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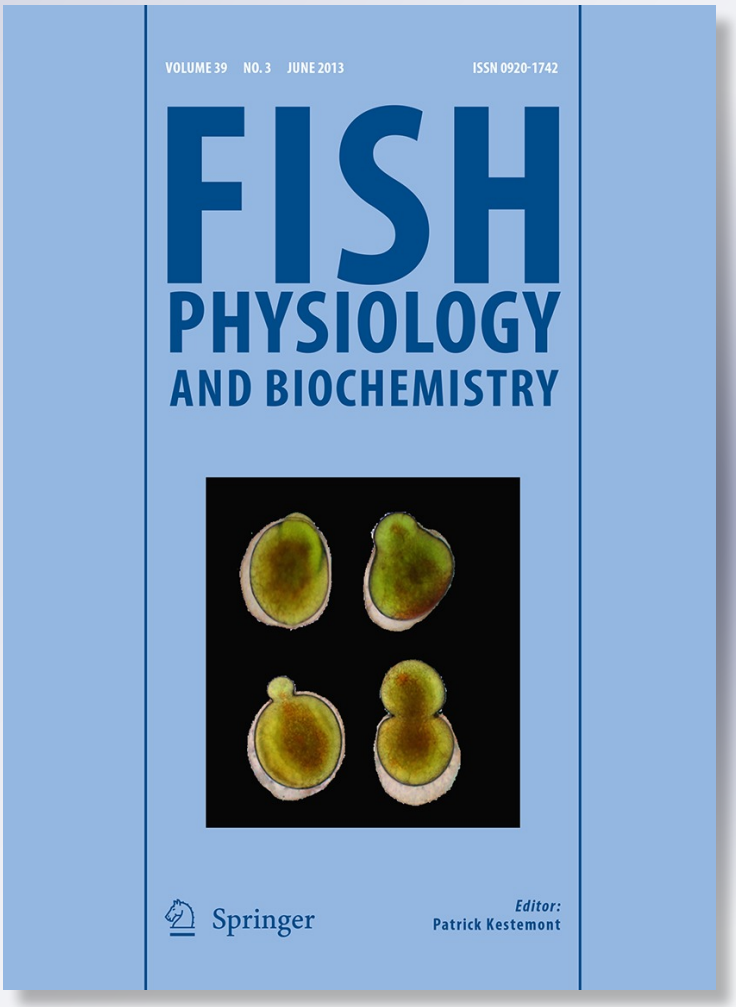

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Beta-glucan: an ideal immunostimulant in aquaculture (a review)

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Abstract The major hindrance in the development and sustainability of aquaculture industry is the occurrence of various diseases in the farming systems. Today, preventive and management measures are central concern to overcome such outbreak of diseases. Immunostimulants are considered as an effective tool for enhancing immune status of cultured organisms. Among different immunostimulants used in aquaculture practices, β-glucan is one of the promising immunostimulant, which is a homopolysaccharide of glucose molecule linked by the glycoside bond. It forms the major constituents of cell wall of some plants, fungi, bacteria, mushroom, yeast, and seaweeds. Major attention on β-glucan was captivated with the gain in knowledge on its receptors and the mechanism of action. The receptor present inside the animal body recognizes and binds to β-glucan, which in turn renders the animal with high resistance and enhanced immune response. This review highlights β-glucan as an immunostimulant, its effective dosages, and route of administration and furthermore provides an outline on role of β-glucan in enhancing growth, survival, and protection against infectious pathogens pertaining to fishes and shellfishes. Study also summarizes the effect of β-glucan on its receptors, recognition of proteins, immune-related enzymes, immune-related gene expression and their mechanisms of action.

Keywords β-Glucan · β-Glucan receptor · β-Glucan binding protein · Prophenoloxidase · Immunostimulant · Aquaculture · Prebiotics

D. K. Meena, Pronob Das, Shailesh Kumar have contributed equally to this work.

D. K. Meena · P. Das (✉) · B. K. Behera
Central Inland Fisheries Research Institute, Barracklpore, Kolkata 700120, West Bengal, India
e-mail: pronobjaan80@gmail.com

S. Kumar · S. K. Singh · K. Kumar · A. K. Pal · S. C. Mukherjee
Central Institute of Fisheries Education, Seven Bungalow, Versova, Mumbai 400061, India

S. C. Mandal
College of Fisheries, Central Agricultural University, Lembucherra 799210, Tripura, India

A. K. Prusty
Project Directorate for Farming System Research (PDFSR), Modipuram, Meerut 250110, UP, India

M. S. Akhtar
Directorate of Coldwater Fisheries Research, Bhimtal, Nainital 263136, Uttarakhand, India

Abbreviations

BG	β-Glucan
LGBP	Lipopolysaccharide and β-glucan binding protein
proPO	Prophenoloxidase
BGR	β-Glucan receptor
LPS	Lipopolysaccharide
TLR	Toll like receptor
WSSV	White spot syndrome virus
RPS	Relative percent survival
FED	Feed efficiency ratio
YYS	Yeast and yeast subcomponents
YCW	Yeast cell wall
YBG	Yeast β-glucan
BYG	Brewer's yeast glucan
CMBG	Carboxymethyl β-glucan
THC	Total hemocyte count
BGBP-HDL	β-Glucan binding protein–high density lipopolysaccharide

Introduction

Intensification of aquaculture practices leads to the emergence of several pathogenic organisms. Rapid and uncontrolled growth of pathogens in aquatic organisms and indiscriminate use of antibiotics to prevent them have resulted in the emergence of several resistant pathogens in aquaculture. Presently, these two factors are the most important concerns for both researchers and farmers. Diseases caused by infectious microorganisms are known to be one of the major constraint in the aquaculture industry for past many years (Scholz et al. 1999) and are impeding the development and sustainability of the industry throughout the world (Bondad-Reantaso et al. 2005). Thus, there is a need to devise suitable tools to control the disease outbreaks in this sector. The concept of functional feed is an emerging paradigm in aquaculture industry to develop diets of balanced nutrition supplemented with feed additives for improving the health and disease resistance of cultured fishes (Gatlin and Li 2004). There is an increased concern over the usage of antibiotics, although some reports have shown that antibiotics may improve growth and feed efficiency by killing the microflora of intestine, thus resulting in enhanced utilization of amino acid by the host organism (Rawles et al. 1997). Use of antibiotics poses considerable threats to the aquatic microorganisms prone to development of antibiotic resistance and accumulation of antibiotic residue from animals of lower food chain to higher one including humans (FAO 2002). These concerns prompted the ban on use of such therapeutics in Europe, USA (Patterson and Burkholder 2003), paving the way in searching for new avenues and alternatives to replace antibiotic use against disease outbreak. Alternative strategies such as use of vaccine, dietary supplement of probiotics, prebiotics, and immunostimulant may help to reduce the susceptibility of fish to diseases. Immunostimulants are the modern and primary tools in aquaculture that help in enhancing resistance against infectious diseases by enhancing innate humoral and cellular defense mechanisms. Various kinds of substances have been used and their suitability as immunostimulant has been studied, but only few of them are found suitable for use in aquaculture (Raa et al.1992; Siwicki et al. 1998). In recent years, many review articles have been published explaining different aspects of relationship between immunostimulants and innate immunity system in fish (Galeotti 1998; Gannam and Schrock 1999). Sohn et al. (2000) discussed the role of immunostimulants in monogastric animals and fishes in details. Efficiencies and dose–effect relationship of immunostimulants in marine fishes have been reviewed by Galindo-Villegas and Hosokawa (2004). Immunostimulants and their biological effects pertaining to fish larval aquaculture were explained by Bricknell and Dalmo (2005), whereas Magnadóttir (2006) gave an overview of the ontogenic development of non-specific immune system with respect to different innate factors influenced by immunostimulants. Later on, Sahoo (2007) and Magnadóttir (2010) described immunological control of fish diseases. More recently, immunological control of fish diseases was discussed by Ringø et al. (2012), in which the authors have discussed in detail about the inherent and external factors affecting fish diseases. Use of prebiotics is limited in aquaculture, which is gaining importance slowly along with immunostimulants. Ganguly et al. (2010) summarized the use of prebiotics and probiotics as immunostimulants in aquaculture.

Various studies in fishes have proven β-glucan as a potent, valuable, and promising immunostimulant for improving immune status and controlling diseases in fish culture (Robertsen et al. 1994; deBaulny et al. 1996; Anderson 1997; Figueras et al. 1998; Kawakami et al. 1998; Robertsen 1999). The immunomodulatory properties of β-glucans were first demonstrated in

mammals wherein induced haemopoiesis and enhanced immunity were observed resulting in an increased resistance to infectious pathogens (Di Luzio 1985). Volman et al. (2008) outlined a broader picture of immunomodulating effects of β-glucans in animals and humans. Soltanian et al. (2009) summarized β-glucan application in vertebrates (mice, guinea pig, rat, pig, sheep, cattle, fish and rabbit) and invertebrates (shrimp and artemia). In recent years, attention has been focused on the use of β-glucan in fishes. Many studies have been carried out on different fish species such as Atlantic salmon (Paulsen et al. 2001), rainbow trout (Jorgensen et al. 1993; Djordievic et al. 2009), Snapper (Cook et al. 2003), African catfish (Yoshida et al. 1995), Prawn (Hai and Fotedar 2009), and Sea bass (Bagni et al. 2005, 2008; Bonaldo et al. 2007). These studies have shown the effect of β-glucan on the growth (Cook et al. 2003; Misra et al. 2006b), survival, resistance, and protection against pathogen (Welker et al. 2007; Sealey et al. 2008), antibody production (Selvaraj et al. 2005; Kamilya et al. 2006), immune-related gene expression (Løvoll et al. 2007; Zhang et al. 2009), and as adjuvant (Rørstad et al. 1993; Kawakami et al. 1998) in wide range of fish species. Considering the multifaceted role of β-glucans in fish immune system, a comprehensive and updated review of effect of various sources of β-glucan through different routes of administration, alone or in combination with other immunostimulants pertaining to fish and shellfishes of both freshwater and marine origin, is presented here. This review provides an in-depth discussion on various roles played by β-glucan in fish and shellfish immunology. Use of β-glucan as immunostimulant in aquaculture (fish and shellfishes) has been summarized in Table 1.

Types, sources, and structure of β-glucans

β-Glucans are naturally occurring polysaccharides with glucose as structural component, linked by β-glycosidic bonds. In nature, β-glucans are wide spread in the cell wall of many plants (wheat, rye, barley, and oat), baker's and brewer's yeast (*Saccharomyces* genus), and Echinaceae members (Tokunaka et al. 2000). Other sources of β-glucan include seaweed like *Laminaria* sp. (Teas 1983), various species of mushrooms such as Shiitake (*Lentinus edodes*), Maitake (*Grifola frondosa*), Reishi (*Ganoderma lucidum*) (Wasser and Weis 1999), Schizophylan (*Schizophyllum commune*), and SSG (*Sclerotinia sclerotiorum*) (Brochers et al. 1999), and certain fungi (*Agaricus subrufesuns*). β-glucans are also the structural constituents of some of the pathogenic fungi, *Pneumocystis carini* (Lebron et al. 2003), *Cryptococcus neoformans* (Reese et al. 2007), and some bacteria belonging to Rhizobiaceae family (Breedveld and Milleri 1994). The common sources of β-glucan are derived from the cell wall of baker's yeast *Saccharomyces cerevisiae* and the most important among all are β-1, 3 and 1, 6 glucan. Glucans are heterogeneous group of glucose polymers, consisting of a backbone of β (1, 3)-linked β-D-glucopyranosyl units with β-(1, 6)-linked side chains of varying distribution and length. β-glucans derived from different sources have differences in their structure. Oat and barley β-glucans are linear with β (1, 4) and (1, 3) linkages. Mushrooms β-glucans have short β (1, 6)-linked branches from β (1, 3) backbone. Yeast β-glucans have β (1, 6) branches further with additional β (1, 3) regions. These structural differences can trigger difficulties in extraction and differences on their activity. Larger molecular weight glucans activate leukocytes, stimulating their phagocytic, cytotoxic, and antimicrobial activities, and production of reactive oxygen species (ROS). Low molecular weight glucans have less cellular effects, whereas very short glucans are considered as inactive (Akramiene et al. 2007). Studies have shown that insoluble (1, 3/1, 6) β-glucans have greater biological activity than that of its soluble (1, 3/1, 4) counterparts (Ooi and Liu 2000).

Basically, glucan molecules are of two types on the basis of glycosidic bonds present in them, that is, α-glucan (dextran with 1,6, starch with α-1,4- and α-1,6-glycosidic bonds) and β-glucan (cellulose with β-1,4, zymosan with β-1,3, laminarin with β-1,3- and β-1,6, lichenin with β-1,3 and β-1,4 glycosidic bond). Because of complex structure in β-glucans, they have superior ability to activate the immune response and act as biological response modifiers (BRM) (Miura et al. 1996). Certain characteristics of this glucan such as ability to function normally on immune system without over activating them (Chihara 1992), ability to lower the elevated levels of cholesterol (Behall et al. 1997; Bell et al. 1999; Braaten et al. 1994), and ability to reduce sugar levels (Wood 1990; Pick et al. 1996) make it unique among immunostimulants.

Table 1 Use of different β-glucan in aquaculture

Sl. no.	Species	Glucan	References
1.	Nile tilapia (*Oreochromis niloticus*)	Yeast and yeast subcomponent	Shelby et al. (2009)
2.	Carp (*Labeo rohita*)	Yeast cell wall	Pal et al. (2007)
3.	Pacific white shrimp (*Litopenaeus vannamei*)	Inactive yeast cell wall	Chotikachinda et al. (2008)
4.	Gilthead seabream (*Sparus aurata* L.)	Whole yeast cell	Cuesta et al. (2007)
5.	Black tiger shrimp (*Penaeus monodon*)	Brewers yeast (β-glucan)	Suphantharika et al. (2003)
6.	Channel catfish (*Ictalurus punctatus*)	YYS/whole cell	Welker et al. (2007)
7.	Black tiger shrimp (*Penaeus monodon*)	Crudlan,zymosan(β-1,3-glucan)	Sritunyalucksana et al. (1999)
8.	Red tail Black shark (*Epalzeorhynchos bicolor*)	MacroGard + Aquagen	Russo et al. (2006)
9.	Pacific white shrimp (*Litopenaeus vannamei*)	β-1,3-Glucan + ascorbic acid	Lopez et al. (2003)
10.	Tiger shrimp (*Penaeus monodon*)	β-Glucan +*Vibrio bacterian*	Pais et al. (2008)
11.	Large yellow croaker (*Pseudosciaena crocea*)	β-1,3-Glucan	Ai et al. (2007)
12.	European sea bass (*Dicentrarchus labrax*)	β-1,3,1,6-Glucan	Bonaldo et al. (2007)
13.	Fathead minnows (*Pimephales promelas*)	β-1,3,1,6-Glucan	Palic et al. (2006)
14.	Atlantic salmon (*Salmo salar L*)	β-1,3,1,6-Glucan (yeast)	Rørstad et al. (1993)
15.	Rainbow trout (*Oncorhynchus mykiss*)	β-1,3,1,6-Glucan (yeast)	Verlhac et al. (1998)
16.	Atlantic cod (*Gadus morhua* L.)	β-1,3,1,6-Glucan	Skjermo et al. (2006)
17.	White shrimp (*Litopenaeus vannamei*)	β-1,3-Glucan (*S. commune*)	Wang et al. (2008)
18.	Black tiger shrimp (*Penaeus monodon*)	Carboxymethyl β-1,3-glucan	Klannukarn et al. (2004)
19.	Black tiger shrimp (*Penaeus monodon*)	β-1,3-Glucan (laminarin)	Sritunyalucksana et al. (2002)
20.	Black tiger shrimp (*Penaeus monodon*)	β-1,3-Glucan (brewers yeast)	Thanardkit et al. (2002)
21.	White shrimp (*Penaeus vannamei*)	β-1,3-Glucan (yeast)	Scholz et al. (1999)
22.	Kuruma shrimp (*Penaeus japonicus*)	β-1,3-Glucan (yeast)	Namikoshi et al. (2004)
23.	Kuruma shrimp (*Penaeus japonicus*)	β-1,3-Glucan (*S. commune*)	Itoh (1997)
24.	Black tiger shrimp (*Penaeus monodon*)	β-1,3-Glucan (*S.commune*)	Chang et al. (1999, 2000, 2003)
25.	Rohu (*Labeo rohita*)	β-1,3-Glucan	Sahoo and Mukherjee (2001, 2002)
26.	Asian catfish (*Clarias batrachus*)	β-1,3-Glucan (yeast)	Kumari and Sahoo (2006a, b)
27.	Carp (*Cyprinus carpio*)	β-1,3-Glucan (yeast)	Selvaraj et al. (2005)
28.	Rainbow trout (*Oncorhynchus mykiss*)	β-1,3-Glucan (laminaran)	Løvoll et al. (2007)
29.	Carp (*Cyprinus carpio*)	β-1,3-Glucan + LPS	Selvaraj et al. (2006)
30.	Indian white shrimp (*Fenneropenaeus indicus*)	Alkali soluble and insoluble β-1,3-glucan	Anas et al. (2009)
31.	Carp (*Cyprinus carpio*)	β-Glucan (yeast)	Selvaraj et al. (2005)
32.	Zebrafish (*Danio rerio*)	β-Glucan(*S. cerviciaea*)	Rodríguez et al. (2009)
33.	Rainbow trout (*Oncorhynchus mykiss*)	β-Glucan (barley)	Sealey et al. (2008)
34.	Sea bass (*Dicentrarchus labrax*)	Yeast β-glucan (Macrogard)	Bagni et al. (2005)
35.	Nile tailapia (*Oreochromis niloticus*)	β-Glucan	Whittington et al. (2005)
36.	Pink snapper (*Pagrus auratus*)	Ecoactiva	Cook et al. (2003)
37.	Catla (*Catla catla*)	Mushroom glucan	Kamilya et al. (2006)
38.	Indian white shrimp (*Fenneropenaeus indicus*)	Marine yeast glucan (Candida sake S165)	Sajeevan et al. (2009)
39.	Black tiger shrimp (*Penaeus monodon*)	Bakers yeast (*S. cerviciae*)	Huang and Song (1999)

Mechanism of action of beta-1,3/1,6-glucan

β-Glucan plays important role in the activation of both innate and acquired immune functions. Innate immune responses stimulated by β-glucans not only act on invading microorganisms but also complement the activation and action of acquired immunity (Sakai 1999). β-glucans are responsible for a multitude of actions which protect and enhance the immune system and provide optimum resistance to any possible health assailants due to its ability to bind directly with macrophages and other white blood cells (neutrophils and natural killer (NK) cells) and to activate them (Gantner et al. 2003; Herre et al. 2004). Macrophage cells are one of the principal cell types involved in natural immunity. When β-glucan receptors are engaged by beta 1,3/1,6 glucans, all immune functions are improved, including phagocytosis (ability to engulf foreign cells and particles), release of certain cytokines (intercellular hormones) IL-1, IL-6, GM-CSF, interferons, and the processing of antigens. These cytokines stimulate formation of new white blood cells (WBC) thus providing immunity to β-glucan binding receptors present in all vertebrates ranging from fish to human. Fishes have both specific and non-specific defense mechanism. The activated phagocytic cells and WBC (B and T cells) produce cytokines and antibodies, respectively, and enhance the efficacy of vaccines (Raa 2000).

Crustacean immune systems have the ability to detect foreign components such as LPS and β-glucan present in the cell wall of microorganisms. In shrimp, specific binding proteins to carbohydrate moieties are present in serum as recognition proteins that activate the cellular functions when they react with the microbial LPS or β-glucan (Vargas-Albores and Yepiz-Plascencia 2000). These microbial components activate the functions of encapsulation, coagulation, melanization, and phagocytosis associated with defense mechanism. β-glucan binding proteins interact with β-glucan molecule and form a complex of BGBP-BG that reacts with the hemocyte surface and releases the hemocytic granules which in turn leads to the activation of proPOs (Vargas-Albores et al. 1996). Johansson et al. (1999) have cloned and characterized the cellular receptors present on the surface of hemocyte. The hemocyte granule plays important role in storing the proPOs enzyme and its release by exocytosis. The enzyme promotes the oxidation of phenol to *o*-phenols to quinines, which have the ability to kill the infectious pathogens (Soderhall et al. 1994).

β-Glucan receptors

Induction of cellular response by β-glucan is associated with specific interactions of one or more cell surface receptors. Glucans are believed to modulate innate immunity by binding to specific receptors on monocyte/macrophages, neutrophils, and natural killer (NK) cells (Muller et al. 2000). Different β-glucans are associated with different or same type of receptors. β-glucan receptor was first identified as opsonin-independent receptor for the activation of alternative complement activation pathway (Czop and Austen 1985). They bind to pattern recognition receptors and modulate innate immunity by activating the macrophage cells. Available reports suggest that there are multiple glucan binding sites on macrophages but the nature of the glucan receptors and exact mechanism for modulating innate host defenses are not clear. Progress made to understand the process of interactions of glucan and its receptors includes binding of type 3 complement receptor (CR3) to glucans by Vetvicka et al. (1996), report of lactosylceramide a glucan binding moiety on immunocytes by Zimmerman et al. (1998), existence of non-CR3 glucan binding (Dectin-1) sites on human monocyte/macrophages and human dermal fibroblasts by Rice et al. (2002), reports on a water-soluble, polyanionic, carboxymethylated (CM) glucan binding to mouse peritoneal macrophages via scavenger receptors (SRs) by Vereschagin et al. (1998), reports of *Drosophila* SR binding to laminarin, a low molecular weight glucan and other microbial cell wall components by Pearson et al. (1995).

Important distinction between innate and adaptive immunity is the difference in their receptors that are responsible for immune recognition (Bendelac and Fearon 2000; Rice et al. 2002). Innate immunity is the first line of defense against microbial infection, where genetically pre-determined pattern recognition receptors (PRRs) recognize bio-molecules (carbohydrates, lipids, and proteins) present in the cell wall of microorganisms. This phenomenon is called as pathogen-associated molecular patterns (PAMPs). However, adaptive immunity requires somatically generated receptors that recognize antigenic patterns to which the host has been previously exposed (Peiser and Gordon 2001; Rice et al. 2002). Different types of β-glucan receptors are as follows:

Scavenger receptor

Based on structural characteristics, scavenger receptors are divided into three classes: SR-A, SR-B, and SR-C. Most of the SRs are non-opsonic receptors that display low-affinity and bind to many polyanionic and modified substances. They may bind to anionic β-glucans (sulphated β-glucans either made chemically or found in certain algae), but does not involve in β-glucan binding and internalization (Dennehy and Brown 2007).

Complement receptor

Complement receptor (CR3) belongs to the family β_2-integrin, a heterodimeric transmembrane glycoprotein, which consists of CD11b and a non-covalently associated CD18. These receptors are highly expressed on neutrophils, monocytes, and NK cells compared to macrophages (Thornton et al. 1996a). The CD11b subunit of CR3 has a functional domain A or I. These domains are important for phagocytosis and binding of iC3b-coated particles, while the other lectin domain located C-terminal to the I-domain is responsible for the non-opsonic binding properties of CR3. The leukocyte β_2-integrin also known as Mac-1, complement receptor type 3 (CR3), and CD11b/CD18 function both as adhesion molecule facilitating diapedesis and as CR3 enabling phagocytosis or degranulation in response to factor I-cleaved C3b fragment of C3 (iC3b)-opsonized microorganisms. The same lectin domain within CD11b regulates both the cytotoxic and adhesion functions of Mac-1/CR3. CR3 (Mac-1, CD11b/CD18, or $\propto$Mβ2-integrin) has been identified as the leukocyte membrane receptor for β-glucans (Thornton et al. 1996b). The role for CR3 in β-glucan-induced tumoricidal activity was also investigated by Vetvicka et al. (1996) who have reported that the soluble CR3-specific polysaccharides such as β-glucan induces a primed state of CR3 which could trigger killing of iC3b-target cells that were otherwise resistant to cytotoxicity.

Lactosylceramide

Lactosylceramide (LacCer; CDw17) is a glycosphingolipid found in the plasma membranes of many cells. Interaction of β-glucan with lactosylceramide receptor can induce macrophage inflammatory protein (MIP)-2 and activate NFkB, which can enhance the neutrophil oxidative burst and antimicrobial functions. It has been identified as a β-glucan receptor from biochemical analyses of the interactions between β-glucan and isolated human leukocyte membrane components (Zimmerman et al. 1998). It was found that lactosylceramide may bind specifically to β-glucan with concomitant production of reactive oxygen metabolites (Chen and Seviour 2007).

Dectin-1 (β-glucan receptor)

Dectin-1 (βGR) is a non-classical C-type lectin that predominantly binds protein ligands and is the primary PRR for glucans (Brown et al. 2003). βGRs have been considered to be the major β-glucan receptor (Dennehy and Brown 2007). It consists of single C-type, lectin like, carbohydrate recognition domain with a short stalk, and a cytoplasmic tail possessing an immunoreceptor tyrosine-based activation motif. It recognizes carbohydrates containing β-1,3 and/or β-1,6 glucan linkages and is expressed on cells of the monocyte/macrophages and neutrophils. Dendrite cells and a subpopulation of T cells are also known to express the βGR, but at a lower level (Taylor et al. 2002). Dectin-1, in association with TLR2, resulted in the activation of a macrophage's proinflammatory response to mycobacterial infection (Yadav and Schorey 2006). Structural relationship of glucan and Dectin-1 has been studied by Adam et al. (2008). They have reported that Dectin-1 is highly specific for glucans that have a (1 $\rightarrow$ 3)-β-D-glucopyranosyl backbone.

Toll-like B-glucan receptors

The innate immune system is an evolutionally conserved host defense mechanism initiated by pattern recognition receptors (PRRs). Among many PRRs, Toll-like receptors (TLRs) are capable of sensing organisms ranging from bacteria, fungi, protozoa to viruses. TLRs recognize pathogens either on the cell surface or in the lysosome/endosome compartment. Recently, cytoplasmic PRRs have been identified to detect pathogens that have invaded cytosols (Uematsu and Akira 2006). These type I transmembrane receptors identify microbial conserved structures or pathogen-associated molecular patterns (PAMPs). Recognition of microbial components by TLRs

initiates signaling transduction pathways and induces gene expression. These gene products regulate innate immune responses and further develop as an antigen-specific acquired immunity. TLR signaling pathways are regulated by intracellular adaptor molecules, such as MyD88, TIRAP/Mal, and provide specificity of individual TLR-mediated signaling pathways. TLR-mediated activation of innate immunity is involved not only in host defense against pathogens but also in the immune disorders (Arancibia et al. 2007). The cytoplasmic portion of a TLR is similar to that of the interleukin (IL)-1 receptor family. It is therefore called the Toll/IL-1 receptor (TIR) domain. A TIR domain is required for initiating intracellular signaling. Moreover, the extracellular region of TLRs and IL-1R are markedly different. However, IL-1R possesses an Ig-like domain where as TLRs contain LRRs in the extracellular domain. LRRs are responsible for the recognition of PAMPs (Akira et al. 2006). Fungal cells and zymosan consist of many different molecular moieties that bind to TLR2 and TLR4 facilitating myD88-dependent intracellular signaling that induce cytokines favoring Th1 cell differentiation (Romagne 2007).

Molecular characterization of BGR, BGBP, proPO in fish

β-Glucan receptors (BGR)

Toll-like receptor 4 (TLR4) is critical for the recognition of LPS, cellular responses, and some viral envelope proteins. Expression of zebra fish *TLR4*, *TLR2,* and *MyD88* gene was reported in the adult stage and was expressed at higher levels in fish infected with the pathogen *Mycobacterium marinum* (Meijer et al. 2004). The same gene from rare minnow, *Gobiocypris rarus* (GrTLR4b) expressed its mRNA in gill, heart, intestine, kidney, liver, muscle, and spleen tissues and was up-regulated after challenging with grass carp reovirus or *Aeromonas hydrophila* (Su et al. 2009a). TLR3 mRNA transcripts from grass carp (ciTLR3) were significantly up-regulated following GCRV infection (Su et al. 2009b) and its polypeptide sequences of subunits β2 (CD18) and aM (CD11b) in rainbow trout exhibited similarity with human, mouse, and zebrafish orthologs. The main source of the trout CD18 and CD11b-like mRNA transcripts was kidney (Mikrou et al. 2009). TLR9 family sense viral and bacterial DNA present in the endosomal compartment. The full-length *TLR9* cDNA was cloned from flatfish species, half-smooth tongue sole (*Cynoglossus semilaevis*) (CsTLR9) which was expressed in spleen and gonads and appeared to be developmentally regulated (Yu et al. 2009). The same receptor (TLR9) was cloned from Gilthead sea bream (*Sparus aurata* L.) and expressed in immune-related organs (spleen, head kidney) and mucosal–epithelial barriers (gills, gut, and skin) where infection with pathogen showed no difference in TLR9 expression compared with control (Franch et al. 2006). Japanese flounder, *Paralichthys olivaceus* TLR 9 cDNA highly expressed in epithelial and lymphoid organs. The mRNA copy number of TLR9 and its adapter protein, MYD88, were enhanced in blood, gill, kidney, and spleen after challenging with *Edwardsiella tarda* and expressed in kidney cells (Takano et al. 2007). TLR9 gene from Atlantic salmon (*Salmo salar*) showed elevated expression in head kidney leukocytes after in vitro treatment with CpG ODNs and recombinant trout interferon (IFN)-γ (Skjæveland et al. 2008).

The full-length TLR from goldfish (Stafford et al. 2003), constitutively expressed in macrophages, spleen, and kidney. Bacterial LPS, heat-killed *Aeromonas salmonicida,* and live *Mycobacterium chelonae* significantly up-regulate the TLR mRNA expression. Japanese flounder, *JF-TLR2* and *JF-TLR22* gene are homologs to human *TLR2* and fugu *TLR22* and mainly expressed in peripheral blood leukocytes (PBLs), kidney, spleen, and gill. Their expression was induced by both peptidoglycan and polyI:C, but number of *JF-TLR*-expressing cells did not change (Hirono et al. 2004). Atlantic salmon (*Salmo salar*) cloned SsTLR8 mRNA expression was reported as tissue-specific, with highest level in the spleen, and SsMyD88 in all the tested tissues. Their expression was up-regulated into cells treated with recombinant IFN a1 and IFN g. Expression of SsTLR8 had no effect following challenge with salmon alpha virus subtype 3 (SAV3) in vivo (Skjæveland et al. 2009). Full-length cDNA and gene sequences of TLR5S, TLR20, and TLR21 from catfish were reported to be single copy genes in catfish (Baoprasertkul et al. 2007).

Toll receptor gene (MjToll) from kuruma shrimp, *Marsupenaeus japonicus*, constitutively expressed in the gill, gut, lymphoid organ, heart, hematopoietic organ, hemocyte, ventral abdominal nerve cord,

eyestalk neural ganglia, and brain tissues. Peptidoglycan enhanced the expression (76-fold) compared to control in lymphoid organ, in vitro (Mekata et al. 2008). Partial Toll receptor gene of giant tiger shrimp, *Penaeus monodon*, expressed in gut, gill, and hepatopancreas on challenging with WSSV and showed clustering with Toll1 and Toll5 gene products (Arts et al. 2007).

Lipopolysaccharide and β-glucan binding protein (LGBP)

Pattern recognition proteins (PRPs) recognize common epitopes present on the surface of invading pathogen and are generally carbohydrate moieties (Fearon and Locksley 1996). Invertebrate protein recognizing carbohydrate from microbial surface has been isolated and showed their involvement in immunity (Sritunyalucksana and Soderhall 2000). A number of PRPs has been isolated and characterized. LGBP gene was cloned from *Fenneropenaeus chinensis*, Fc-LGBP, and its expression was down-regulated 24 h post-injection of bacteria. Bacterial binding assays showed strong binding activity of LGBP to Gram-negative bacteria (Du et al. 2007). The same was expressed only in the hemocyte and hepatopancreas of Chinese shrimp *Fenneropenaeus chinensis* and transcription of LGBP was enhanced in response to bacterial infection (Liu et al. 2009). The Chinese mitten crab *Ericdheir sinensis*, LGBP gene (Es-LGBP) was cloned and highly expressed in hemocytes and the lowest in the stomach. Es-LGBP gene expression was up-regulated on binding to LPS and β-1,3-glucan and bacterial infection (Zhao et al. 2009). The same gene transcript in the hemocyte of white shrimp *Litopenaeus vannamei* was increased in 3 and 6 h of post-*Vibrio alginolyticus* injection (Cheng et al. 2005). Disk abalone (*Haliotis discus discus*), Pattern recognition protein (HD-PRP) was expressed in gill, mantle, digestive tract, hepatopancreas and hemocytes, and the expression was enhanced with the administration of *Vibrio alginolyticus*, LPS, β-1,3-glucan (Nikapitiya et al. 2008). A high-density lipoprotein, β-glucan binding protein (βGBP-HDL) cloned from white shrimp *Litopenaeus vannamei* showed its expression in hepatopancreas, muscle, pleopods, and gills and involved in immune response (Romo-Figuero et al. 2004). Black tiger shrimp *Penaeus monodon* β-1,3-glucan binding protein (GBP) expressed in hemocyte and showed no significant change in mRNA expression of shrimps injected with curdlan or heat-killed bacterial cell of *Vibrio harveyi* within 12 h post-injection (Sritunyalucksana et al. 2002).

Prophenoloxidase (proPO)

Crustaceans lack normal defense mechanism as it occurs in fishes, but involves proPO, which is one of the main defenses functioning as a non-self recognition system. This system is triggered by lipopolysaccharides (LPS) or peptidoglycans (PGN) from bacteria and β-1, 3-glucans from fungi. There are proteins involved in the recognition of LPS, peptidoglycans, and β-1, 3-glucan, which have been named as pattern recognition proteins (PRPs) and are involved in various ways in the humoral defense mechanisms in crustaceans. The proPO activation pathway, like the vertebrate complement system, is a proteolytic cascade containing several serine proteases and their inhibitors. It is known that one of the serine protease in the cascade, named as proPO-activating enzyme (ppA), will cleave proPO to generate the active enzyme, phenoloxidase (PO). This enzyme can produce toxic compounds to microorganisms by oxidizing phenols to melanin.

In crustaceans, several proteins of this enzyme cascade have been isolated and partially characterized (Soderhall et al. 1990). ProPO, the latent inactive proform of phenoloxidase, has been purified from crayfish blood and found to possess a molecular mass of 76 kDa (Aspan and Soderhall 1991). It is converted into its active form by a serine protease, ppA, and the resulting active forms of phenoloxidase has molecular masses of 62 and 60 kDa (Aspan and Soderhall 1991). The proPO activation enzyme, ppA, has also been purified and characterized of having molecular mass of 36 kDa (Aspan et al. 1990). These proPO system proteins are contained in the blood cells of crayfish (Soderhall 1981) and can be released by a regulated exocytosis (Johansson and Soderhall 1989).

Phenoloxidase (PO) is the terminal enzyme of the whole cascade system. Once generated, it oxidizes dihydroxy phenylalanine (DOPA) to dopaquinone, which is converted to melanin (a brown pigment) through several non-enzymatic steps (Soderhall 1982; Sritunyalucksana and Soderhall 2000). A direct antimicrobial activity has been described for melanin and

its precursors (Nappi and Vass 1993). Besides, the production of reactive oxygen species such as super oxide anions and hydroxyl radicals during the generation of quinoids (Song and Hsieh 1994; Nappi et al. 1995) also has an antimicrobial role. The generated PO plays an important role in invertebrate defense, as it can melanize pathogens (Soderhall and Cerenius 1998), sclerotize the cuticle (Sugumaran 1991), and heal wounds (Lai-Fook 1966). In addition, PO also performs other biological activities such as mediating the cell adhesion of crayfish blood cells (Johansson and Soderhall 1988) and promoting encapsulation (Kobayashi and Soderhall 1990).

The activity of PO should be tightly regulated to avoid melanin formation at sites other than those of infection. Proteinase inhibitors are present in plasma such as a trypsin inhibitor, with a mass of 155 kDa (Hergenhahn et al. 1987) and α_2 macroglobulin (Hall et al. 1989). Both these high molecular mass proteinase inhibitors can inhibit ppA and thus inhibiting the activation of the proPO system, with the trypsin inhibitor being more efficient (Aspan et al. 1990).

Besides several proteins of this cascade, proPO gene from several species has been cloned and characterized and demonstrated specific expression. Similarly, expression varied with life stages of animals, health status, and environmental conditions. In white shrimp (*L. vannamei*), proPO gene was cloned and its mRNA expression was confirmed in hemocyte, but not in the hepatopancreas or muscle (Lai et al. 2005). Similarly, proPo gene in giant fresh water prawn (*Macrobrachium rosenbergii*) was observed in hemocyte only and its transcript expression was observed highest at B stage and lowest at D2/D3 stage (Liu et al. 2006), and proPO mRNA showed highest level of expression in vivo, with 5 μg of CpG oligodeoxynucleotide injection (Lu et al. 2006). Red swamp cayfish (*Procambarus clarkii*) proPO sequence showed similarity with arthropod proPO and it was closely related with freshwater crayfish *P. leniusculus* (Li et al. 2009). All the proPOs sequence cloned illustrated some common regions. Mud crab (*Scylla serrata*) proPO gene showed strongest expression in hemocyte and its level of expression was significantly increased within 12–14 h of post-injection with LPS (Ko et al. 2007).

A new proPO was cloned from white shrimp *Litopenaeus vannamei* (Ai et al. 2008, 2009; Yeh et al. 2009). proPO-b was detected mainly in hemocyte as well as in gill, midgut, heart, stomach, posterior midgut cecum, and cuticular epidermis. Sequence of both proPO-a and proPO-b showed microsatellite site at the 3' end of open reading frame (ORF) (Ai et al. 2008). Similarly, LvproPO-2 showed similarity with LvproPO-1 of white shrimp and expression was observed on the surface and nucleus of the hemocytes, but not in plasma (Ai et al. 2009). ProPO-I (cloned early) and proPO-II mRNAs of shrimp are expressed in hemocytes and located on different loci. Shrimps fed with diet containing sodium alginate alone or in combination with *Vibrio alginolyticus* showed mRNA expression of both proPOs at different molt stages (Yeh et al. 2009).

β-Glucan on protein and gene expression

Role and potential influence of β-glucan on immune-related gene and protein expression in different fish species have been reported by many authors. Head kidney macrophages cells from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon immunostimulated with LPS and β-glucan showed enhanced levels of ILs, but transcription of C3 was not induced in both trout and salmon. Complement C3 subtypes were differentially regulated after 48 h in vivo stimulation with LPS and β-glucan. These results supported the earlier findings of absence of C3 in macrophages of the spotted wolf fish (*Anarhichas minor*) (Løvoll et al. 2007). Similarly, oral administrated β-1, 3-glucan in tilapia (*Oreochromis niloticus*) for 5 days stimulated production of cytokine-like proteins of tumor necrosis factor-α (TNF-α), IL (interleukin)-1β, IL-10, IL-12 in fish plasma (Chansue et al. 2000). Many authors have reported the presence of IL-Iβ (Secombes et al. 1996; Ellsaesser and Clem 1994; Verburg van Kemenade et al. 1995) and TNF-α (Zelinkoff et al. 1990; Ahne 1993) in fishes. However, Hardie et al. (1994) have reported specific TNF-α receptor in rainbow trout lymphocytes. β-glucan administered through bath treatment in rainbow trout fry at two different doses (0.1 and 1.0 μM) for 45 min, four times with an interval of 1 week, showed enhanced gene expression with regard to the pro-inflammatory cytokines IL-1β, TNF-α, IL-6, the anti-inflammatory cytokines IL-10 and TGF-β at first bath.

However, no significant change in the pro-inflammatory cytokine IL-17A transcripts, compared to control, was observed. Gene expression levels in β-glucan treated fish after the fourth bathing showed no significant differences compared to control (Zhang et al. 2009). The same were fed with lentinan, a mushroom β-glucan, for 37 days and administered with bacterial LPS, an inflammation inducing agent. Use of lentinan showed decrease in the expression of genes involved in acute inflammatory reaction to inflammatory agents compared to fish fed with bacterial LPS, but no change in expression of gene associated with major immune response was observed (Djordievic et al. 2009).

Grass carp treated with β-glucan for 15 days prior to grass carp hemorrhage virus (GCHV) injection showed increased MX gene expression levels during early stages (12 and 36 h) of GCHV infection and significantly improved the survival rate to 60 %. Increased superoxide dismutase (SOD) and catalase (CAT) activities of erythrocytes and Mx gene expression were observed compared to the group not pretreated with β-glucan, indicating that β-glucan enhances anti-viral responses (Kim et al. 2009). No significant response on the expression of TNF-α or IL-1β was reported in fish fed with 5 mg/ml of β-glucan (*S. cerevisiae*), but modulation of IFN-γ and chemokine expression in kidney were reported (Rodríguez et al. 2009).

Feeding Pacific white shrimp with different dosages viz. 0, 0.2, 1.0 % of β-glucan (*Schizophyllum commune*) for 1 week showed up- and down-regulations of gene within 24 h. Penaeidin 3 (*Litvan* PEN3) was down-regulated (0–24 h), β-glucan binding protein–high density lipoprotein (BGBP-HDL) and lipopolysaccharide/β-glucan binding protein (LGBP) showed a delayed up-regulation (3–7 days), but hemocyanin, crustin, prophenoloxidase (proPO), and transglutaminase (TGase) showed no response. Diet with 2 g glucan/kg feed was sufficient for gene expression (Wang et al. 2008). Gene expression in Pacific white shrimp was observed to be site specific such as BGBP-HDL, LGBP and hemocyanin are expressed in hepatopancreas. Propo, TGase, penaeidin 3, crustins, and lysozyme showed highest expression in hemocyte and lowest in hepatopancreas; moreover, cMnSOD showed its highest expression in stomach and muscle while lowest in hemocyte, midgut, neural ganglion, and hepatopancreas (Wang et al. 2007a, b).

β-Glucan as immunostimulant

β-Glucan as immunostimulant has been widely studied in several vertebrate and invertebrate species and discussed in detail by Soltanian et al. (2009). Dietary administration of β-glucan to Atlantic salmon (*Salmon salar*) showed stimulation of respiratory burst activity (RBA) of head kidney macrophage in vitro. In vivo experiment showed negative effect on RBA, serum lysozyme production, and disease resistance to amoebic gill disease (AGD) on challenging them with the infectious pathogen (Bridle et al. 2005). A number of reports reveal that dietary β-glucan administration increases resistance to infection for short time periods in Chinook salmon, *Onchoryhnchus tshawytscha* (Nikl et al. 1993), African catfish, *Clarius gariepinus* (Yoshida et al. 1995), gilthead sea bream, *Sparus aurata* (Ortuno et al. 2002), and Indian carp, *Labeo rohita* (Sahoo and Mukherjee 2002). Time of administration of β-glucan plays significant role in induction of resistance against microsporidian, *Loma salmonae*, in the rainbow trout (Guselle et al. 2007).

Bagni et al. (2005) reported duration-dependant effect of dietary glucan where significant elevation of serum complement activity in sea bass fed with alginic acid and glucans at 15 days was reported; however, serum lysozyme, gill and liver HSP concentrations were enhanced at 30 days. Increased complement activity was reported only in fishes fed with Ergosan diet. Long-term period had no significant impact on innate and specific immune parameters, survival, growth performances, and conversion index in treated and control fish. The quality of immunostimulant (β-glucan) supplied plays crucial role in enhancing the immune responses. Use of commercial β-glucan products has showed their effects on immune response. In an in vitro study, head kidney macrophage of pink snapper (*Pagrus auratus*) pre-incubated with commercial β-glucan (EcoActiva^{TM}) and subsequently induced either by phorbol myristae acetate (PMA) or lipopolysaccharide (LPS) resulted in significant stimulation of superoxide anions and respiratory burst activity compared to induction of macrophage with EcoActiva alone (Cook et al. 2001). Result of this study demonstrates that feeding of β-glucan may enhance the recognition of LPS present in the cell wall of Gram-negative fish pathogenic bacteria resulting in improved killing efficiency of macrophage to these pathogens. In another study,

oral administration of EcoActiva in Pink snapper increased macrophage O_2 radicals especially in wintertime only, but no enhancement in classical and alternative pathway activity was seen, indicating winter time to be the most favorable to feed snappers for disease resistance (Cook et al. 2003). Glycans Bar, krestin, scleroglucan, and zymosan in diets showed significant increase in the survival rates of tilapia and grass carp after injection with *Aeromonas hydrophila* (Wang and Wang 1997). The effects of feeding 1,3/1,6 β-glucans on the innate and the adaptive immune responses were studied in European sea bass (*Dicentrarchus labrax*) (Bonaldo et al. 2007).

Yeast β-1, 3/1, 6-glucan have been used for in vitro and in vivo experiments to study degranulation of primary granules in fish neutrophils (Palic et al. 2006). β-glucan supplied to non-stress (NS), acute stress (AS), and chronically stressed (CS) fish showed increase degranulation in NS and prevented decrease of degranulation in AS, whereas in CS fish, degranulation reached to NS level after 3 days of feeding in fathead minnows (*Pimephales promelas*, *Rafinsesque*). Results indicated that β-glucan supplementation prior to AS and during CS in fish diet can enhance neutrophils function and increase disease resistance and survival rate. Immunomodulatory effect of dietary β-1, 3/1, 6-yeast glucan showed enhanced effect on concanavalin A, which induced proliferation of lymphocytes and antibody response after vaccination with formalin-killed *Yersinia ruckeri* against red mouth disease in rainbow trout. It had also showed enhanced macrophage, lysozyme, and complement activity, but no effect was observed on oxidative burst, pinocytosis, and alternative complement pathway activation (Verlhac et al. 1998). Wang et al. (2007a, b) used five different glycans (Barley, Krestin, MacroGard, Scleroglucan and Zymosan) to study the immune response in hybrid Tilapia and Japanese eels *Anguilla japonica*. They showed increase lysozyme activity and phagocytic activity in both anterior kidney and peripheral blood phagocytes. In vitro and in vivo conditions, classical complement pathway (CCP), hemolytic complement titer, and alternative pathway-hemolytic complement titer improved in glucan treated tilapia. Intramuscular injection of β-glucan (*Saccharomyces cerevisiae*) in zebrafish (*Danio rerio*) on 6th day before challenge with *Aeromonas hydrophila* showed significant reduction in the mortality, increase in the myelomonocytic cell population in the kidney, and ability of kidney cells to kill *A. hydrophila* (Rodríguez et al. 2009). Protective effect of β-glucan injection against several infections (Selvaraj et al. 2005; Misra et al. 2006a; Anderson and Siwicki 1994) and dose-dependent response to intramuscular injection with β-glucan (Selvaraj et al. 2005) have been demonstrated in different species.

Similar studies have been done in invertebrates. Indian white shrimp fed with marine yeast glucan once in every 7 days showed significant survival against WSSV (Sajeevan et al. 2009). However, increased ProPO and reactive oxygen intermediate activity were demonstrated with alkali insoluble glucan (AIG, rich in (1 → 3)-β-D-glucan) from a filamentous fungi *Acremonium diospyri*, whereas alkali soluble glucan (ASG, rich in (1 → 3)-α-glucan) did not show significant immune response. Their results revealed that glucan can be used as a potential immunostimulant for shrimp, where it should contain (1 → 3)-β-D-glucan as the major fraction (Anas et al. 2009). Dietary supplementation of β-1, 3/1, 6-glucan from *Saccharomyces cerevisiae* was used in spawners of *Penaeus monodon* to study maternal transmission of immunity to white spot syndrome caused by WSSV. Glucan fed shrimp showed clinical symptoms of red body coloration and white spot on the shell, while larvae showed protection against WSSV when challenged and showed enhanced RPS of larva compared to the control, clearly indicates maternal transmission of immunity in invertebrates (Huang and Song 1999).

Soltanian et al. (2007a) challenged *Artemia* nauplii with *Vibrio campbellii* under gnotobiotic conditions to study anti-infectious potential of six commercial β-glucans. Their results explained that the quality of glucans (molecular weight, structure ratio of 1, 3/1, 6 glucan ratio and branching) is more important than the quantity of the product offered in diet. Application of commercial β-glucan sources together with chitin has been reported to enhance disease resistance in *Artemia* (Soltanian et al. 2007b).

Feeding β-glucans to clam (*Ruditapes decussatus*) and Mediterranean mussel (*Mytilus galloprovincialis*) showed improved immune response. Increased nitric oxide production was observed in both the species but release of free oxygen radicals and phorbol 12-myristate 13-acetate (PMA) was enhanced in mussel hemocytes. However, high dose of β-glucans combined with zymosan decreased there respiratory burst activity in mussel. Hemolymph treated with several

doses of β-glucans restricted the growth of *Vibrio algynolyticus*, *Vibrio splendidus,* and *Escherichia coli*, but the antibacterial activity modulation was limited to clams (Costa et al. 2008).

Yeast and yeast subcomponents as immunostimulant

Yeast and yeast components, that is, glucans and mannans have been reported to boost immunity in many fish species. Brewers yeast (*Saccharomyces cerevisiae*) is a natural product from the brewing industry and contains various compounds such as glucans, nucleic acids as well as mannan oligosaccharides, capable of enhancing immune responses (Ortuno et al. 2002) and growth (Lara-Flores et al. 2002) of various fish species. Experimental supplementation of yeast and yeast subcomponents (YYS) was used to study the physiological performance of juvenile channel catfish, *Ictalurus punctatus*. Experimental diets for 4 weeks followed by 2 weeks on control diet showed no response on the growth performance, hematology, or immune function but partial resistance to stress was observed on exposure to low-water stress with no improved resistance against *E. ictaluri* (Welker et al. 2007). Similarly, Nile tilapia showed minimal effect on immune function and no effect on growth and mortality when challenged with *Streptoccocus iniae* (Shelby et al. 2007). YYS fed Nile tilapia juveniles did not show any effect on growth, serum component, antibody response, and resistance to *Streptoccocus iniae* and *Edwardsiella tarda* (Shelby et al. 2009). Oral administration of yeast cell wall (YCW) from *Saccharomyces cerevisiae* in *Labeo rohita* against bacterial pathogen, *Aeromonas hydrophila,* was studied by feeding them on experimental diet for 15 days and then switched back to control diet. Challenging them with intraperitoneal injection of virulent *A. hydrophila* showed low mortality and enhanced immunostimulatory and protective effect (Pal et al. 2007), whereas feeding YCW showed minimum protection against columnaris disease caused by *Flavobacterium columnare* in early life stages of *Labeo rohita* (Kunttu et al. 2009). Li and Gatlin (2003) established the beneficial effects of partially autolyzed brewers yeast on immune responses of hybrid striped bass and resistance to *S. iniae* infection.

In vitro studies with spent brewer's yeast β-glucan (BYG) showed enhanced phenoloxidase activity, the same was demonstrated on oral administration of BYG in diet for 3 days in black tiger shrimp (Suphantharika et al. 2003) and improved hemocytes number and the bacterial killing activity against the pathogen *Vibrio harveyi* (Thanardkit et al. 2002). Pacific White shrimp fed with diet containing inactive yeast cell wall showed no significant difference in weight, survival, and growth rate, but showed better effects on immune parameters (total and granular hemocyte count, bacterial clearance) compared to control (Chotikachinda et al. 2008).

β-Glucan with other immunostimulants

Combinations of β-glucan with other immunostimulants are equally effective in improving immunity against antigens. β-glucan plus O-antigen-treated turbot leukocytes destroyed an avirulent strain of *V. damsela* efficiently and increased respiratory burst in leukocytes on 7 days post-infection. Lysozyme activity in serum of fish injected with glucan and O-antigen, alone or in combinations, was enhanced compared to the control (Santarém et al. 1997). Administration of β-glucan and O-antigen of *Vibrio damsela* enhanced several non-specific immune responses in turbot (*Scophthalmus maximus*). Immune response of seven different immunostimulants in Coho Salmon to *Aeromonas salmonicida* was studied by injecting them along with bacterin. Fish fed with lentinan, formalin-killed *Renibacterium salmoninarum* cells, and Vita-Stim-Taito for 27 days and challenged with *Aeromonas salmonicida* by cohabitation and immersion method showed continued increase in the protection (Nikl et al. 1991). In a study, combined effect of β-glucan, ascorbic acid (Aa), and α-tocopherol (α-T) was performed on sea bass. Initially, fishes were adopted to diet supplemented with Aa, α-T for 5 weeks but later moved to diet containing β-1, 3/1, 6-glucan and high dose of Aa and α-T for 2 weeks in every 3 months. Immune parameters such as alternative pathway of complement activation and lysozyme activity were enhanced in experimental fish (Bagni et al. 2008).

Vaccinated juveniles of red tail black sharks (*Epalzeorhynchos bicolor*) were fed with β-glucan or nucleotide for 24 days and then infected with *S. iniae* by intracoelemic injection. Both vaccinated and

non-vaccinated fishes were fed with glucan or nucleotide showed low mortality compared to control but no difference was noticed among fishes fed with β-glucan and nucleotide (Russo et al. 2006). β-1,3/1,6-glucan from chrysolaminaran was fed to two sets of Atlantic cod (*Gadus morhua* L.) larvae. In one experiment, effect of microalgal glucan on immunity was compared with MacroGard (Yeast glucan) and the results showed increased survival rate in fish fed with microalgal glucan compared to control, but MacroGard had no significant effect on survival. In another experiment, fish fed with microalgal glucan showed positive growth rate during weaning to dry feed compared to high alginate (Mannuronic acid) fed fish (Skjermo et al. 2006).

β-Glucan had shown its positive effects on growth, survival, and immune response of fish species against infectious pathogens alone or in combination with other immunostimulants or probiotics. Effect of β-1,3/1,6 glucan and probiotics (*Vibrio alginolyticus*) inclusion in shrimp (*Penaeus vannamei*) larva at three different stages early, middle, and late was studied. Shrimp larva fed with probiotics and β-1,3/1,6 glucan in larviculture, where β-1,3/1,6 glucan showed highest survival against WSSV in early stage compared to other stages. Probiotics showed negative effect on plasmatic protein (PP), but increased antibacterial activity (AA) and THC. However, β-1,3/1,6 glucan showed negative effect on the O_2 generation. In challenge study, interaction between β-1,3/1,6 glucan and probiotics was found beneficial for improving immune parameters (Rodregeuz et al. 2007). Similarly, intramuscular injection of inactivated WSSV alone or in combination with β-1, 3-glucan and inactivated *Vibrio penaeicida* was given to Kuruma shrimp (*P. japonicus*). Shrimps were challenged by intramuscular injection of WSSV on the 10th and 30th day of post-vaccination with formalin-inactivated WSSV where no significant difference in mortality was observed when shrimp was treated with inactivated WSSV alone. However, survival was significantly improved in treatment group containing inactivated WSSV together with β-1, 3-glucan or inactivated *Vibrio penaeicida* (Namikoshi et al. 2004). This result corroborates the finding that glucan and inactivated *Vibrio* enhance the resistance against *Vibrio* as well as WSSV infections (Sung et al. 1994; Teunissen et al. 1998; Sritunyalucksana et al. 1999). Formalin-killed *V. harveyi* (bacterin) and Carboxymethyl β-glucan (CMBG) was administrated to black tiger shrimp, *Penaeus monodon* to protect against *V. harveyi* infection (Klannukarn et al. 2004). In one case, bacterin and CMBG were provided with feed to fishes for 10 days, and in other case, their combination was used in feed for 2 months in commercial ponds. The relative percent survival (RPS) and shrimp hemolymph parameters studies were demonstrated that bacterin and CMBG can induce internal defense and provide protection against *V. harveyi* when given separately rather than in combinations. They also determined the mechanism of cellular and humoral protections in shrimp.

Feeding β-glucan- or vitamin C-containing diet to *Litopenaeus vannamei* juvenile enhanced the growth and increased numbers of blood cells after salinity shock in fish fed with β-glucan and decreased in Vit C fed shrimp. Decrease in the proPo activity was observed in both β-glucan and Vit C fed shrimps, but the proPO granular cell ratio was enhanced in Vit C and decreased in β-glucan fed shrimp (Lopez et al. 2003). Immersion of juvenile of American white shrimp (*Litopenaeus vannamei*) for 6 h out of 1, 3, 6 h in aerated sea water along with β-glucan and sulphated polysaccharide solutions showed increased superoxide anion (SOD) activity in hemocytes and muscle compared to control or untreated samples. The total hemocyte counts (THC) were decreased in first 24 h of post-challenge with immunostimulants, but THC and total soluble hemocyte protein reached to its normal value after 48–120 h, thus showing induced immunostimulatory activity in muscle and hemocytes (Campa-Córdova et al. 2002).

Dosage of β-glucan in aquaculture

Dosages of immunostimulant given to aquatic organisms play an important role in the stimulation of immune response. It is essential to know correct dosages of immunostimulants and appropriate administration route to be followed to achieve the desired results. Steam pellet diet containing 0, 0.01, 0.1, and 1.0 % VST (VitaStim-Taito) was fed for 7 days, and bath challenged with *Aeromonas salmonicida* (As) vaccine. Diet containing 0.1 and 1.0 % VST resulted in significant protection against the *Aeromonas salmonicida* when challenged through oral administration. In the immersion trials, vaccine and VST were

administered either alone or in combinations. Following bath challenge with virulent at 21 day of post-vaccination, no significant protection was noted in any of the groups tested, indicating that VST was inactive as an immunopotentiator by this route (Nikl et al. 1993). A diet containing 0.5 g β-1.3/1.6-glucan (Macrogard)/100 g of pellets was fed to rainbow trout (*Oncorhynchus mykiss*) daily for a week and were immunized by immersing them in anti-*Yersinia ruckeri* vaccine. This resulted in an increased number of antibody-secreting cells (ASC) and specific Ig levels in serum, thus enhanced the effectiveness of *Yersinia ruckeri* vaccine in fish (Siwicki et al. 2004). However, feeding them with 0.1 % β-glucan for 4 weeks and exposing to 2 h of transportation stress showed elevated innate immune response (phagocytosis and oxidative radical production) in treated fish and helped to prevent negative effect of stress and protection against *Flexibacter columnaris*. Stress-induced elevated cortisol levels in plasma and hyperglycemia were lowest in 0.1 % fed β-glucan group (Jeney et al. 1997). Multiple injections of barley β-glucans at different dosages 0, 5, 10, 15 mg/kg of body weight were used to enhance the immune response and disease resistance against *Aeromonas hydrophila* and *Edwardsiella tarda* in *Labeo rohita* fingerlings. The results showed that injection of glucan 10 mg/kg body weight, three times, was advocated to enhance the immune response (Misra et al. 2006a). Likewise β-glucan at a dose of 250 mg/kg diet was recommended for enhancing immunity, growth, and survival against opportunistic pathogens such as *Aeromonas hydrophila* and *Edwardsiella tarda* in rohu. Immune parameters (leukocyte count, phagocytic ratio, phagocytic index, lysozyme activity, complement activity and serum bactericidal activity) rose to its highest levels on 42 days (Misra et al. 2006b). Oral administration of three barley genotypes with different quantity of β-glucan low (38 g/kg), average (52 g/kg), high (82 g/kg) showed decrease in the growth but increase in the IHNV neutralizing antibody and enhanced survival against IHNV; however, respiratory burst, lysozyme, TNF-α showed no response. Survival rates in fish fed with average or high dose of barley glucan were similar to yeast glucan (MacroGard) at 2 g/kg fed fish (Sealey et al. 2008).

Efficacies of different routes (intraperitoneal injection, bathing, and oral administration) were tested on *Cyprinus carpio*, where fish was fed with β-glucan and LPS to study survival and immune response on challenging with *Aeromonas hydrophila*. Intraperitoneal injection showed 100 % relative percentage survival (RPS) at all concentrations of β-glucan, whereas oral administration showed high RPS at higher concentrations (1 % β-glucan + 0.25 % LPS), but bathing did not improved RPS levels. Minimum dose (100 mg β-glucan + 10 mg LPS) per fish had a significant increase in total blood leukocyte, neutrophils, monocytes counts, and superoxide anion activity. Neither dose nor the route of administration of compounds had a significant effect on increase in expression of interleukin-1 beta mRNA and on classical and alternative complement pathways (Selvaraj et al. 2006). Baker's yeast β-glucan was administered though same routes in different concentrations 100, 500, 1,000 μg, but intraperitoneal injection of 500 μg showed enhanced RPS, whereas bathing and oral administration showed no effect on RPS. All the three concentrations showed increase in total blood leukocyte counts, neutrophils, monocytes, and elevated expression of interleukin-1beta mRNA on 7th day in fish injected with glucan (Selvaraj et al. 2005). Immunized and non-immunized Nile tilapia were fed with different concentrations of β-glucan 0, 50, 100, 200 mg for 14 weeks. β-glucan, immunization, and their combination showed no effect on growth and survival after 10 weeks, but fish fed with 100 and 200 mg β-glucan showed partial decrease in feed efficiency ratio (FER) compared to 50 mg glucan fed fish. Immunized fish showed reduced percentage mortality (PM) and increased RPS against *S. iniae*; moreover, their combination had no effect on PM and RPS (Whittington et al. 2005).

Large yellow croaker diets supplemented with 0 % (control), 0.09 % (low), and 0.18 % (high) of β-1, 3 glucan were fed for 8 weeks, low glucan levels enhanced fish growth, while high levels significantly enhanced the lysozyme activity. Respiratory burst activity in head kidney macrophage was enhanced with low concentration of β-glucan. Overall growth, lysozyme, phagocytosis, respiratory burst, and protection against *V. harveyi* were enhanced but there was no effect on alternative complement pathway (Ai et al. 2007). Feeding 0 and 10 g whole wild yeast or fks-1 mutant strain per kg diet for 2, 4, and 6 weeks to gilthead seabream (*Sparus aurata* L.) showed decrease in serum peroxidase and complement activity after 6 weeks and increase in lysozyme activity after

2 weeks. Phagocytosis was increased to a significant degree during all the time, but only with fks-1 strain-supplemented diet, while respiratory burst activity and natural cytotoxicity (after 4 and 6 weeks) were enhanced with either yeast strain; moreover, intracellular content was not changed with either of the strain (Rodríguez et al. 2003). Head kidney phagocytic cells from blue gourami (*Trichogaster trichopterus*) injected intraperitoneally with laminaran β-1,3-glucan in different dosages of 5, 10, 20, and 40 mg/kg were studied for chemiluminescent response (CL). Fish injected with 20 mg/kg dose showed enhanced CL and resistance to *A. hydrophila* up to 22 days. However, 5, 10, and 40 mg/kg dose showed enhanced CL for shorter duration up to 14 days and low resistance to *Aeromonas hydrophila*. Fish treated with 10 mg/kg glucan plus formalin-killed bacteria showed higher CL response and resistance compared to bacteria injected fish. Fish fed with diet containing 20 mg/kg of laminaran plus bacteria showed higher response and resistance at 22 days. Thus, 20 mg/kg of laminaran can protect the fish effectively from *A. hydrophila* up to 29 days (Samuel et al. 1996). Laboratory developed feed stimulant, BAISM, and dietary supplementation of β-1,3-glucan were used to study their effect as feed additives on juvenile olive flounder, *Paralichthys olivaceus* (Yoo et al. 2007), and it was concluded from the study that optimum level of β-1,3-glucan and BAISM could be approximately 0.1 % β-1,3-glucan + 0.9 % BAISM (G0.1B0.9) of diet for better growth and survival.

Similarly, feeding 0, 0.5, 1.0 % yeast culture supplement diamond VXP YC to pacific white shrimp (*Litopenaeus vannamei*) daily for 4 weeks has shown protection against bacterial diseases (Burgents et al. 2004). Oral administration of fungal polysaccharide schizophyllan at a dose of 50 or 100 mg/kg body weight per day showed higher survival rates after challenging with the virus (RV-PJ) compared to the control. Phagocytic activities were found to be higher in schizophyllan fed shrimps. These results indicate that schizophyllan can confer effective protection against viral infection by increasing antiviral immune responses in invertebrates (Itoh 1997). Similarly, *Schizophyllum commune* glucan fed for 40 days to grass prawn in indoor and outdoor rearing tanks. Irrespective of rearing tanks, enhanced survival compared to control was recorded in shrimps fed with β-glucan at a dose of 20 g/kg diet. Hemocyte phagocytic activity, cell adhesion, and superoxide anion production were also elevated (Chang et al. 2000). The same glucan (0, 1, 2, 10, 20 g/kg diet) was fed to *Penaeus monodon* for 20 days and challenged with WSSV injection. Shrimps fed with 10 g/kg glucan showed highest survival ($P < 0.05$) than other groups, whereas hemolymph THC, PO, O_2, and SOD were dropped initially in all group except in 10 g/kg fed glucan group, on challenging and reached normal later (Chang et al. 2003). Different concentrations of marine yeast β-glucan (0.05, 0.1, 0.2, 0.3, 0.4 g/100 gm) were fed to post-larvae of Indian white shrimp for 21 days. Larval survival was observed against oral administration of white spot syndrome virus and shrimps fed with 0.2 % glucan showed maximum survival, while in second experiment, 0.2 % glucan was fed at different days intervals and study showed that 0.2 % glucan when given once every 7 days recorded best result (Sajeevan et al. 2009). Post-larvae of tiger shrimp were immersed in aerated β-glucan suspension for 3 h in four different concentrations 0.25, 0.5, 1.0, 2.0 mg/ml. Enhanced growth of larvae was observed in 0.5, 1.0, and 2.0 mg/ml β-glucan suspension, but no response in 0.25 mg/ml glucan was observed. After 43 days when larvae were immersed in bacterial suspension of *Vibrio vulnificus* cells (5×10^7 CFU/ml for 12 h), increased protection against the pathogen along with enhanced phenoloxidase activity in shrimp hemocyte was reported (Sung et al. 1994). Effective doses of various β-glucan used in aquaculture have been summarized in Table 2.

β-Glucan and immunocompromised fish

Several experiments have been carried out to study the effect of β-glucan on immunocompromised fish in enhancing resistance to infectious pathogens. Immunity of immunocompromised fish is reduced using certain toxins or chemicals. Effect of aflatoxin B_1, β-1,3-glucan, and their combination on non-specific immunity and disease resistance was studied (El-Boshy et al. 2008). Dietary supplementation of aflatoxin (AFB_1) in fish showed reduced immunity with affected biochemical parameters related to organ damage. Feeding 0.5 % β-1,3-glucan to immunocompromised (200 µg/feed aflatoxin B1) Nile tilapia for 21 days showed enhanced resistance against *S. iniae* and improved non-specific immunity levels compared

Table 2 Effective doses of various β-glucan used in aquaculture

Sl. no.	Types of β-glucan	Dosages used	Route of administration	Species studied	Remarks	References
1.	β-Glucan (*Saccharomyces cerevisiae*)	5, 2 and 0.5 mg/ml	Intraperitonial injection	*Danio rerio*	5 mg/ml showed reduction in the mortality to *A. hydrophilla* and increased myelomonocytic cell population. Modulation of IFN-γ and chemokine expression in kidney	Rodríguez et al. (2009)
2.	Marine yeast glucan	0.2 g/kg feed once in a week	Oral	*Fenneropenaeus indicus*	Improved survival against WSSV	Sajeevan et al. (2009)
3.	Brewer's yeast β-glucan	0.2 % (w/w) for 3 days	Oral	*Penaeus monodon*	Enhanced phenoloxidase activity	Suphantharika et al. (2003)
4.	Brewer's yeast β-glucan	0.2 % (w/w) for 3 days	Oral	*Penaeus monodon*	Increased number of haemocytes and the bacterial killing activity against *Vibrio harveyi*	Thanardkit et al. (2002)
5.	Inactive yeast cell wall	1 and 2 g/kg feed	Oral	*Litopenaeus vannamei*	Improved immune parameters	Chotikachinda et al. (2008)
6.	Inactive yeast cell wall	1 and 2 g/kg feed	Oral	*Litopenaeus vannamei*	No effect on weight, survival and growth rate	Chotikachinda et al. (2008)
7.	β-glucan	0.1 % of feed	Oral	*Oncorhynchus mykiss*	Lowest cortisol levels in plasma against transportation stress	Jeney et al. (1997)
8.	Barley β-glucans	5, 10, 15 mg/kg of body weight	Injection	*Labeo rohita*	10 mg/kg body weight, three times showed enhanced immune response and disease resistance against *Aeromonas hydrophila* and *Edwardsiella tarda*	Misra et al. (2006a)
9.	β-glucan	250 mg/kg feed for 42 days	Oral	*Labeo rohita*	Enhance immunity, growth and survival against opportunistic pathogens such as *Aeromonas hydrophila* and *Edwardsiella tarda*	Misra et al. (2006b)
10.	β-glucan	38 g, 52 g and 82 g/kg feed	Oral	*Oncorhynchus mykiss*	Showed decrease in the growth but enhanced survival against IHNV	Sealey et al. (2008)
11.	Baker's yeast β-glucan	100, 500, 1,000 µg/fish	Injection	*Cyprinus carpio*	500 µg showed enhanced RPS and all three showed increase in total blood leukocyte counts, neutrophils, monocytes and elevated expression of interleukin-1beta mRNA	Selvaraj et al. (2005)
12.	β-1, 3 glucan	0.09 and 0.18 %	Oral	*Pseudosciaena crocea*	Low glucan levels enhanced fish growth, while high levels significantly enhanced the lysozyme activity	Ai et al. (2007)
13.	Wild yeast or fks-1 mutant strain	10 g/kg diet for 2, 4 and 6 weeks	Oral	*Sparus aurata* L.	Decreased serum peroxidase and complement activity after 6 weeks and increased lysozyme activity after 2 weeks	Rodríguez et al. (2003)
14.	VXP YC	0.5 and 1.0 %	Oral	*Litopenaeus vannamei*	Protection against bacterial diseases	Burgents et al. 2004

Table 2 continued

Sl. no.	Types of β-glucan	Dosages used	Route of administration	Species studied	Remarks	References
15.	Fungal polysaccharide schizophyllan	50 or 100 mg/kg body weight	Oral	*Litopenaeus vannamei*	Higher survival rates after challenging with the virus (RV-PJ)	Itoh (1997)
16.	Schizophyllum commune glucan	20 g/kg diet	Oral	*Penaeus monodon*	Enhanced survival and immunity	Chang et al. (2000)
17.	Schizophyllum commune glucan	1, 2, 10, 20 g/kg diet	Oral	*Penaeus monodon*	10 g/kg glucan showed highest survival and immunity against WSSV infection	Chang et al. (2003)
18.	β-glucan	0.25, 0.5, 1.0, 2.0 mg/ml	Immersion	*Penaeus monodon* (PL)	Enhanced growth and protection to *Vibrio vulnificus* was observed in 0.5, 1.0 and 2.0 mg/ml glucan	Sung et al. (1994)
Dosages of β-glucan with other immunostimulants						
19.	β-glucan and nucleotide	1 g/kg +2 g/kg feed	Oral	*Epalzeorhynchos bicolor* juveniles	Increased resistance against *S. iniae*	Russo et al. (2006)
20.	Coomercially available β-glucan, VitaStim-Taito (VST)	0.01, 0.1, and 1.0 % for 7 days	Oral and immersion	*Oncorhynchus tshawytscha*	0.1 and 1.0 % VST resulted in significant protection against the *Aeromonas salmonicida*	Nikl et al. (1993)
21.	β-1.3/1.6-glucan (Macrogard) and immunized by anti-yersinia ruckeri vaccine	0.5 g/100 g of pellets for a week	Oral	*Oncorhynchus mykiss*	Increased number of antibody secreting cells (ASC) and specific Ig levels in serum	Siwicki et al. (2004)
22.	β-glucan and LPS	1 % + 0.25 %	Oral	*Cyprinus carpio*	Improved RPS to *Aeromonas hydrophila*	Selvaraj et al. (2006)
23.	β-glucan and LPS	100 mg +10 mg/fish	Injection	*Cyprinus carpio*	Increased total blood leukocyte, neutrophils, monocytes counts and superoxide anion activity	Selvaraj et al. (2006)
24.	β-glucan and immunization	50, 100, 200 mg/kg feed	Oral	*Oreochromis niloticus*	No effect on growth and survival after 10 weeks	Whittington et al. (2005)
25.	Head kidney phagocytic cells and Laminaran β-1,3-glucan	5, 10, 20 and 40 mg/kg feed	Injection + oral	*Trichogaster trichopterus*	20 mg/kg feed showed enhanced chemiluminescent response	Samuel et al. (1996)
26.	BAISM and β-1,3-glucan	0.9 % + 0.1 %	Oral	*Paralichthys olivaceus*	Better growth and survival	Yoo et al. (2007)

to AFB_1 non-treated fish. An artificial immunosuppressor, cyclophosphamide was used in Asian catfish, *Clarias batrachus* to make them immunocompromised, the cyclophosphamide fed fish showed lower respiratory burst, myeloperoxidase, phagocytic and hemagglutination activity in blood; however, use of β-1,3-glucan as feed supplement resulted in enhanced immune response in immunocompromised fish. Superoxide anion, myeloperoxidase, phagocytic activity, and hemagglutination were also increased (Kumari and Sahoo 2006b). In same immunocompromised fish, use of β-glucan enhanced antibody production and protection against *Aeromonas hydrophila* when challenged with formalin-killed *A. hydrophila* bactericin (Kumari and Sahoo 2006a). Feeding β-1,3 glucan to immunocompromised *Labeo rohita* for 7 days showed significant enhancement in the resistance against *A. hydrophila*. The non-specific immunity levels such as leukocyte number, phagocytic index, and serum bactericidal activity were also enhanced compared to non-treated AFB_1-exposed fish (Sahoo and Mukherjee 2001). Aflatoxin B_1-induced immunocompromised Indian Major Carp, vaccinated with formalin-killed *Edwardsiella tarda,* showed enhanced specific immunity and reduced mortality, but failed to enhance specific immunity and protection in healthy fish. The increased bacterial agglutination titer and reduced mortality by feeding β-1,3-glucan in healthy vaccinated fish compared to control indicate glucan as an potent immunostimulant (Sahoo and Mukherjee 2002).

β-Glucan as adjuvant

Adjuvants are compounds that enhance the specific immune response against co-inoculated antigens. Oils and bacterial lipopolysaccharides have been shown to induce elevated antibody production when administered in fish, but oil emulsions as adjuvant may lead to side effects such as adhesions of internal organs, reduced growth, skeleton deformations, etc. (Berg et al. 2006). Effect of β-1,3-glucan on enhancing the specific immune response when added together with immunogens and administered through any route (injection, bath or dietary incorporation) in fish was demonstrated by Anderson (1997). Yeast β-glucan (YBG) has been used as adjuvant from past many years in aquaculture; *Vibrio damsela* vaccine was injected prior, together, and post-application of yeast (*Saccharomyces cerevisae*) β-1,3-glucan in turbot (*Scophthalmus maximus* L.). The highest activity among all the immune parameters was obtained when glucans were injected after the bactericin application. Finding of this study indicates that the sequence of glucan administration is critical when used as a vaccine adjuvant (Figueras et al. 1998). β-glucan of same species showed adjuvant effect on antibody production, when pretreated by injecting 100–1,000 μg glucan/fish, resulted in enhancing the production of highest antibody titer against *A. hydrophila* following vaccination in *Cyprinus carpio* (Selvaraj et al. 2005). YBG along with *A. salmonicida* vaccine showed protective response in *Atlantic salmon* (Rørstad 1993); moreover, similar protective effect was also observed against furunculosis disease (Midtlyng et al. 1996). Feeding Japanese flounder with formalin-killed bactericin *E. tarda* along with curdlan, a β-glucan showed no antibody response, but the survival rate was higher compared to the control fish when challenged with *E. tarda* (Ashida et al. 1999). Mushroom glucan as adjuvant in conjunction with a formalin-killed *Aeromonas hydrophila* vaccine in *Catla catla* showed significant enhancement in antigen-specific proliferation, 'macrophage activating factor' (MAF), and antibody production leads to increased protection against *A. hydrophila* (Kamilya et al. 2006). β-1,3-glucan adjuvant in the Aeromonas vaccine enhanced production of antibodies to whole or outer membrane of bacteria and LPS and stimulated production of protein reactive antibodies, but no sufficient protection and survival against challenge with *Aeromonas salmonicida* was observed (Aakre et al. 1994). Injection of 100 mg β-glucan and 10 mg LPS/fish had an adjuvant effect on antibody production, resulted in higher antibody titer against *A. hydrophila* following vaccination, and enhanced resistance in carp (Selvaraj et al. 2006).

In contrast, some reports discuss on negative effect of β-glucan adjuvant. Oral administration of yeast β-glucan following *Streptococcus iniae* vaccine showed no protection against *S. iniae* in *Oreochromis niloticus* (Whittington et al. 2005) and against Streptococcus bacterin in turbot (Romalde et al. 1999). When yeast β-glucan was co-injected along with *Pasteurella piscicida* vaccine, the glucan showed a negative adjuvant effect in yellow tail (Kawakami et al. 1998).

Glucans as prebiotics

Prebiotics are non-digestible feed ingredients that benefit the host by stimulating growth and activity of beneficial bacteria in gastrointestinal (GI) tract (Gibson and Roberfroid 1995). The main advantage of prebiotics over probiotics is that they are natural feed ingredients thus regulatory control over dietary supplementation is limited (Gatelin III et al. 2006). Use of most of the antibiotics is banned due to potential development of antibiotic-resistant bacteria (FAO 2002), presence of their residues in seafood, destruction of microbial populations in the aquaculture environment, and suppression of the aquatic animal's immune system. Report has shown that antibiotics may impede growth and feed efficiency by killing intestinal microflora and thus reducing amino acid utilization by the host in some animal species (Rawles et al. 1997). These adverse effects and cost of antibiotics to overcome the diseases encouraged investigators to explore alternatives and application of prebiotics is one among such options.

GroBiotic-A^R, a commercial prebiotics, is a mixture of partially autolyzed brewer's yeast, dairy components, and dried fermentation products. It has shown enhanced growth performance and disease resistance in hybrid striped bass (Gatlin and Li 2004; Li and Gatlin 2004, 2005). Feeding GroBiotica-A^R-supplemented diet enhanced protein and organic digestibility in striped bass (Gatlin et al. 2006) and in rainbow trout improved resistance against different pathogens (Sealey et al. 2007). Dairy yeast prebiotics in golden shiners (*Notemigonus crysoleucas*) have enhanced the growth and survival (Lochmann et al. 2009), while mortality against *Flavobacterium columnare* was reduced significantly (Sink and Lochmann 2008). The survival, growth, and immune response were enhanced in juvenile western king prawns (*Penaeus latisulcatus*) by feeding Bio-Mos® in combination with β-1,3-D-glucan (Hai and Fotedar 2009). However, it improved the survival, health status, and immunity of marron, *Cherax tenuimanus,* against bacterial infection and under stress conditions with NH_3 exposures (Sang et al. 2009).

Use of Fermacto prebiotic improved growth and feed efficiency ratio of common carp (*Cyprinus Carpio*) fry (Mazurkiewicz et al. 2008). Feeding crucian carp (*Carassius auratus*) with prebiotic xylooligosaccharides showed no response on the growth and survival, but difference in the protease and amylase activity compared to the control was reported (Xu et al. 2009). Feeding short-chain fructooligosaccharide (scFO) supported growth of microbial flora of GI tract and enhanced the immunity in Pacific white shrimp (Li et al. 2007). Similarly fructooligosaccharide and mannan oligosaccharides showed positive effect on growth of Atlantic salmon (*Salmo salar*) (Grisdale-Helland et al. 2008), and mannan oligosaccharide showed enhanced salinity tolerance and development of micovilli in gut of cobia, *Rachycentron canadum* larva (Salze et al. 2008).

Conclusions

Surging human population has created spiraling demand of food, which needs to be met through innovative approaches without ignoring the context of human health and environmental sustainability. In the background of such concerns, β-glucan has tremendous potentials to be used in fish culture, disease management, and fish products development through biotechnical approach. An agent for prophylaxis and disease management, β-glucan, imparts immunity against various fish pathogens. Research reports have shown β-glucan dosages, quality, route, and time of administration and duration of treatments play significant role in enhancing various parameters related to growth, survival, and immunity. There is a need to give emphasis on optimizing the dosage and improve the quality of β-glucan to be used in different fish species. The adjuvant effect of β-glucan needs to be further addressed. The possible use of β-glucan from other sources like seaweed, plants, etc., should also be explored. Application of β-glucan as prebiotics in aquaculture is another emerging avenue, which requires further attention. So, further research on unexplored roles of β-glucan should be undertaken to adopt β-glucan as a potent immunostimulant in aquaculture.

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