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MANAGEMENT OF FISH /SHELLFISH DISEASES USING IMMUNOSTIMULANTS ISOLATED FROM MARINE NATURAL PRODUCTS AND OTHER ADDITIVES

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INTRODUCTION

Application of antibiotics and other chemicals in aquaculture has its own intricate problems. For example, regular use of antibiotics in fish or shrimp hatchery or grow out system may lead to development of not only antibiotic resistant fish/shrimp bacteria, but also human bacteria. Information is still lacking on the absorption and distribution of antibiotics in fish and shrimp and persistence of residues or effects of them in the environment. Hence, promoting the holistic systems approach in managing fish/shellfish health problems needs special attention.

Considering the potential threat of diseases on the one hand and the environmental issues on the other hand, disease management aspects should concentrate on environment friendly biotechnological methods like: prophylaxis (vaccines, immunostimulants, bioremediation of environment – including the methods of administration) and disease treatment protocols.

BACTERINS/VACCINES

A vaccine could be defined as a substance that causes a specific immune response. Vaccination as a part of standard fish culture programme is relatively new although the impact of vaccination is found to be dramatic. For example, the culture of salmon in brackish water and marine environment was made possible by usage of the Vibriosis vaccine, which led to a great expansion in pen-rearing of Atlantic salmon in Norway and Chile. Several studies have been undertaken on the possibility of using live attenuated vaccines.

Considering the importance of vaccination, biotechnologists are trying to develop subunit vaccines, i.e., vaccines consisting of the major protective antigens of the pathogen. The sub-unit vaccines have evident advantages: The important advantage is that the vaccine contains only a component of the pathogen and is therefore, more chemically defined and likely to be more stable. The other advantage is that the vaccine can be produced by direct synthesis or recombinant DNA technology. Thus these vaccines may be genetically engineered to express further protective antigens from other fish pathogens and thereby yield multivalent vaccines.

Techniques in administering vaccines, bacterins and immunostimulants

The route of exposure of the immunizing antigen has a direct impact on the levels and types of protective immunity that develops. Currently four methods are commonly used to deliver antigens:

1. Injection (intraperitoneal, intramuscular or subcutaneous) induces highest levels of protection; but is very labour-intensive and stressful. Semi-automated devices, which can immunize 4000 fish per hour, have been developed which reduce the stress on fish and risk of exposure to the worker.

2. Vaccination by immersion is perhaps the most widely used method. In this method, fish are dipped for 20 seconds in a well-aerated vaccine suspension. Thus a litre of vaccine can be used to vaccinate 10,000X10g fish. Dip vaccination would be stressful, but the problem is overcome by bath vaccination where fish are vaccinated by being exposed to higher dilutions of vaccines (e.g. 1:100) for times ranging from 20 minutes to several hours. Vaccine can be added directly to hatchery troughs or transport bags.

3. Spray vaccination is another modification of direct immersion but in this method fish must be handle thus making it stressful. The level of protection, though variable has been reported to be comparable to immersion.

4. Immunization by oral route by incorporating the bacterin in the feed is a potentially useful method.

IMMUNOSTIMULANTS

Immunostimulants are substances, which elicit non-specific defense mechanisms and enhance the barrier of infections against pathogens. They are isolated from natural sources and then synthesised chemically. (Ex: cell wall preparations from bacteria, fungi, mushroom).Most of the research on immunostimulants has been directed towards treatment of cancer in humans. Immunostimulating compounds induced production of cytokine proteins like interleukins, interferon, tumor necrosis factor and colony stimulating factors. Injection of such preparations resulted in improvement of symptoms from malignant tumors and prolongation time of cancer patients.

The active principles of immunostimulatory cell wall preparations are various muramylpeptide fragments, lipopolysaccharides, lipopeptides, acyloligopeptides and specific ides composed of glucose units which are linked through b-1, 3 and b-1, 6 bonds. These glucans can exist in various structural forms, water soluble oligomers; water insoluble macromolecules and particulate matters.

In fishes, the killed mycobacteria and muramy1 dipeptide enchanced resistane of coho salmon, *Oncorhynchus kisutch* against several bacterial pathogens. Injection of the synthetic lactolyl tetra peptide FK-565, increased the phagocytic activity and non-specific resistance of rainbow trout against *Aeromonas salmonicida* infections. Resistance of carp to infections by *Edwardsiella tarda* by activating the non-specific was achieved by administration of schizophyllan, scleroglucan and lentinar. The non-specific disease resistance in Atlantic salmon was enhanced by glucan preparation from *Saccharomyces cerevisiae*. Since then several researchers have suggested the possible use of glucans against viral infections in fish and shrimps.

In shrimps, the Wheatgerm Agglutinin (WGA), a lectin, administered as feed additive has promoted the bacterial resistance of *Penaeus orientalis*.M-Glucan (a mixture of insoluble *b*-1, 3 and b-1, 6 poly glucoses) was found as a short-term immunostimulant for the shrimp, *Penaeus monodon*. Immersion treatment with yeast beta-glucan was demonstrated to enhance growth and vibriosis resistance in tiger shrimp *P. monodon*. In the treated shrimps, the disease resistance could be correlated with enhanced phenoloxidase activity and intrahemocytic production of superoxide anion.

In shrimps the prophenoloxidase ('Propo'), the defense enzyme system, is activated by immunostimulants. The activation of 'Propo' results in recognising pathogens and providing resistance.

In fish, the non-specific defense system is activated by the immunostimulants. The first line of defense - i.e., non specific humoral defense or proteases, lysins and agglutinins in mucous cell secretion; The second line of defense provided by the mucosal lining cells and the third line of defense achieved by blood cells, especially granulocytes and monocytes which destroy microbes present in the circulation are activated. Endocytically active cells such as endothelial cells, macrophages and granulocytes in organs and tissues, which degrade microbes or microbial products, take up the final defence. The final endocytic and degradation process strongly depend on the effectiveness of reticulo endothelial system, which consist of endothelial cells, and macrophages, which line the small blood vessels (sinusoids and ellipsoids). The central cells in the production of antimicrobial substances are macrophages and granulocytes, which are activated by the immune enhancers.

Hemocytes are also activated by immunostimulants. In addition, they enhance the clotting activities and produce bactericidins. In tiger shrimp *Penaeus monodon,* increased bacterial clearance was noted after injection with glucan. The bacterial clearance ability of haemolymph drawn from the tiger shrimp *Penaeus monodon* immersed in a viable cell suspension of *Vibrio vulnificus* showed that *Vibrio* cells were largely eliminated from shrimp haemolymph within 12 h following invasion and completely undetectable at 24 h. The anti-*E. coli* activity of plasma, phenoloxidase (PO) activity, as well as the production of superoxide anion (O^2) were significantly enhanced due to administration of glucan and zymosan. Immunostimulants can promote recovery from the status of immunosuppression caused by stress. The peptidoglycan- fed black tiger shrimp exhibited a higher tolerance to dissolved oxygen, salinity and stress than those fed with the controlled diet.

The immunostimulants have several advantages:

1. Being natural products, there is no environmental hazard.
2. Unlike vaccines, which give protection to a specific pathogen, immunostimulants provide a wide range of protection against several pathogens.
3. Most of the immunostimulants can be synthesized and the problem of residual effect on shrimps or fish is not encountered.
4. Fish depend more heavily on non-specific defense mechanisms than mammals and therefore immunostimulants have a significant role in health management strategies in aquaculture.
5. When glucans were administered along with *Aeromonas hydrophila* vaccine, the response was even more enhanced, suggesting that yeast glucans have important role in disease management in warm water aquaculture.

In shrimps, three main types of circulating haemocytes have been identified and isolated by isopycnic cetrifugation on Percoll gradient. Semi granular cells respond to microbial polysaccharides such as lipopolysaccharides and B-1,3-glucans by degranulation. Since the degranulated cells attach and spread on foreign surfaces, they have an important role in encapsulation. Granular haemocytes with large granules are a repository for the prophenoloxidase (pro-PO) activating system. In crustaceans, clotting is mediated by coagulogens present in the plasma and also compartmentalized within circulating cells. The plasma factor is converted to covalently linked polymers of coafulin by Ca^{2+}-dependent transaminase whereas the cell factor is converted to a gel by a serine protease proclotting enzyme, which may be triggered, by microbial molecules such as lipopolysaccharide (LPS) and -1,3-glucans.

BIOREMEDIATION OF THE FARMING ENVIRONMENT

One of the important prophylactic measures against the disease management in aquaculture is proper water management. In culture conditions, the disease problems are linked to the stress factors arising out of inadequate physico-chemical and microbiological quality of water. Ammonia and hydrogen sulfide are two important factors of great significance to the well being of the cultivable species. As the culture progresses and biomass increases, the water quality deteriorates due to accumulation of metabolic waste of cultured organisms, decomposition of unutilized feed and decay of other biotic materials. It is reported that many of the pathogens isolated from diseased shrimp were the normal flora of the culture system, which become opportunistic pathogens. For the eco-friendly environment and disease management, the concept of 'probiotics' is gaining importance.

Presently a variety of commercial products of water additive probiotics are available. The 'probiotic organisms' work on the principle of competitive exclusion. This ecological process modifies the microbial species composition of the host and its environment. The probiotic application also acts as a "bio control", through which pathogens can be killed or reduced in number in the aquatic environment. Thus the concept of "bioremediation" is initiated, when microbes are used to treat pollutants or waste, which break down undesirable substances.

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Table 1

List of immunostimulants with promising immunostimulatory effect in lab experiments

Immunostimulants	*Species*	Pathogen	References
Glucan	P. monodon	Vibrio	Raa et al., 1996
β 1-3 Glucan	P. monodon	WSSV	Chang *et al.*, 1999
β1-3 Glucon	Marine Shrimp	Virus	Dugger and Jory 1999
Glucan	Crustacean	Disease	Takaharhi and Itami 1997
Glucan Zymoson Heat killed Vibrio LPS Trypsin	*P.monodon* *M.rosenberigi*	*Vibriosis*	Sung *et al.*, 1998
Glucan	*P.monodon*	Vibriosis	Teunissen *et al.*, 1998
β 1-3 glucan peptido glucan	Crustacea	Disease	Takahashi *et al.*, 1995
β glucan	*P.monodon*	Microbicidal activity	Sung *et al.*, 1996
β Glucan	*P.monodon*	Vibrio	Liao *et al.*, 1996
Vibrio aliginolyticus bacterin	*P.monodon*	Bacteria	Adams, 1991
Glucan	*P.monodon*	*Vibrio* vulnificus	Sung *et al.*, 1994;
Vibrio bacterin, Yeast β1-3 glucan	*P.monodon*	*Vibrio* sp.	Davaraj *et al.*, 1998;
Peptidoglycan	*P.monodon*	Virus	Itami *et al* ., 1998
Lipopoly saccharide & β1-3 glucan	*Pacifastacus leniusculus*		Lee *et al.*, 2000.
Yeast β1-3 glucan Lipopoly Saccharide	*P.monodon*	White spot Baculo virus	Karunasagar *et al.*, 1996;
Vibrio bacterin	*Shrimp*	Vibrio	Horne *et al* ., 1995.
Pepidoglucan	*Shrimp*	Yellow head Baculo virus	Boonyaratpalin *et al.*, 1995.
Yeast glucan	*Shrimp*		Song and Hsieh, 1994.

β1-3 glucan	Brown Shrimp		Vergas *et al.*, 1996.
Glucan	Brown Shrimp		Hernandez *et al.*, 1996
β1-3 glucan	Crustacean		Cerenius *et al.*, 1994.
β1-3 glucan	Crustacean		Sugumaran and Nellaiappan.1991.
β1-3 glucan	Shore Crab		Smith *et al.*, 1984.
β1-3 glucan	Horse shoe crab		Soederhaell *et al.*, 1985.
Chitosan Levamisole	*Panulirus homarus*		Huxley *et al.*, 2000

Table 2

Marine natural products with promising immunostimulatory effect

Source of organism	**Experimental organism**	**Assay/inhibitory activity**
Porphyra yezoensis (Seaweed)	Murine	Phagocytic assay
Undaria pinnaftifida (Seaweed)	NS	Immunomodulatory
Ecteinascida turbinata (Tunicate)		
	Eel	Phagocytic assay
Haliotis discus hannai (Abalone)		
	Trout	Phagocytic assay NK cell assay
Hyrtiss erecta (Sponge)		
	NS	Immunomodulatory
Briareum exavatum (Gorgonid)	NS	Immunomodulatory
	Shrimp and fish	Immunostimulatory
Ulva fasciata (Seaweed)		

NS – Not Specified