disease, fin rot disease *etc.* are one of the important limiting condition on which the success or failure of aquaculture mostly depends.). To prevent the occurrence of diseases, vaccination is an important strategy to induce specific humoral immune response through the production of antibodies. However, fish does not have well developed immune system. As a result, vaccination

induces only a single class of immunoglobulin (**IgM**), which is of short duration. Therefore, several pathogens like bacteria, fungus, protozoa and viruses cause disease in fish and shellfish. In absence of well-defined immune system, innate immune system plays a major role in protecting fish against diseases. Toll-like receptors (TLRs) are important signaling molecules, which induces innate immunity when they are activated by the pathogen associated molecular patterns (PAMPs). To date, the functions of several Toll-like receptors (TLR) are significantly clear in humans and animals. With the identification of similar TLR orthologs (TLR3, TLR5, TLR9, TLR20, TLR 21 *etc.*) in various exotic fishes : *Danio rerio*, *Fugu rubripes*, *Carassius auratus*, *Paralichthys olivaceus*, *Onchorhynchus mykiss*, *Ictalurus punctatus* and *Penaeus monodon*, the role of TLRs in contributing the innate immunity in fish are becoming important though much is not known about Indian spp. To understand the role of TLRs in innate immunity of fishes, we project to analyze three phylogenetically different types of fish, like scaled fish (carps), non scaled fish (catfish) and cartilaginous fish (shark), where no information is available on the presence of TLRs, their expression profiles in different tissues and also their nucleotide sequences. Once we obtain these data, we will be able to understand the evolutionary relationship among these fishes in respect to innate immune profiles contributed by TLR-network.

Major innovations to be attempted

- Understanding evolutionary lineages of TLR genes in phylogenetically divergent cartilaginous, non-scaled and scaled bony fishes.
- Identification and elucidation of the role of novel TLR genes of which have no mammalian counterparts and understanding the associated PAMPs in the innate immune system of fishes.
- Exploiting TLR signaling pathways across species barriers.

8. Outputs:

- Basic information on species / tissue specific TLR gene expression induced by pathogens / ligands in phylogenetically divergent fishes.
- Evolutionary relationship of TLR-genes in fish species and mammals.
- Sequences of novel TLR genes
- Development of basic information fish disease management alternatives based on the TLR and PAMPS based innate immune systems

9. Expected outcome and impact:

- Understanding of the innate immune system in fishes will help not only the scientists involved in disease management in fish but also will add to the knowledge of immune systems of animals in general
- The knowledge of fish management disease management strategies alternatives may be developed further for development of appropriate low-cost vaccine like products