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Polysaccharides from marine macroalga, *Padina gymnospora* improve the nonspecific and specific immune responses of *Cyprinus carpio* and protect it from different pathogens

Priyatharsini Rajendran ^a, Parasuraman Aiya Subramani ^b, Dinakaran Michael ^b  

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Highlights

- Polysaccharides (PF) were extracted from *Padina gymnospora*.
- PF administered to *Cyprinus carpio* improved selected immune parameters.
- PF significantly reduced mortality with a RPS of 80.
- PF upregulated expression of IL-1 β and Lysozyme-C.

Abstract

Immunostimulation by plant-derived compounds presents a fascinating alternative to vaccines and antibiotics in aquaculture. Fish farmers are longing for immunostimulants that activate both specific and nonspecific immune responses of fish and protect fishes from all possible infections. In this study, we observed that polysaccharide fraction from marine macroalga, *Padina gymnospora* stimulated the immune response of common carp *Cyprinus carpio* (Filed for patent, Indian patent no. 201641027311 dated:10-Aug-2016). Our results indicate that fish fed with polysaccharides as feed supplement improved all the immune parameters tested which include serum lysozyme, myeloperoxidase activities and antibody response. Further, polysaccharide fraction protected the fish from its common bacterial pathogens namely *Aeromonas hydrophila* and *Edwardsiella tarda* with relative percent survival (RPS) values of 80 and 60 respectively. Gene expression studies, indicate that the immunostimulation by *P. gymnospora* might be at least in part due to the upregulation of the cytokine interleukin-1 β (IL-1 β) and antimicrobial peptide lysozyme-C.

Introduction

Per capita consumption of fish by the world population has increased from 9.9Kg in the 1960s to 19.2Kg in 2012 [19] and the increase in fish consumption is not accompanied by the increase in fishes captured. Therefore, aquaculture has become indispensable to feed these teeming world population. It is no wonder that aquaculture accounts for nearly 50% of fishes that are consumed worldwide [19]. However, the growth of aquaculture industry is severely hampered by infectious diseases of fish [23].

Though different prophylactic methods like immunostimulation, vaccination, genetic improvement etc. and therapeutic methods like usage of disinfectants and antibiotics are available for fishes, immunostimulation by plant-derived natural products are highly preferred [24]. The reasons are many which include very narrow range of protection conferred by vaccines, overuse of antibiotics that may lead to undesired consequences like antibiotic resistance [41] and finally the polluting nature of hazardous chemical disinfectants used. Fishes greatly rely on nonspecific immunity for protection from infections because sufficient antibody synthesis in fish typically takes about two weeks that also temperature dependent. The advantages of plant-derived immunostimulants are its little or relatively fewer side effects and residual problems, easier administration procedures and more economic feasibility [25].

Seaweeds have been identified as a rich source of bioactive compounds that are not found in terrestrial plants [7]. *Padina gymnospora* is a brown alga, seaweed commonly found in the

coastal regions around the world. *Padina gymnospora* is reported to possess antimicrobial [35], antiviral [29], antioxidant and anticancer activities [36].

Interleukin-1 beta (IL-1 β) is an important mediator of inflammatory response in fishes [6] and innate immune mechanism [39]. IL-1 β as a defensive cytokine is often upregulated during pathogenic infection [8]. IL-1 β is a key mediator in response to microbial invasion and tissue injury and can stimulate immune responses by activating lymphocytes or by inducing the release of other cytokines capable of triggering the activities of macrophages, NK cells and lymphocytes. Macrophages are the primary source of IL-1 β although it is produced by a wide variety of other cell types as well [32].

Lysozyme is a hydrolytic enzyme that cleaves β -(1,4) glycosidic bonds. Lysozyme is involved in defence against bacteria, parasites and viruses [34]. Lysozyme is an important opsonizing agent of both Gram positive and negative bacteria. It also activates numerous downstream immunological processes like phagocytosis, complement activation etc. in fishes [55].

We have earlier reported about the immunostimulating properties of many terrestrial plants [18], [15], [1] and a seaweed [4]. The present study reports on the immunostimulating and disease resistance properties of polysaccharide fraction obtained from the seaweed *Padina gymnospora* in the common carp, *Cyprinus carpio*.

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Section snippets

Fish

Fish weighing 100 \pm 5g were used for all the assays. Fish were procured from a local fish farmer and acclimated for two weeks in fibre-reinforced plastic (FRP) tanks (vol. 500L) at a density of 4g/L. Canister external water filters (Eheim, Deizisau, Germany) were fitted to each tank for removal of ammonia and oxygenation of water. Uneaten feed was siphoned out periodically to avoid possible microbial growth. The fishes were maintained in natural, ambient photoperiod and temperature of

Results

The experiments were designed and done to study the immunomodulatory effect of PF administered orally as feed supplement for one, two or three weeks. At the end of each week, nonspecific and specific immune parameters and disease resistance were examined.

Discussion

Fishes mostly rely upon nonspecific immune response for clearance of foreign substances [33]. Lysozyme is an important serum nonspecific defence factor against invading pathogen [22]. Previous studies have shown that increase of lysozyme activity in *C. carpio* through the administration of immunostimulants had protected the fish from *A. hydrophila* infection [2]. *C. carpio* that were naturally resistant to *A. hydrophila* showed enhanced lysozyme activity upon experimental challenge with *A.*

Conclusion

PF of *P. gymnospora* enhanced all the immune parameters and the expression of immune response genes tested in *C. carpio*, consequently protected the fish from experimental infection with two main pathogens namely *A. hydrophila* and *E. tarda*. So, it is proposed that PF of *P. gymnospora* is a good immunostimulant and upon confirming its activity in culture conditions, it can be used as a routine feed additive to *C. carpio* and perhaps other carps to protect it from bacterial infection. Future studies

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Citation Excerpt :

...In the current study, the positive effect of dietary seaweed inclusion on IL-1 β and TNF- α genes expression of rainbow trout was observed. In agreement to our results, [39], showed the IL-1 β gene was up-regulated by *P. gymnospora* derived polysaccharides in common carp [43]. also reported that green tea (*Camellia sinensis*) increased IL-1 β transcription in the kidney of rainbow trout....

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