Yeast β-Glucans as Fish Immunomodulators

Subjects: Immunology

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Administration of immunostimulants in fish is a preventive method to combat infections. A wide variety of these biological molecules exist, among which one of the yeast wall compounds stands out for its different biological activities. The β-glucan that forms the structural part of yeast is capable of generating immune activity in fish by cell receptor recognition. The most frequently used β-glucans for the study of mechanisms of action are those of commercial origin, with doses recommended by the manufacturer. Nevertheless, their immune activity is inefficient in some fish species, and increasing the dose may show adverse effects, including immunosuppression. Conversely, experimental β-glucans from other yeast species show different activities, such as antibacterial, antioxidant, healing, and stress tolerance properties.

Keywords: biomolecules ; functional carbohydrates ; immunity ; infectious diseases

1. Introduction

Given the accelerated growth of the aquaculture industry, the use of whole yeasts and their derived compounds as immunostimulants has been shown to be an excellent approach [1]. Yeasts are unicellular organisms distributed worldwide in a wide range of environments [2][3]. Their benefits are so extensive that they are also used in feed production as partial protein replacers [4][5]. Yeasts play a biological role within microbial communities in the fish intestine, including nutrient supply, pathogen control, and mucosal immunity maintenance [6][7]. Additionally, compounds of interest in the yeast cell wall promote biological activities in fish, such as mannan-oligosaccharide (immunostimulant) [8] and β-glucan (wound healing, stress resistance, immunostimulant, and disease protection) [9][10][11][12][13]. For instance, cell wall β-glucans have generated immunobiological activities in various animal taxonomic groups (birds, crustaceans, mammals and fish) [14][15][16][17]. Furthermore, β-glucans support other biological activities, including antibacterial [18], antioxidant [19], wound healing [11], and stress tolerance [12] effects. β-glucans are polysaccharides composed of glucose monomers joined by glycosidic bonds [20]. Their immunostimulant activities have been attributed to chemical composition, structural conformation, and molecular weight, among other factors [21]. All these characteristics depend on the yeast strain's origin, and may affect their immunostimulant properties (**Table 1**). Meanwhile, β-glucans are recognized by several immune cell receptors [17][18] and generate immune responses that strengthen resistance to pathogenic bacteria, fungi, parasites, and viruses [11][19]. β-glucans have been shown to promote disease resistance by stimulating the immune system in fish species [12][21]. However, a possible immune signaling pathway, dose, and effective route of administration have not yet been indicated. Added to this is the potential of experimental β-glucans extracted from other yeasts, which can be used to benefit freshwater and marine fish production.

Table 1. Molecular weights of β-glucans from different yeast species.

Species	*Mw* *	Reference
Cystobasidium benthicum	**2.32 kDa**	[22]
Saccharomyces cerevisiae **(bakery)**	**175 kDa**	[23]
Saccharomyces uvarum	**220 kDa**	[24]
Saccharomyces cerevisiae **(brewery)**	**240 kDa**	[25]
Debaryomyces hansenii **(BCS004)**	**689.35 kDa**	[26]

* *Mw* = Molecular weight. kDa = KiloDaltons.

2. Yeast Cell Wall and β-Glucan Composition

2.1. Yeast Cell Wall

The yeast cell wall is a 100 to 150-nm thick cell armor (hard and rigid), representing approximately 15 to 32% of the dry weight [14][27] and 25 to 50% of the cell volume [15]. It is composed of approximately 85% polysaccharides (β-glucan and chitin) and 15% proteins (manno and transmembrane proteins) [16] (**Figure 1**). The yeast cell wall composition and organization form a layered ultrastructure that can be observed by electron microscopy [17][28].

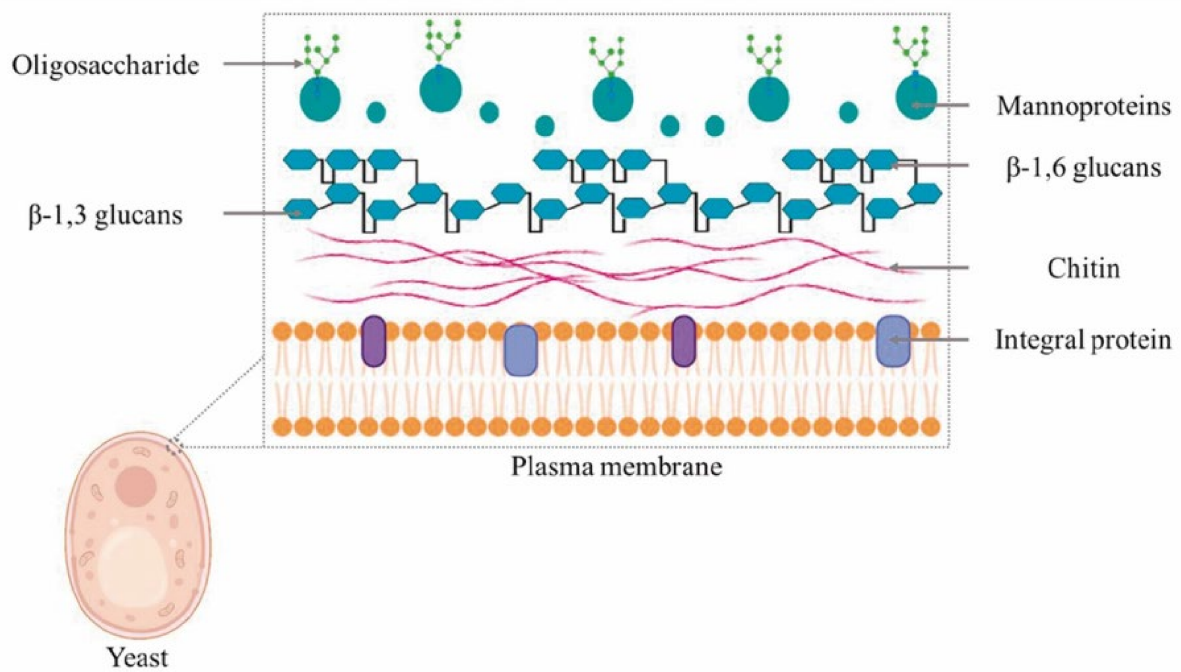

Figure 1. Conformation of the yeast cell wall. The cell wall is the largest, most resistant, and rigid organelle affecting the interaction with the external environment and the protection of the intracellular organelles, where compounds of great biotechnological interest are found including β-glucans.

2.2. Yeast β-Glucans

Yeast β-glucans are structured polysaccharides formed by monosaccharides (glucose), called “beta” (β) because of the specific glucosidic bonds (β-1,3 and 1,6) to which they are linked [29]. Due to the interaction of intermolecular polyhydroxyl groups, their structure can be single helix or triple helix, which makes them insoluble in water and in organic solvents (i.e., ethanol) [25]. This structural complexity endows β-glucans with high molecular weight that can vary according to the yeast species from which the β-glucan is extracted (**Table 1**).

Various studies have shown that insoluble β-glucans (β-1,3 and β-1,6) have superior capacity as biological response modifiers compared to soluble β-glucans (β-1,3 and β-1,4) [30]. Research reports have demonstrated that the most effective immunoenhancing activities, such as cell proliferation, were attributed to β-glucans with triple helix structures [24][30][31]. Observed effects included cell proliferation, phagocytic, antibacterial, and antioxidant activities, and immune-related gene expression [32][33].

3. Yeast β-Glucan Extraction

The extraction method has a significant influence on the physicochemical properties of β-glucans. Various methods have been proposed for β-glucan extraction, including physical [34][35][36], chemical [37][38], and enzymatic methods [39][40] (**Figure 2**). In the following, extraction methods using different processes are described, mainly based on cell disruption to release the cell contents and separate the β-glucan.

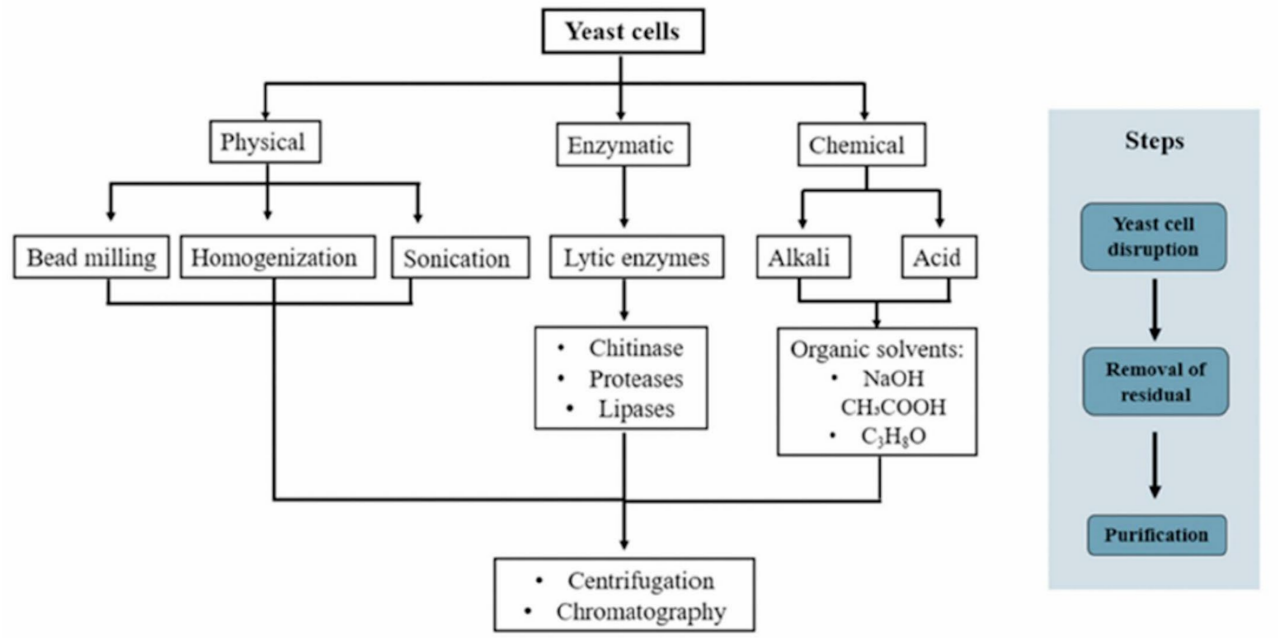

Figure 2. Representative scheme of the methods used for β-glucan extraction from yeast. Methodologies used for β-glucan extraction from yeast mainly differ in the method of breaking down the cell wall to release the internal components, and in the use of organic solvents to separate them. Finally, a purification process by centrifugation and chromatography is performed to obtain β-glucan from yeast.

3.1. Physical Method

Physical disruption is a non-contact method that uses external force to achieve cell membrane rupture [38]. The different methods include sonication, homogenization, and bead milling. Among these methods, sonication has been among the most popular for obtaining β-glucans [36]. Cell wall disruption by sonication is caused by ultrasonic vibrations that produce a high-frequency sound, causing physical modifications that permit the solvent to penetrate into solids, increasing the diffusion rate of the desired molecule to the solvent [34]. Homogenization and bead milling lysis provide kinetic energy for cellular disruption and the release of intracellular components [36]. The latter method is old, and little used in research: cell disruption is prompted by hydraulic pressure (Frances press) and the method continues to be used in industry because of the low cost of operation. This method consists of applying direct pressure to release the intracellular contents [41].

3.2. Chemical Method

Chemical cell lysis can be achieved by using specific chemicals to disrupt the cell wall, forcing it to release its contents [34]. This method is gentler than the physical approach and is suitable for lysing bacterial, fungal, and yeast samples [35]. Therefore, the chemical method is one of the most frequently employed to obtain β-glucans from yeast and other species [40]. Chemicals used for β-glucan extraction include alkali and/or acid organic solvents, such as acetic acid and sodium hydroxide, among others [42]. Organic solutions break down the cell wall through the difference in electronic charge, and also cause residues that contain chitin, glycogen, and proteins to be dropped [39]. It was recently reported that with this method a β-glucan of high quality, quantity, and biological activity was obtained.

3.3. Enzymatic Method

In recent years, biotechnological isolation methods with enzyme treatment have been developed [43]. Enzyme-based β-glucan extraction from yeast is a potential alternative to conventional solvent-based extraction methods, and possesses the advantages of being environmentally friendly, highly efficient, and a simplified process. Currently, enzymes including chitinase, proteases, and lipases have been widely used to degrade yeast cell walls and improve β-glucan isolation [39].

The final step of β-glucan extraction is purification, which consists of separating certain components found in yeast cell walls. In this sense, centrifugation and chromatography have been used for removing lipids and proteins from the cell wall, leading to more purified fractions of β-glucans [40].

4. Effects of Yeast β-Glucans on Fish Immune System

Fish have innate and adaptive immune systems. β-glucans are considered a type of pathogen-associated molecular pattern (PAMP) [44]. As such, they generate a signaling pathway in fish, but this has yet to be described in specific detail.

The signaling pathway is described and exemplified in **Figure 3**, based on the latest research with β-glucans in fish.

Figure 3. Signaling pathway for yeast β-glucans in teleost fish organisms. Proposed scheme of the β-glucan activation pathway in fish. (1) Intestinal epithelial enterocytes synthesize metabolic proteins activated by yeast β-glucan that secretes them into the systemic circulation. (2) Recognition of β-glucan by pathogen-associated molecular pattern receptors (PAMPs) that generate innate cellular immune responses and gene expression through translocation of the nuclear factor kappa beta (NF-κB) by phosphorylation, ubiquitination, and protein degradation. (3) Production of pro- and anti-inflammatory cytokines, receptors, and other proteins that activate the communication and activity of the adaptive immune system. (4) Production of immunoglobulins by B cells activated by the recognition of β-glucan.

After orally administration, β-glucans reach the intestine of the teleost fish; epithelial enterocytes synthesize apolipoprotein A-IV (apoa4) related to carbohydrate and lipid metabolism that probably captures β-glucan and secretes it into systemic circulation. Cytoplasmic actin 1 (actb) is permanently present in the intestinal microvilli, which together with transgelin (tagln) participate in actin-dependent β-glucan uptake [45]. Additionally, the presence of TLR-like receptors (Tlr2) in intestinal enterocytes could participle in the recognition of yeast β-glucans [46]. When β-glucans enter the systemic circulation, they are recognized by certain receptors, such as the three types of lectin C (a, b, and c) found in innate immune cells, and together with the spleen tyrosine kinase (Syk) generate intracellular signal transduction downstream by the mitogen-activated protein kinase (mapkin2) and nuclear factor kappa B (NF-κB) pathways [45][47][48]. Toll-like receptors (TLR 2/6) together with the myeloid differentiation primary response adapter protein 88 (myd88) also generate signaling cascades that cause activation of NF-κB [49]. The complement receptor (CR3) is a heterodimeric integrin that constitutes a critical link between cells and the extracellular matrix, functioning as anchoring sites and central elements for detection, processing, and transduction of the information received by β-glucans [50]. When NF-κB is activated, it initiates the expression of several pro- and anti-inflammatory cytokines [51]. Some of these cytokines have activities in the adaptive immune system, such as IL-6 and IL-10 that play important roles in the humoral immune response and induce differentiation of B lymphocytes [52][53]. IL-11 is another cytokine involved with anti-inflammatory characteristics, and is only characterized in a certain number of teleost fish [54]. When B lymphocytes recognize β-glucans, they begin to secrete immunoglobulins, such as IgM and IgT, involved in mucosal immunity [55]. IgT or IgZ is specific in teleost fish and is related to the intestinal mucosa [56][57][58]. Finally, yeast β-glucans could be involved in adaptive immune responses [59][60], but additional studies are required to better understand their signaling pathways in fish species.

Currently, the majority of studies (in vitro and in vivo) have used commercial β-glucans, and very few have assessed experimentally extracted yeast β-glucans. Up to now, the main yeast strains for β-glucan extraction have been those belonging to *Saccharomyces cerevisiae*. However, non-*Saccharomyces cerevisiae* strains have also been tested with promising results, such as *Saccharomyces uvarum* [31], *Yarrowia lipolytica* N6 [61], *Sterigmatomyces halophilus* [18], *Debaryomyces hansenii* BCS004 [26], and *Cystobasidium benthicum* [22]. Remarkably, many studies have related β-glucan supplementation to increased disease resistance in fish.

References

1. Øverland, M.; Skrede, A. Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture: Yeast from lignocellulosic biomass as a feed in aquaculture. J. Sci. Food Agric. 2016, 97, 733–742.
2. Gianni, L. The fascinating and secret wild life of the budding yeast S. cerevisiae. eLife 2015, 4, e05835.
3. Sagot, I.; Laporte, D. The cell biology of quiescent yeast—A diversity of individual scenarios. J. Cell Sci. 2019, 132, jcs213025.
4. Martin, A.M.; Goddard, S.; Bemibster, P. Production of Candida utilis biomass as aquaculture feed. J. Sci. Food Agric. 1993, 61, 363–370.
5. Reveco-Urzua, F.E.; Hofossæter, M.; Kovi, M.R.; Mydland, L.T.; Ånestad, R.; Sørby, R.; Press, C.M.; Lagos, L.; Øverland, M. Candida utilis yeast as a functional protein source for Atlantic salmon (Salmo salar L.): Local intestinal tissue and plasma proteome responses. PLoS ONE 2019, 14, e0218360.
6. Dimitroglou, A.; Merrifield, D.L.; Spring, P.; Sweetman, J.; Moate, R.; Davies, S.J. Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (Sparus aurata). Aquaculture 2010, 300, 182–188.
7. Navarrete, P.; Tovar-Ramírez, D. Use of Yeasts as Probiotics in Fish Aquaculture. Sustain. Aquac. Tech. 2014, 1, 135–172.
8. Gonçalves, A.; Gallardo-Escárate, C. Microbiome dynamic modulation through functional diets based on pre- and probiotics (mannan-oligosaccharides and Saccharomyces cerevisiae) in juvenile rainbow trout (Oncorhynchus mykiss). J. Appl. Microbiol. 2017, 122, 1333–1347.
9. Yuan, X.-Y.; Liu, W.-B.; Liang, C.; Sun, C.-X.; Xue, Y.-F.; Wan, Z.-D.; Jiang, G.-Z. Effects of partial replacement of fish meal by yeast hydrolysate on complement system and stress resistance in juvenile Jian carp (Cyprinus carpio var. Jian). Fish Shellfish Immunol. 2017, 67, 312–321.
10. Boonanuntanasarn, S.; Ditthab, K.; Jangprai, A.; Nakharuthai, C. Effects of Microencapsulated Saccharomyces cerevisiae on Growth, Hematological Indices, Blood Chemical, and Immune Parameters and Intestinal Morphology in Striped Catfish, Pangasianodon hypophthalmus. Probiotics Antimicrob. Proteins 2018, 11, 427–437.
11. Voloski, A.P.D.S.; Soveral, L.D.F.; Dazzi, C.C.; Sutili, F.; Frandoloso, R.; Kreutz, L.C. β-Glucan improves wound healing in silver catfish (Rhamdia quelen). Fish Shellfish Immunol. 2019, 93, 575–579.
12. Divya, M.; Gopi, N.; Iswarya, A.; Govindarajan, M.; Alharbi, N.S.; Kadaikunnan, S.; Khaled, J.M.; Almanaa, T.N.; Vaseeharan, B. β-glucan extracted from eukaryotic single-celled microorganism Saccharomyces cerevisiae: Dietary supplementation and enhanced ammonia stress tolerance on Oreochromis mossambicus. Microb. Pathog. 2020, 139, 103917.
13. Ji, L.; Fu, S.; Ji, R.; Li, X.; Liu, Y. β-glucan mitigated trinitrobenzene sulfonic acid-induced enteritis in the rainbow trout (Oncorhynchus mykiss). Aquaculture 2019, 513, 734393.
14. Orlean, P. Architecture and Biosynthesis of the Saccharomyces cerevisiae Cell Wall. Genetics 2012, 192, 775–818.
15. Aon, J.C.; Sun, J.; Leighton, J.M.; Appelbaum, E.R. Hypoxia-elicited impairment of cell wall integrity, glycosylation precursor synthesis, and growth in scaled-up high-cell density fed-batch cultures of Saccharomyces cerevisiae. Microb. Cell Factories 2016, 15, 1–16.
16. Lesage, G.; Bussey, H. Cell Wall Assembly in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 2006, 70, 317–343.
17. Ozhovan, S.M.; Knorre, D.A.; Severin, F.F.; Bakeeva, L.E. Ultrastructure of yeast cell Saccharomyces cerevisiae after amiodarone treatment. Cell Tissue Biol. 2010, 4, 90–95.
18. Reyes-Becerril, M.; Guardiola, F.A.; Sanchez, V.; Maldonado, M.; Angulo, C. Sterigmatomyces halophilus β-glucan improves the immune response and bacterial resistance in Pacific red snapper (Lutjanus peru) peripheral blood leucocytes: In vitro study. Fish Shellfish Immunol. 2018, 78, 392–403.
19. Guzmán-Villanueva, L.T.; Ascencio-Valle, F.; Macías-Rodríguez, M.E.; Tovar-Ramírez, D. Effects of dietary β-1,3/1,6-glucan on the antioxidant and digestive enzyme activities of Pacific red snapper (Lutjanus peru) after exposure to lipopolysaccharides. Fish Physiol. Biochem. 2013, 40, 827–837.
20. Elder, M.J.; Webster, S.J.; Chee, R.; Williams, D.L.; Gaston, J.S.H.; Goodall, J.C. β-Glucan Size Controls Dectin-1-Mediated Immune Responses in Human Dendritic Cells by Regulating IL-1β Production. Front. Immunol. 2017, 8, 791.
21. Petit, J.; Wiegertjes, G.F. Long-lived effects of administering β-glucans: Indications for trained immunity in fish. Dev. Comp. Immunol. 2016, 64, 93–102.

22. Reyes-Becerril, M.; Angulo, M.; Sanchez, V.; Machuca, C.; Méndez-Martínez, Y.; Angulo, C. β-Glucan bioactivities from Cystobasidium benthicum in Totoaba macdonaldi thymus cells. Fish Shellfish Immunol. 2021, 119, 542–553.
23. Khan, A.A.; Gani, A.; Masoodi, F.; Amin, F.; Wani, I.A.; Khanday, F.A.; Gani, A. Structural, thermal, functional, antioxidant & antimicrobial properties of β- d -glucan extracted from baker's yeast (Saccharomyces cereviseae)—Effect of γ-irradiation. Carbohydr. Polym. 2016, 140, 442–450.
24. Araújo, V.B.D.S.; De Melo, A.N.F.; De Souza, N.T.; Da Silva, V.M.B.; Castro-Gomez, R.H.; Silva, A.S.; De Souza, E.L.; Magnani, M. Oral Intake of Carboxymethyl-Glucan (CM-G) from Yeast (Saccharomyces uvarum) Reduces Malondialdehyde Levels in Healthy Men. Molecules 2015, 20, 14950–14958.
25. Yuan, H.; Lan, P.; He, Y.; Li, C.; Ma, X. Effect of the Modifications on the Physicochemical and Biological Properties of β-Glucan—A Critical Review. Molecules 2019, 25, 57.
26. Reyes-Becerril, M.; Angulo, M.; Sanchez, V.; Guluarte, C.; Angulo, C. β-D-glucan from marine yeast Debaryomyces hansenii BCS004 enhanced intestinal health and glucan-expressed receptor genes in Pacific red snapper Lutjanus peru. Microb. Pathog. 2020, 143, 104141.
27. Nguyen, T.H.; Fleet, G.H.; Rogers, P.L. Composition of the cell walls of several yeast species. Appl. Microbiol. Biotechnol. 1998, 50, 206–212.
28. Leger-Silvestre, I.; Gas, N. The Nucleolus. In The Nucleolar Ultrastructure in Yeast; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2004; pp. 21–28.
29. Zhu, F.; Du, B.; Bian, Z.; Xu, B. Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. J. Food Compos. Anal. 2015, 41, 165–173.
30. Bohn, J.A.; BeMiller, J.N. (1→3)-β-d-Glucans as biological response modifiers: A review of structure-functional activity relationships. Carbohydr. Polym. 1995, 28, 3–14.
31. Gopalakannan, A.; Arul, V. Enhancement of the innate immune system and disease-resistant activity in Cyprinus carpio by oral administration of β-glucan and whole cell yeast. Aquac. Res. 2009, 41, 884–892.
32. Adams, E.L.; Rice, P.J.; Graves, B.; Ensley, H.E.; Yu, H.; Brown, G.D.; Gordon, S.; Monteiro, M.A.; Papp-Szabo, E.; Lowman, D.W.; et al. Differential High-Affinity Interaction of Dectin-1 with Natural or Synthetic Glucans Is Dependent upon Primary Structure and Is Influenced by Polymer Chain Length and Side-Chain Branching. J. Pharmacol. Exp. Ther. 2008, 325, 115–123.
33. Liu, Y.; Tang, Q.; Zhang, J.; Xia, Y.; Yang, Y.; Wu, D.; Fan, H.; Cui, S.W. Triple helix conformation of β-d-glucan from Ganoderma lucidum and effect of molecular weight on its immunostimulatory activity. Int. J. Biol. Macromol. 2018, 114, 1064–1070.
34. Benito-Román, Ó.; Alonso, E.; Cocero, M.J. Ultrasound-assisted extraction of β-glucans from barley. LWT-Food Sci. Technol. 2013, 50, 57–63.
35. Bystryak, S.; Santockyte, R.; Peshkovsky, A.S. Cell disruption of S. cerevisiae by scalable high-intensity ultrasound. Biochem. Eng. J. 2015, 99, 99–106.
36. Sourki, A.H.; Koocheki, A.; Elahi, M. Ultrasound-assisted extraction of β-d-glucan from hull-less barley: Assessment of physicochemical and functional properties. Int. J. Biol. Macromol. 2017, 95, 462–475.
37. Ahmad, A.; Anjum, F.M.; Zahoor, T.; Nawaz, H.; Ahmed, Z. Extraction and characterization of β-d-glucan from oat for industrial utilization. Int. J. Biol. Macromol. 2010, 46, 304–309.
38. Islam, M.S.; Aryasomayajula, A.; Selvaganapathy, P.R. A Review on Macroscale and Microscale Cell Lysis Methods. Micromachines 2017, 8, 83.
39. Varelas, V.; Liouni, M.; Calokerinos, A.C.; Nerantzis, E.T. An evaluation study of different methods for the production of β-D-glucan from yeast biomass. Drug Test. Anal. 2015, 8, 46–55.
40. Javmen, A.; Grigiskis, S.; Gliebutė, R. β-glucan extraction from Saccharomyces cerevisiae yeast using Actinomyces rutgersensis 88 yeast lyzing enzymatic complex. Biologija 2012, 58, 51–59.
41. Dallies, N.; François, J.; Paquet, V. A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of Saccharomyces cerevisiae. Yeast 1998, 14, 1297–1306.
42. Zhu, F.; Du, B.; Xu, B. A critical review on production and industrial applications of beta-glucans. Food Hydrocoll. 2016, 52, 275–288.
43. Robinson, P.K. Enzymes: Principles and biotechnological applications. Essays Biochem. 2015, 59, 1–41.
44. Pionnier, N.; Falco, A.; Miest, J.J.; Shrive, A.K.; Hoole, D. Feeding common carp Cyprinus carpio with β-glucan supplemented diet stimulates C-reactive protein and complement immune acute phase responses following PAMPs injection. Fish Shellfish Immunol. 2014, 39, 285–295.

45. Kiron, V.; Kulkarni, A.; Dahle, D.; Vasanth, G.; Lokesh, J.; Elvebo, O. Recognition of purified beta 1,3/1,6 glucan and molecular signalling in the intestine of Atlantic salmon. Dev. Comp. Immunol. 2016, 56, 57–66.
46. Lauriano, E.; Pergolizzi, S.; Capillo, G.; Kuciel, M.; Alesci, A.; Faggio, C. Immunohistochemical characterization of Toll-like receptor 2 in gut epithelial cells and macrophages of goldfish Carassius auratus fed with a high-cholesterol diet. Fish Shellfish Immunol. 2016, 59, 250–255.
47. Pietretti, D.; Vera-Jimenez, N.; Hoole, D.; Wiegertjes, G. Oxidative burst and nitric oxide responses in carp macrophages induced by zymosan, MacroGard® and selective dectin-1 agonists suggest recognition by multiple pattern recognition receptors. Fish Shellfish Immunol. 2013, 35, 847–857.
48. Angulo, C.; Sanchez, V.; Delgado, K.; Reyes-Becerril, M. C-type lectin 17A and macrophage-expressed receptor genes are magnified by fungal β-glucan after Vibrio parahaemolyticus infection in Totoaba macdonaldi cells. Immunobiology 2018, 224, 102–109.
49. Ji, L.; Sun, G.; Li, X.; Liu, Y. Comparative transcriptome analysis reveals the mechanism of β-glucan in protecting rainbow trout (Oncorhynchus mykiss) from Aeromonas salmonicida infection. Fish Shellfish Immunol. 2019, 98, 87–99.
50. Mikrou, A.; Marioli, D.; Papanastasiou, A.D.; Zarkadis, I.K. CR3 complement receptor: Cloning and characterization in rainbow trout. Fish Shellfish Immunol. 2009, 26, 19–28.
51. Zhang, C.; Li, C.; Jia, X.; Wang, K.; Tu, Y.; Wang, R.; Liu, K.; Lu, T.; He, C. In Vitro and In Vivo Anti-Inflammatory Effects of Polyphyllin VII through Downregulating MAPK and NF-κB Pathways. Molecules 2019, 24, 875.
52. Wei, X.; Li, B.; Wu, L.; Yin, X.; Zhong, X.; Li, Y.; Wang, Y.; Guo, Z.; Ye, J. Interleukin-6 gets involved in response to bacterial infection and promotes antibody production in Nile tilapia (Oreochromis niloticus). Dev. Comp. Immunol. 2018, 89, 141–151.
53. Huo, H.J.; Chen, S.N.; Li, L.; Nie, P. Functional characterization of IL-10 and its receptor subunits in a perciform fish, the mandarin fish, Siniperca chuatsi. Dev. Comp. Immunol. 2019, 97, 64–75.
54. Zhu, Q.; Fan, Z.-J.; Cai, S.-X.; Yao, C.-L. Molecular and immunological characterizations of interleukin-11 in large yellow croaker (Larimichthys crocea). Fish Shellfish Immunol. 2020, 100, 9–17.
55. Piazzon, M.C.; Galindo-Villegas, J.; Pereiro, P.; Estensoro, I.; Calduch-Giner, J.A.; Gómez-Casado, E.; Novoa, B.; Mulero, V.; Sitjà-Bobadilla, A.; Pérez-Sánchez, J. Differential Modulation of IgT and IgM upon Parasitic, Bacterial, Viral, and Dietary Challenges in a Perciform Fish. Front. Immunol. 2016, 7, 637.
56. Danilova, N.; Bussmann, J.; Jekosch, K.; Steiner, L.A. The immunoglobulin heavy-chain locus in zebrafish: Identification and expression of a previously unknown isotype, immunoglobulin Z. Nat. Immunol. 2005, 6, 295–302.
57. Hansen, J.D.; Landis, E.D.; Phillips, R.B. Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout: Implications for a distinctive B cell developmental pathway in teleost fish. Proc. Natl. Acad. Sci. USA 2005, 102, 6919–6924.
58. Zhang, Y.-A.; Salinas, I.; Li, J.; Parra, D.; Bjork, S.; Xu, Z.; La Patra, S.E.; Bartholomew, J.; Sunyer, J.O. IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nat. Immunol. 2010, 11, 827–835.
59. Salah, A.S.; El Nahas, A.F.; Mahmoud, S. Modulatory effect of different doses of β-1,3/1,6-glucan on the expression of antioxidant, inflammatory, stress and immune-related genes of Oreochromis niloticus challenged with Streptococcus iniae. Fish Shellfish Immunol. 2017, 70, 204–213.
60. Angulo, C.; Alamillo, E.; Ascencio, F.; Reyes-Becerril, M. Characterization of nuclear factor of activated T-cells-c3 (NFATc3) and gene expression of upstream-downstream signaling molecules in response to immunostimulants in Pacific red snapper cells. Dev. Comp. Immunol. 2018, 78, 149–159.
61. Alamillo, E.; Reyes-Becerril, M.; Cuesta, A.; Angulo, C. Marine yeast Yarrowia lipolytica improves the immune responses in Pacific red snapper (Lutjanus peru) leukocytes. Fish Shellfish Immunol. 2017, 70, 48–56.