Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 1441-1447
Received: 20-09-2018
Accepted: 21-10-2018

Faiqa Syeed Farooqi
KVK-Ganderbal, SKUAST-K, Shalimar, Jammu and Kashmir, India

Wasim Ul Haq Qureshi
Sheri Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar Campus, Srinagar, Jammu and Kashmir, India

Correspondence
Faiqa Syeed Farooqi
KVK-Ganderbal, SKUAST-K, Shalimar, Jammu and Kashmir, India

Immunostimulants for aquaculture health management

Faiqa Syeed Farooqi and Wasim Ul Haq Qureshi

Abstract
The production of fish and shellfish by aquaculture has become the fastest growing animal food sector in the world. The advances in culture techniques and the introduction of new species have contributed to the rapid growth of the aquaculture industry. The practice of intensification has become common in both finfish and shellfish culture to optimize the returns. High stocking densities, artificial feeding and fertilization have become common husbandry practices in both carp and shrimp culture systems. Due to intensification of culture practices, various diseases of microbial etiology have surfaced in rearing and grow out ponds, being a major threat to the sustainability of the aquaculture industry. Use of synthetic chemicals and antibiotics to prevent or treat various diseases has achieved some success. However, the use of antibiotic therapy in aquaculture inevitably leads to the emergence of resistance in the target bacteria. Vaccination is a useful prophylaxis for infectious diseases of fish, but the development of vaccines against intracellular pathogens has not so far been successful. An alternative approach has been the application of various compounds to boost or stimulate the innate immune system of farmed fish and shrimp. These compounds, termed Immunostimulants are considered an attractive and promising agent for the prevention of diseases in fish and shellfish. Immunostimulants comprise a group of biological and synthetic compounds that enhance the innate or non-specific cellular and humoral defence mechanism. Immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Several immunostimulants also stimulate lysosomes and the antibody responses of fish.

Keywords: Immunostimulants; immune system; aquaculture; health management

1. Introduction

Aquaculture has been growing rapidly for food production in the last few decades. Several commercial fish species have been cultured intensively in narrow or enclosed spaces such as ponds, cages or tanks under overcrowding or high density leading to adversely affect the health of cultured fish with a potentially stressful environment and infectious diseases. The infectious disease-outbreaks have emerged as constraints for the development of aquaculture. The occurrences have spread through the uncontrolled movement of live aquatic animals resulting in the transfer of pathogenic organisms among the countries. Antibiotics and chemotherapeutics have been used to prevent or control bacterial infections in aquaculture for more than twenty years. Unfortunately, the use of antibiotics for treatment of fish diseases is not successful and sustainable due to increase in antibiotic-resistant bacteria, negative effect on the indigenous micro flora of juveniles or adult fish [22], and the accumulation of antibiotic residues in fish tissue and environment causing human and animal health issues. Antibiotic use is gradually increasing in aquaculture due to the urgent needs of the industry but their use has resulted in several drug residues accumulating within aquatic products, thus raising food safety concerns. In addition, several pathogenic microorganisms have also developed drug resistance. However, many of the current economically disastrous diseases are due to intracellular pathogens, and the production of effective vaccines against these pathogens has not been an easy task. As the demand for an increase in food quality continues and the side effects of antibiotics become an increasing threat to humans, antibiotic use must be eliminated or reduced and replaced with newly developed products. These new products could enhance environmental protection by acting in a manner more conducive to the health of humans as well as animals [24].

The manipulation and control of fish health and production by natural substances has been identified as an important area for future developments in aquaculture. Immunostimulants are dietary additives which enhance the innate (non-specific) defence mechanisms and increase resistance to specific pathogens. An immunostimulant is defined as a chemical, drug, stressor or action that enhances the innate or non-specific immune response by interacting directly with cells of the immune system and activating them. Immunostimulants can be grouped under chemical agents, bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines [27]. As there is no memory component, the response is likely to be of short duration. Use of the immunostimulants is an effective means of increasing the immunocompetency and disease resistance of fish. The term immunostimulant applies to any compound that modulates the immune system by increasing the host's resistance to disease. Immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Several immunostimulants also stimulate lysosomes and the antibody responses of fish [27].

The pathogens that have already been controlled successfully by using immunostimulants in fish/shrimp, include bacteria *Aero monas salmonicida, A.hydrophila, Vibrio Anguilla rum, V. vulnificus, V. salmonicida, Yersinia ruckeri, Streptococcus* spp.; viruses causing infectious such as hematopoietic necrosis, yellow head virus, viral hemorrhagic septicemia and the parasite like *Ichthyophthirius multifiliis* [4].

2. Nutritional factors and the immune response

It is well known that nutrition may affect fish health and immune responses. Nutritional factors are components of the diet essential for normal growth and development of the fish. Many of these undoubtedly have roles in the immune response. Vitamins C, B6, E and A, and the minerals iron and fluoride have been identified as micronutrients that can affect disease resistance. With respect to the strict definition of immunostimulants; vitamins and minerals are not immunostimulants as they enhance the immune system by providing substrates and serving cofactors necessary for the immune system to work properly, but controversy exists [27,31]. Nutrients, essential or non-essential, either singly or in combination, directly or indirectly can influence immune functions and fish health. Amino acids have a central role in the defence mechanisms since they are involved in the synthesis of an array of proteins such as antibodies and in the control of key immune regulatory pathways. Studies on different fish species have revealed that dietary nucleotide supplementation enhanced their resistance to parasites, bacteria and virus. Several investigations have revealed that dietary fat is essential for the health of warm-blooded animals. It has been found that the deficiency of vitamin C leads to immune suppression and susceptibility to infectious diseases. Besides, it has been studied that the inadequate amounts of dietary vitamin E results in non-specific cell degenerative conditions.

3. Various Immunostimulants used in Aquaculture

3.1 β-Glucans

Glucans mainly exists in the cell walls of bacteria and yeast and possess a helical or spiral backbone due to specific intramolecular hydrogen bonding. Glucans are recognized by the immune system of aquatic animals as a foreign molecular pattern. The application of glucans has been extensively studied in aquatic animals, and findings indicate that b-glucans promote growth in certain types of aquatic animals. Glucans are high molecular-weight substances composed of glucose as building blocks, usually isolated from cell walls of bacteria, mushrooms, algae, cereal grains, yeast and fungi. Glucans in general comprise a great variety of substances common in nature (such as cellulose, glycogen, and starch), most of which do not interact with the immune system. Pharmacologically, they are classified as biological response modifiers (BRM). The common feature of immunomodulatory glucans is a chain of glucose residues linked by β-1.3-linkages, also called beta-glucans. Of the different β-glucans, the products known as β-1.3/1.6-glucans, derived from baker's yeast, are suggested to be the most potent immune-system enhancers. β-1.3/1.6-glucans is characterized by side-chains attached to the backbone that radiate outward like branches on a tree. The frequency and nature of side-chains strongly affect the ability of the glucan to mediate binding to surface receptors on the target cells influencing the effectiveness of the glucan as an immunostimulant.

Lopez, Cuzon, Gaxiola, Taboada, Valenzuela, Pascual, Sanchez and Rosas [20] demonstrated that β-glucans increase the growth rate of *Litopenaeus vannamei* juveniles when added to feed. Additionally, Ai, Mai, Zhang, Tan, Zhang, Xu and Li [1] demonstrated that a basal diet supplemented with 0.09% β -glucans significantly enhances the growth of large yellow croakers. Dalmo and Bogwald [11] argued that β -glucans improve intestinal immune responses, increasing disease resistance in aquatic animals and promoting their growth. Many studies indicate that b-glucans promote the growth of aquatic animals in relation to the amount included in the feed, the duration of feeding, culture temperature and species being raised. β -glucans activate phagocytic cells in fish, improving phagocytosis and the ability of the cells to kill pathogenic organisms [1]. They also increase lysozyme and complement activity [1] in the serum of fish and enhance the resistance of fish to pathogens in the water [26]. Glucans are also believed to modulate innate immunity by binding to specific receptors on monocyte/macrophages, neutrophils and natural killer (NK) cells the role and potential influence of β-glucans on immune-related gene and protein expression in different fish species have been reported by many authors.

3.2 Bioactive alginate (high-M alginate) and Ergosan

The adaptive immune system is poorly developed in early developmental stages of fish, which is the reason that immunostimulants and probiotics have been used in an attempt to increase survival of larvae against microbial pathogens. In this respect, alginate has been proposed as a potential candidate. Alginate is a polysaccharide composed of β-1, 4-D-mannuronic acid (M) and C5-epimer α-Lglucuronic acid (G). The monomers are usually arranged in M-blocks, G-blocks and alternating MG-blocks. Alginate also binds various cations found in the seawater such as Mg^{2+}, Sr^{2+}, Ba^{2+}, and Na^{+}. Commercial alginates are extracted from three species of brown algae; *Laminaria hyperborean*, *Ascophyllum nodosum* and *Macrocystis pyrifera*, in which alginate comprises up to 40% of the dry weight. Furthermore, bacterial alginates, have also been isolated from *Azotobacter vinelandii* and several *Pseudomonas* species.

Ergosan is an algal based product and contains 1 % alginic acid extracted from *Laminaria digitata*. The first study on Ergosan in aquaculture, was reported by Miles and coauthors on striped snakehead (Channa striata) [21]. In a study with rainbow trout, Peddie *et al*. reported that a single injection of 1 mg of Ergosan significantly augmented the proportion of neutrophils in the peritoneal wall, increased the degree of phagocytosis, respiratory burst activity and expression of interleukin-1β (IL-1β), interleukin-8 (IL-8) and one of the two known isoforms of tumour necrosis factor-alpha (TNF-α) in peritoneal leucocytes one day post-injection [23].

3.3 Plant extracts

Some immunostimulants cannot be used because of various disadvantages, such as high cost and limited effectiveness upon parenteral administration. Numerous plants have on the other hand long been used in traditional medicine for the treatment and control of several diseases. As herbs have little side effects and are easily degradable and abundantly available in farm areas, numerous investigations have investigated the effect of plant products on innate and adaptive immune response and to prevent and control fish and shellfish diseases

Dügenci and co-authors investigated the effects of mistletoe, nettle and ginger on dietary intake of rainbow trout [14]. The diets contained lyophilized extracts of these plants at two inclusions levels, 0.1 and 1%, in a 3 week experiment. At the end of the experimental period, various parameters of innate defence mechanisms, including extracellular and intracellular respiratory burst activities, phagocytosis in blood leukocytes, total plasma protein level, specific growth rates and condition factors were examined. Inclusion of the plant materials increased the extracellular respiratory burst activity ($P<0.001$) compared to control. Furthermore, fish fed the diet containing 1% ginger roots exhibited a significant innate immune response. Phagocytosis and extracellular burst activities of blood leukocytes were significantly higher in this group compared to the control group. All plant extracts increased plasma protein level except for the 0.1% ginger supplemented diet. The highest level of plasma proteins was observed in the group fed with 1% ginger extract.

Oral administration of the medical plant, Eclipta alba, on the non-specific immune responses and disease resistance of tilapia (*Oreochromis mossambicus*) has been investigated [9]. The results indicated that E. *alba* administrated in the diet significantly enhanced the non-specific immune parameters tested. Furthermore, when tilapia was challenged with Aeromonas hydrophila mortality was significantly reduced in E. *alba* treated fish.

Lectins are proteins or glycoprotein substances, usually of plant origin, and they are sugar-binding proteins which are highly specific for their sugar moieties. Lectins are also known to play important roles in the immune system by recognizing carbohydrates that are found exclusively on pathogenic bacteria, or that are inaccessible to host cells [12].

3.4 Animal extracts

Ete (Tunicate) and Hde (Abalone): Ete is an extract from the marine tunicate, *Ecteinascida turbinate* and Hde is a glucoprotein fraction of water extract from abalone, *Haliotis discus hannai*. It can enhance the killing of tumor cells *in vitro* and inhibits tumor growth *in vivo*. Ete (Tunicate) can enhance the phagocytosis and increased survival of Eel when injected against *A.hydrophila* [13]. In addition, when rainbow trout is injected with Hde against *V. anguillarum* infection showed increased survival along with enhanced phagocytic activites [28].

3.4.1 Firefly squid

Firefly squid, *Watasenia scintillans*, can stimulate the immune system of rainbow trout such as the production of superoxide anion, potential killing activities by macrophages and the lymphoblastic transformation of lymphocytes *in vitro*.

3.5 Chitin and Chitosan

Both chitin and chitosan have a major role in aquaculture. They are non-specific Immunostimulatory which are effective on a short term basis. Chitin is a polysaccharide which constitutes the principal component of exoskeletons of crustaceans and insects and cell walls of few fungi. It can stimulate macrophage activity and give resistance from certain bacteria [18]. Anderson and Swicki [3] administered chitosan to brook trout (*Salvenus fontinalis*) by injection and immersion and found that high levels of protection occurred 1, 2, 3 days afterwards, but protection was greatly reduced by day 14. Injection of chitosan was also more effective than simple immersion. Chitosan is a deacetylation product of chitin. The influence of chitosan on immune response of healthy and cortisol treated *Labeo rohita* was demonstrated. After treatment with chitosan sufficiently higher responses in almost all assays of non-specific immunity were observed in comparison to their healthy control or cortisol treated counterparts respectively without chitosan treatment [27]. In aquaculture, chitosan has been used as an immunostimulant for protection against bacterial disease in fish, for controlled release of vaccines, and as a diet supplement reveal that dietary β-glucan administration increases [6].

3.6 Vitamins used as Immunostimulants

3.6.1 Vitamin C

Vitamin C, also known as ascorbic acid, can be used as a hydrogen acceptor or hydrogen donor in animals. It cannot be synthesized in aquatic animals or obtained from food. Aquatic animals fed high doses of Vitamin C exhibit improved immunity and resistance to disease. Vitamin C plays an important role in several physiological functions including growth, resistance to infections, wound healing, response to various stressors and possibly lipid metabolism through its action on carnitine synthesis by administering with feed. Vitamin C (Ascorbic acid) is a co-factor in many biological processes including collagen synthesis and cellular functions related to neuromodulation, hormone and immune systems. It has been observed by Tewary and Patra [33] that higher levels of dietary vitamin C significantly increased the protection against A. hydrophila.

3.6.2 Vitamin E

Vitamin E, also known as tocopherol, is a group of biologically active phenolic compounds. The appropriate dose of Vitamin E can enhance the generation of antibodies and complement activity in response to antigens, promote the proliferation and differentiation of lymphocytes and cytokine production and improve cytotoxicity and phagocytosis. Vitamin E can enhance specific and cell-mediated immunity against infection in Japanese Flounder Paralichthys olivaceus [34] and macrophage phagocytosis in fish such as channel catfish *Ictalurus punctatus* [36] and turbot *Scophthalmus maximus*. Blazer and Wolke [5] found that Vitamin E deficiencies result in reduced protection in trout against *Y. ruckeri*.

3.7 Microorganisms

Probiotics, the beneficial microorganisms that enhance the utilization of food and the disease resistance of the host, optimize the surroundings of aquatic animals through the colonization of the microflora in the habitat of aquatic animals. Numerous types of probiotics have been applied in aquaculture, including *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Pseudomonas, Clostridium and Saccharomyces* spp. Screening probiotics is the first step for their application in aquaculture, and probiotics should have the following characteristics [15]: (i) antagonism to pathogens, which is one property of probiotic bacteria (ii) beneficial to the host animal; (iii) capable of surviving in or colonizing the gut of an aquatic organism via adhesion (iv) long-term stability under storage and field conditions; (v) non-pathogenic and non-toxic so that undesirable side effects are avoided when administered to aquatic organisms; and (vi) of an animal-species origin. The application of probiotics has been widely investigated in aquaculture, and probiotics are typically used as feed additives or are directly added to the water. Furthermore, probiotics improve the balance of digestive tract flora and the utilization rate of feed, promote the growth of aquatic animals, enhance immune function and disease resistance and optimize the quality of water by absorbing or degrading organisms or toxic substances [19]. Cao, Li, Yang, Wen and Huang [7] demonstrated that periodically releasing *Bacillus licheniformis* into ponds with Yellow sea bream *(Sparus latus)* can significantly reduce the concentration of nitrites and phosphates in the water, as well as the mass fraction of organic carbon in the sediment. Additionally, Dalmin, Kathiresan and Purushothaman [10] demonstrated that *Bacillus* can optimize the quality of water [35].

Table 1: Immunostimulants evaluated in fish and shrimp.

Synthetic chemicals	**Levamisole, FK-565 (Lactoyltetrapep tide from Streptomyces olvaceogriseus), Quaternary ammonium compounds (QAC).**
Biological substances	
1) Bacterial derivatives	MDP (Muramyl dipeptide from *Mycobacterium* species), Lipopolysaccharide (LPS), Freund's complete adjuvant (FCA), EF203 (fermented egg product), Peptidoglucan (from *Brevibacterium lacto fermentum* and *Vibrio* sp.), *Clostridium butyricum* cells, *Achromobacter stenohalis* cells, *Vibrio Anguilla rum* cells (*Vibrio* vaccine)
2) Yeast derivatives	b-1, 3 glucan, b-1, 6 glucan
3) Nutritional factors	Vitamin C and E, n-3 fatty acid
4) Hormones	Growth hormone, Prolactin, tri-iodothyronine
4) Cytokines	Interferon, Interleukin
5) Polysaccharides	Chitosan, Chitin, Lentinan, Schizophyllan, Oligosaccharide
6) Animal and plant extracts	Ete (tunicate), Hde (Abalone), Glycyrrhizin, Firefly squid, Quillaja, Saponin (Soap tree)
7) Others	Lactoferrin, Soyabean protein, Quil A, Spirulina, *Achyranthesaspera* (Herb), *Mucorcircinelloides* (Fungi)

4. Vaccines vis-a-vis Immunostimulants
Vaccination is an important tool in preventing infectious disease in humans and animals and both passive and active vaccinations are extensively employed in fish. It is a term that should strictly be applied only when the purpose is long lasting protection through immunological memory. A vaccine targets the specific immune response. It requires primary challenge with antigen and is dependent upon the clonally derived lymphocytes subsets to be implemented [30]. However, most commercial vaccine usually enhances resistance to only one or two specific pathogens and confers only a temporary resistance to disease. Immunostimulants by contrast, can boost immunity to a wide variety of pathogens, thus are nonspecific. Immunostimulations can be achieved in a more general sense by, for instance, targeting complement activation, phagocytosis and cytokines secretion, without necessary or purposefully requiring a specific response to a defined antigen. Examples include zymosan, glucans and lipopolysaccharides and these are best called as true immunostimulants. Comparisons of characteristics of vaccines vis-a-vis immunostimulants are given in Table 2.

Table 2: A comparison of characteristics of vaccines and immunostimulants.

Sl. No.	Vaccine	Immunostimulants
1.	Prophylactic for long duration with only one or two treatments.	Prophylactic for short duration, require more treatments.
2.	Efficacy of vaccine is excellent	Efficacy of immunostimulants is good
3.	Limited spectrum of activity.	Wide spectrum of activity
4.	No toxic side effects	No toxic side effects
5.	No accumulation of toxic residues	No accumulation of toxic residues
6.	No environmental impact	No environmental impact
7.	Enhance specific and nonspecific immune response	Mainly enhance nonspecific immune system of larvae before specific immune system matures.
8.	Difficult to vaccinate larvae of fish and shrimp	Easy to vaccinate larvae of fish and shrimp
9.	Costly	Cost effective

5. Method of administration
The immunostimulants potentiate the immunity of the host itself, enabling it to defend more strongly against pathogens. Several immunostimulants also stimulate the natural killer cells, complement, and lysozyme and antibody response of fish [17]. There are mainly three ways to deliver immunostimulants viz. injection, immersion and oral uptake. Injection of immunostimulants can produce strong non-specific response but it is costly affairs involving a lot of time and labour intensive as well, applicable only for large size of fish more than 10-15g in body weight in intensive aquaculture system. It has been reported that injection has wide protection against a range of pathogens like intra-peritoneal injection with glucan injected to channel catfish shows increased in phagocytic activity reducing fish mortality challenge with *Edwardsiella ictaluri* [29]. For small fish vaccination is impractical. Immersion produces less non-specific immune response, but more cost effective than injection, increase more stress to fish while handling, applicable in intensive culture system. Immersion method is very effective during acclimation of juveniles to ponds in field condition. Using immersion of levamisole showed increase in circulating leukocytes, phagocytic rate and increase protection against *P. damselae* sub s *P. piscicida* in European Seabass [8].
Oral ingestion produces good non specific immune response and can be the most cost effective method with economically

viability. It is mostly suited for extensive aquaculture systems. Immunostimulant powders are mixed with feed using a fish oil coating. Now a days, bio encapsulation method is also followed to immunize the fish larvae during their early larval stages with live fed organisms.

6. Timing of administration

It is very important to apply the immunostimulants at the right time. Anderson [2] proposed that the application of immunostimulants should be implemented before the outbreak of disease to reduce disease related losses. The *effective dosage* and *exposure time* will be further more complicated based on different culture systems with feeding regime [16]. In Atlantic salmon, injection with high dose of glucans @ 100 mg/ kg led to absence of protection for 1 week, but maximum benefits only occur after 3-4 weeks. Also, at low dose of injection @ 2-10 mg/kg, give protection only one week [25]. Similarly, it has been noticed that increase in the number of NBT positive cells in African Catfish fed with glucan or oligosaccharide over 30 days, but not over 45 days [32].

7. Mode of action

The mode of action of immunostimulants is to activate the immune systems of organisms, to enhance the immunity level against invading pathogens. The approach is very diverse in nature or may be poorly understood and also depends on the type of immunostimulants, dosage, route of administration, time and length of exposure. Following are some of the mechanism of actions:

- Stimulators of T-lymphocytes- Levamisole, Freund's Complete Adjuvant (FCA), Glucans, *Muramyl dipeptide*, FK-565 (Lactoyl tetrapeptide from *Streptomyces olivaceogriseus*).
- Stimulates of B-cells- Bacterial endotoxions, Lipopolysaccharides.
- Macrophage activator- Glucans, Chitin and Chitosan
- Inflammatory agents including chemotoxins
- Cell membrane modifiers- Detergents and Sodium dodecyl sulphate, Quaternary ammonium compounds (QAC), Saponins
- Nutritional factors- Vitamin C and E, n-3 fatty acids
- Cytokines- Leukotriene, Interferon
- Heavy metals- Cadmium
- Animal and fish extracts- Mitogens
- In general immunostimulants enhance the phagocytosis and bacterial killing ability of macrophage, complements, lymphocytes and nonspecific cytotoxic cells, resulting in resistance and protection to various diseases and invading microorganisms.

8. Evaluating the efficacy of immunostimulants

There are two main procedures for evaluating the efficacy of an immunostimulant: (i) *in vivo*, such as a challenge test using fish pathogens, and (ii) *in vitro*, such as the measurement of the efficiency of cellular and humoral immune mechanisms. Knowledge of the immune system is often very limited for most fish species, and information on the mode of action of most immunostimulatory substances is even more restricted. The evaluation of an immunostimulants based on *in vitro* methods that test the effects of that substance on the immune system is preferred in preliminary studies. Nevertheless, *in vitro* tests should be performed together with *in vivo* experiments, if possible, to elucidate the basic mechanisms responsible for any protection provided. *In vitro* evaluations should be based on at least one of the following parameters: serum lysozyme activity, complement activation, total leucocyte and erythrocyte counts, RBA phagocytosis, chemotaxis, chemokinesis and lymphocyte proliferation. Several other recommendations of parameters to monitor include C-reactive protein levels, natural cytotoxic activity and macrophage-activating factor (MAF) levels. Techniques involved in these evaluations range from relatively simple and inexpensive methods to the use of immunoassays, flow cytometry and other bio-molecular approaches.

9. Discussion

An alternative technique to prevent diseases is the strengthening of fish immune systems through the application of Immunostimulants which has proven to be one of the most promising methods. Immunomodulation by contrast, is a consequence of a change in the number or function of the cells involved in the immune response. The most proven effect of immunostimulants is to facilitate the function of phagocytic cells and increase their bactericidal and fungicidal activities. Immunostimulants can promote recovery from immunosuppressive states caused by any form of stress. Immunostimulants appear to be most promising and useful tools for prophylactic treatment of farmed fish and shell fish. It is safer than chemotherapeutics and their range of efficacy is wider than vaccination. However, these compounds will not replace vaccines, proper nutrition or good management techniques. The strength of these compounds appear to lie in their ability to enhance larval culture before the specific immune system matures and the animals can be vaccinated and able to improve non-specific immune function against a broad spectrum of pathogens.

Thus, application of immunostimulants to aquatic health management has immense potential, but in order to capitalize on this issue a lot of scientific research is necessary to understand the mode of action. Many of the *in vitro* tests are expensive to conduct, limiting the ability to rapidly screen potential immunostimulants regimens for efficacy. Additional research is needed to define the specific dosage rates and efficacy of various compounds for a variety of aquatic species and their pathogens and to decrease costs of the immunostimulants. It is expected that during coming years immunostimulants will find more application to make aquaculture sustainable.

However, the development of effective immunostimulants should be approached by combining the search for biological factors from animals together with the application of genetic engineering that maximizes the immunogenicity for a desired immune response. These immunostimulants may trigger specific immunological processes without producing a generalized response with strong side effects. The gene expression of biological factors used as immunostimulants within an animal has the following advantages: the factors (i) are stable and efficient immune-enhancing molecules; (i) they contain a specific antibacterial spectrum; (iii) they are harmless to the host; and (iv) drug resistance is unlikely to occur. New immunostimulants have the potential to become alternative bioactive agents, possibly replacing traditional antibiotics, and future research may provide new immunostimulant sources and ideas for their application. Therefore, immunostimulants developed via gene engineering may become an important new commodity in aquaculture as a candidate for use in new applications.

9. References

1. Ai Q, Mai K, Zhang L, Tan B, Zhang W, Xu W, *et al*. Effects of dietary β1,3-glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea.* Fish and Shellfish Immunology. 2007; 22:394-402.
2. Anderson DP. Immunostimulants, adjuvant, and vaccine carriers in fish: applications to aquaculture. Ann Rev Fish Dis. 1992; 2:281-307.
3. Anderson DP, Siwicki, AK. Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. Prog. Fish Culturist. 1994; 56:258-261.
4. Barman D, Nen P, Mandal SC, Kumar V. Immunostimulants for Aquaculture Health Management. J Marine Sci Res Dev. 2013; 3:134.
5. Blazer VS, Wolke RE. The effects of α-tocopherol on the immune response and non-specific resistance factors of rainbow trout (*Salmo gairdneri* Richardson). Aquaculture. 1984; 37:1-9.
6. Bullock G, Blozer V, Tsukunda S, Summerfelt S. Toxicity of acidification of chitosan for cultured rainbow trout (*Oncorhynchus mykiss*). 2000; 185(3-4):272-280.
7. Cao YC, Li ZJ, Yang YY, Wen GL, Huang HH. Effects of *Bacillus licheniformis* strain de on growth of *Sparus latus* and main environmental factors in aquaculture pond. South China Fisheries Science. 2010; 6:1.
8. Chen D, Ainsworth AJ. Glucan administration potentiates immune defense mechanisms of channel catfish, *Ictalurus punctatus* Rafinesque. J Fish Dis. 1992; 15:295-304.
9. Christybapita D, Divyagnaneswari M, Michael RD. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish Shellfish Immunol. 2007; 23:840-852.
10. Dalmin G, Kathiresan K, Purushothaman A. Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem. Indian Journal of Experimental Biology. 2001; 39:939-942.
11. Dalmo RA, Bogwald J. IB-glucans as conductors of immune symphonies. Fish and Shellfish Immunology. 2008; 25:384-396.
12. Dam TK, Brewer CF. Lectins as pattern recognition molecules: The effects epitope density in innate immunity. Glycobiol. 2010; 20:270-279.
13. Davis JF, Hayasaka SS. The enhancement of resistance of the American eel, *Anguilla rostrata* Le Sueur, to a pathogenic bacterium *Aeromonas hydrophila*, by an extract of the tunicate *Ecteinasci diaturbinate*. Journal of Fish Diseases. 1984; 7:311-316.
14. Dügenci SK, Arda N, Candan A. Some medicinal plants as immunostimulants for fish. J Ethnopharmacol. 2003; 88:99-106.
15. Farzanfar A. The use of probiotics in shrimp aquaculture. FEMS Immunology and Medical Microbiology. 2006; 48:149–158.
16. Galeotti M, Volpatti D, Jeney G. The nature of non-specific immune response of sea bass (*Dicentrarchuslabrax*) to *Pasteurella piscicida* following bath exposure to levamisole. European Assoc Fish Pathologist Seventh Int Conf Palma de Mallorca, Spain. 1995.
17. Johansson MW, Soderhall K. Cellular immunity in crustaceans and the pro PO system. Parasitol Today. 1989; 5:171-176.
18. Kawakami H, Shinohara N, Sakai M. The non-specific immunostimulation and adjuvant effects of *Vibrio anguillarum* Bacterin, M-glucan, Chitin and reund's complete adjuvants against *Pasteurella piscicida* infection in Yellowtail. Fish Pathol. 1998; 33:287-292.
19. Liu J, Song XL, Chen ZX. Progress on the function of probiotics in Aquaculture. Progress in Veterinary Medicine. 2009; 30:78.
20. Lopez N, Cuzon G, Gaxiola G, Taboada G, Valenzuela M, Pascual C, *et al*. Physiological, nutritional, and immunological role of dietary b 13 glucan and ascorbic acid 2-monophosphate in *Litopenaeus vannamei* juveniles. Aquaculture. 2003; 224:223-243.
21. Miles DJC, Polchana J, Lilley JH, Kanchanakhan S, Thompson KD, *et al*. Immunostimulation of striped snakehead *Channa striata* against epizootic ulcerative syndrome. Aquaculture. 2001; 195:1-15.
22. Misra CK, Das BK, Mukherjee SC, Pattnaik P. Effect of multiple injections of Beta-glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings. Fish and Shellfish Immunology. 2006; 20:305-319.
23. Peddie S, Zou J, Secombes CJ. Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. Vet Immunol Immunopathol. 2002; 86:101-113.
24. Quesada SP, Paschoal JAR, Reyes FGR. Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones–a review. Journal of Food Science. 2013; 78:1321-1333.
25. Robertsen B, RØrstad G, Engstad R, Raa J. Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. J Fish Dis. 1990; 13:391-400.
26. Sahoo PK, Mukherjee SC. Effect of dietary β-1,3 glucan on immune responses and disease resistance of healthy and aflatoxin B1-induced immunocompromised rohu (*Labeo rohita* Hamilton). Fish and Shellfish Immunology. 2001; 11:683-695.
27. Sakai M. Current research status of fish immunostimulants. Aquaculture. 1999; 172:63-92.
28. Sakai M, Kamiya H, Atusta S, Kobayashi M. Immunodulatory effects on rainbow trout, *Oncorhynchus mykiss*, injected with the extract of abalone, *Haliotis discushannai*. J *Appl Ichthyol.* 1991; 7:54-59.
29. Smith VJ, Söderhäll K, Hamilton M. ß-1.3-glucan induced cellular defense reaction in the shore crab, *Carcinusmaenas*. Comp Biochem Physiol. 1984; 77:636-639.
30. Smith VJ, Brown JH, Hauton C. Immunostimulation in crustaceans: does it really protect against infection? Fish Shellfish Immunol. 2003; 15:71-90.
31. Suchner U, Kuhn KS, Fürst P. The scientific basis of immunonutrition. Proc Nutr. 2000; 59:553-563.
32. Sung HH, Kou GH, Song YL. Vibriosis resistance induced by glucan treatment in tiger shrimp (*Penaeus monodon*). Fish Pathol. 1994; 29:11-17.
33. Tewary A, Patra BC. Use of Vitamin C as an immunostimulant- effect on growth, nutritional quality, and immune response of *Labeo rohita* (Ham.). Fish Physiol Biochem. 2008; 34:251-259.
34. Villegas JG, Fukada H, Masumoto T, Hosokawa H. Effect of dietary immunostimulants on some innate immune responses and disease resistance against *Edwardsiella tarda* infection in Japanese Flounder

(*Paralichthys olivaceus*). Aquaculture Sci. 2006; 54:153-162.
35. Wang Y, Han J. The role of probiotic cell wall hydrophobicity in bioremediation of aquaculture. Aquaculture. 2007; 269:349-354.
36. Wise DJ, Tomasso JR, Gatlin DM, Bai SC, Blazer VS. Effects of dietary selenium and vitamin E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. J Aquat Anim Health. 1993; 5:177-182.