Chapter 5

Use of Yeasts as Probiotics in Fish Aquaculture

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1. Introduction

According to the UN Food and Agriculture Organization (FAO), "aquaculture, probably the fastest growing food-producing sector, now accounts for nearly 50 percent of the world's food fish" [1]. However, production is hampered by unpredictable mortalities that may be due to the negative interactions between fish and pathogenic bacteria. Intensive fish farming has resulted in a problematic growth in bacterial diseases, prompting the necessary and intensive use of antimicrobials for their treatment.

Because of the rapid expansion of aquaculture, a limited supply of fishmeal has the potential to impede the future growth of this industry. Consequently, much effort has been given to studying other protein and oil sources, but finding a suitable alternative has proved to be challenging. Among the alternatives, plant-based formulations are the least expensive, and many such formulations have a suitable protein profile and long-term availability. Oilseeds, in particular soybean and grain products, have great potential as alternative sources of fish feed. Soybeans are rich in protein and represent the most commonly used plant protein source on the world market. Soybean meal (SBM) has already become an important protein source in fish feed. However, the inclusion of some vegetable proteins, such as SBM, in the diets of fish at levels of >20% may induce intestinal disorders including pathomorphological changes in the distal intestinal epithelium accompanied by diarrhea [2, 3], sometimes caused by the anti-nutritional factors that are present in SBM. The addition of probiotics (acid lactic bacteria) to starter diets appeared to improve SBM utilization in first feeding rainbow trout [4].

In this context, two of the major challenges in fish aquaculture facilities are 1) the control of diseases, especially during the earliest life stages, and 2) the improvement of nutrition by optimizing food utilization, especially for new fish species.

It is well recognized that the bacterial microbiota of fish is beneficial to the host and affects important biological processes, including nutrient processing and absorption, the develop-

ment of the mucosal immune system, and angiogenesis, as was demonstrate in gnotobiotic mice. In larval gnotobiotic zebrafish studies, was shown that the microbiota also influences enterocyte morphology and epithelial renewal, host-transcriptional responses to the microbiota regarding epithelial proliferation, nutrient and xenobiotic metabolism, and immune responses [5].

Yeast have been identified as part of the normal microbiota of both wild and farmed fish, and their role in fish health and nutrition has been addressed in the literature, as yeast have been used either alive to feed live food organisms or after processing as a feed ingredient after demonstrating an artificial colonization of the intestinal host.

Even when accounting for less than 1% of the total microbial isolates in the host, yeast can represent a major physiological contribution beyond what has been observed for probiotic bacteria; in fact, cell volumes from yeast may be larger than those of bacteria by a hundredfold [6]. In contrast to bacteria, yeast cells utilize a wide spectrum of simple and more complex organic compounds. This phenomenon results from the extensive metabolic potential of yeast, which is reflected by the production of diverse enzymes. Polyamines secreted by yeasts are also involved in the maturation of the digestive tract of fish larvae. Furthermore, some yeast species and their components, such as β-glucans and mannoproteins, can stimulate the immune and antioxidant systems of the host. Understanding the participation of yeast microbiota in fish health and nutrition may improve both the sanitary conditions and the production performance of fish.

The aim of this chapter is to describe the current knowledge regarding the use of yeasts as probiotics in aquaculture systems. The chapter will include a recent review on the presence and diversity of yeast in marine and aquaculture systems, focusing on the yeast diversity found in the fish gut microbiota. The chapter will also include basic information on the molecular methods used for yeast identification. Finally, the chapter will emphasize topics related to the essential role of probiotic yeasts used in disease control and nutritional improvements in aquaculture, with a special focus on the beneficial effects of yeast β-glucans.

2. Yeast identified in the marine and other aquatic environments

Yeasts are unicellular eukaryotic microorganisms that are taxonomically placed within the phyla Ascomycota and Basidiomycota within the Kingdom Fungi [7]. Ascomycete yeasts comprise approximately 1,000 phylogenetically diverse species that have recently been assigned to 14 different lineages on the basis of multigene sequence analysis [8]. The other species of yeasts are classified as the basidiomycetes [9]. Some of the general characteristics and ecological properties of each phylum include the following: 1) the cell wall polysaccharide composition is dominated by chitin in the basidiomycetes and by β-glucans in the ascomycetes; 2) the guanine + cytosine (G + C) composition of the nuclear DNA tends to be higher than 50% in basidiomycetes and lower than 50% in ascomycetes; 3) ascomycetes yeasts are generally more fermentative, more copiotrophic (but at the same time nutritionally specialized), more fragrant, and mostly hyaline, while basidiomycete yeasts more often form mucoid colonies,

display intense carotenoid pigments and tend to use a broader range of carbon compounds more efficiently at lower concentrations [7]; and 4) ascomycete yeasts are often found in specialized niches involving interactions with plants and insects or other invertebrate animals that they rely upon for dispersal, while basidiomycete yeasts seem to be adapted to the colonization of nutrient-poor solid surfaces and may not rely to the same extent on animal vectors for their dispersal [10].

Marine samples	Identified yeast	Identification method	Reference
Northern Biscayne Bay	*Candida tropicalis* *Candida guilliermondii* *C. parapsilosis* *Rhodotorula rubra*	identification of cultivated yeasts by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]
Marine grass flats (Soldier Key)	*Rhodotorula pilimanne* *R. rubra* *Cryptococcus*	identification of cultivated yeasts by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]
Gulf Stream 15 miles east of the coast of South Florida.	*R. graminis* *R. glutinis*	identification of cultivated yeasts by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]
Marine vegetation	*Cryptococcus albidus*	identification of cultivated yeasts by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]
Suwannee Florida estuary (water)	*Candida guilliermondii* *Candida krusei* *Candida valida* *Cryptococcus laurentii var. laurentii* *Cryptococcus laurentii var. flavescens* *Hansenula saturnus var. saturnus* *Hansenula spp.* *Rhodotorula marina* *Rhodotorula minuta var. minuta* *Rhodotorula rubra* *Rhodotorula spp.* *Torulopsis candida* *Trichosporon cutaneum*	identification of cultivated yeasts by the methods described by Lodder [26].	[27]

Marine samples	Identified yeast	Identification method	Reference
Suwannee Florida estuary (sediment)	*Brettanomyces intermedius* *Candida boidinii* *Candida diversa* *Candida glaebosa* *Candida ingens* *Candida krusei* *Candida lambica* *Candida maritima* *Candida melibiosica* *Candida silvae* *Candida solani* *Candida valida* *Candida spp.* *Cryptococcus dimennae* *Cryptococcus laurentii var. laurentii* *Cryptococcus laurentii var. flavescens* *Debaryomyces cantarellii* *Debaryomyces phaffii* *Hansenula beijerinckii* *Hansenula saturnus var. saturnus* *Kluyveromyces polysporous* *Leucosporidium capsuligenum* *Pichia membranaefaciens* *Pichia ohmeri* *Rhodotorula glutinis* *Rhodotorula graminis* *Rhodotorula lactosa* *Rhodotorula marina* *Rhodotorula rubra* *Rhodotorula spp.* *Saccharomyces spp.* *Torulopsis candida* *Torulopsis inconspicua* *Torulopsis mogii* *Torulopsis spp.* *Trichosporon aculeatum* *Trichosporon cutaneum* *Trichosporon penicillatum*	identification of cultivated yeasts by the methods described by Lodder [26].	[27]

Marine samples	Identified yeast	Identification method	Reference
Western coast of Baja California Sur, Mexico	*Sporobolomyces roseus* *Sporobolomyces puniceus* *Sporobolomyces hosaticus*	morphological and biochemical identification	[28]
Suruga and Sagami Bay (sediments, crab and *Calyptogena*)	*Kluyveromyces nonfermentans*	18S rDNA, 5.8S rDNA and ITS sequencing	[29]
Northwest Pacific Ocean (benthic animals)	*R. aurantiaca* *R. glutinis* *R. minuta* *R. mucilaginosa* *Sporobolomyces salmonicolor* *S. shibatanus*	5.8S-ITS rDNA sequencing of cultivated yeasts	[30]
Northwest Pacific Ocean (sediments)	*R. glutinis* *R. mucilaginosa*	5.8S-ITS rDNA sequencing of cultivated yeasts	[30]
Sagami bay (deep-sea tubeworm)	*R. lamellibrachii*	sequencing of ITS, 5.8S rDNA, and D1/D2 of the 26S rDNA	[31]
Sagami Bay and Iheya Ridge (deep-sea tubeworm)	*R. bentica*	sequencing of ITS, 5.8S rDNA, and D1/D2 of the 26S rDNA	[32]
Sagami Bay (deep-sea clam)	*R. calyptogenae*	sequencing of ITS, 5.8S rDNA, and D1/D2 of the 26S rDNA	[32]
Deep-sea hydrothermal systems of the Mid-Atlantic Rift	*C. atlantica* *C. atmosphaerica* *C. lodderae* *C. parapsilosis* *D. hansenii* *P. guilliermondii* *Rhodosporidium babjevae* *R. diobovatum* *R. kratochvilovae* *R. sphaerocarpum* *R. toruloides* *Rh. Mucilaginosa* *Rh. minuta* *S. dacryoides*	26SrRNA gene sequencing of cultured yeasts	[33]
Northwest Pacific ocean	*R. pacifica*	sequencing of ITS, 5.8S rDNA, and D1/D2 of the 26S rDNA	[34]
11 deep-sea samples	*Pichia fermentans* *Saccharomyces cerevisiae* *Debaryomyces hansenii*	Cloning and sequencing	[35]

Marine samples	Identified yeast	Identification method	Reference
Japan Trench (deep-sea sediments)	*Dipodascus tetrasporeus*	Sequencing of 18S rDNA, ITS1, 5.8S rDNA, ITS2 and D1/D2 of the 26S rDNA	[36]
Coastal waters of northeastern Taiwan	*Candida tropicalis* *Pichia anomala* *Issatchenkia orientalis* *C. glabrata* *Saccharomyces yakushimaensis* *Kodamaea ohmeri* *Hanseniaspora uvarum* *Kazachstania jiainicus* *Torulaspora delbrueckii*	5.8S-ITS rDNA sequencing of cultivated yeasts	[37]
Deep-sea shrimps Deep-sea mussels	*R. mucilaginosa*	26SrRNA gene sequencing of cultured yeasts	[14]
R. exoculata exuviae in decomposition on smoker rocks, *B. azoricus* and a sponge	*Rhodosporidium diobovatum* *Sporobolomyces roseus*	26SrRNA gene sequencing of cultured yeasts	[14]
carbonate colonization module	*Cryptococcus uzbekistanensis*	26SrRNA gene sequencing of cultured yeasts	[14]
B. azoricus mussel	*Leucosporidium scottii*	26SrRNA gene sequencing of cultured yeasts	[14]
R. exoculata, *M. fortunata*, a deep-sea coral and the gills of the gastropod *Ifremeria nautilei*	*Debaryomyces hansenii*	26SrRNA gene sequencing of cultured yeasts	[14]
R. exoculata exuviae in decomposition *B. azoricus*	*Candida atlantica*	26SrRNA gene sequencing of cultured yeasts	[14]
Deep-sea sponge	*Pichia guilliermondii* *Candida viswanathii*	26SrRNA gene sequencing of cultured yeasts	[14]
Deep-sea coral	*Candida sp.*	26SrRNA gene sequencing of cultured yeasts	[14]
B. azoricus	*Phaeotheca triangularis* *Hortaea werneckii*	26SrRNA gene sequencing of cultured yeasts	[14]
Arabian sea (200 m depth)	*Candida* *Lipomyces* *Yarrowia*	morphological and biochemical identification	[38]

Marine samples	Identified yeast	Identification method	Reference
	Rhodotorula *Pichia*		
Arabian sea (500 m depth)	*Candida* *Yarrowia* *Lipomyces* *Rhodotorula* *Debaryomyces* *Pichia* *Wingea* *Dekkera*	morphological and biochemical identification	[38]
Arabian sea (1000 m depth)	*Lypomyces* *Candida* *Wingea* *Dekkera* *Rhodotorula*	morphological and biochemical identification	[38]
Bay of Bengal (200 m depth)	*Wingea* *Candida* *Cryptococcus* *Rhodotorula* *Bullera* *Lipomyces* *Oosporidium* *Dekkera*	morphological and biochemical identification	[38]
Bay of Bengal (500 m depth)	*Candida* *Rhodotorula* *Cryptococcus* *Yarrowia* *Pichia* *Bullera* *Wingea* *Dekkera* *Oosporidium*	morphological and biochemical identification	[38]
Bay of Bengal (1000 m depth)	*Candida* *Wingea* *Rhodotorula* *Bullera* *Lipomyces* *Trichosporon*	morphological and biochemical identification	[38]

Table 1. Yeast identified in the marine and other aquatic environment

Yeasts are widely distributed in several natural environments such as soil, freshwater, and seawater. Their numbers and species distributions are dependent on the concentrations and types of available organic materials. Nearshore environments are usually inhabited by 10 to 1000 of yeast cell/L of water, whereas low organic surface to deep sea oceanic regions contain 10 or fewer cells/L. Marine yeasts are divided into "obligate" and "facultative" groups. When yeast are able to grow on a marine substrate and are frequently collected from the marine environment, they are called "obligate marine" yeasts; in contrast, "facultative marine" yeasts can also be recovered from terrestrial habitats. Marine yeasts participate in several ecological processes in the sea, especially in estuarine and nearshore environments, such as the decomposition of plant substrates [11], nutrient recycling [12], and the biodegradation of oil/recalcitrant compounds [13]; they are also part of the microbiota of marine and aquaculture animals [6, 14]. This functional diversity is due, in part, to the fact that yeasts have extraordinary metabolic potential. This potential is available for exploitation [15-20], but notably, the vast majority of this potential has yet to be discovered. Several yeast compounds have significant biological value as reagents, cell proteins, vitamins, pigments, and enzymes. Different yeast species have been identified in several marine locations (Table 1). For excellent reviews on marine yeasts, see [21, 22]. The ascomycete yeasts *Debaryomyces hansenii*, and *Candida* spp. are typical ubiquitous species found in oceanic, and other aquatic environments. Basidiomycete yeasts often account for the majority of the total yeast population found in oligotrophic oceanic water. Among the basidiomycete yeasts, some species of *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* are widespread across various oceanic regions [22].

3. Yeast as part of the gut microbiota of fish

Most of the literature on the yeast microbiota of fish is based on the identification of cultivable yeast (Table 2). Yeast have been isolated from the gills, skin, mouths, feces and guts of different fish species. The occurrence of yeast in the fish gut is variable and can fluctuate from non-detectable levels to 10^7 CFU/g of intestinal content [6]. Both ascomycete and basidiomycete yeasts have been isolated from fish intestines (Table 2): among ascomycetes, *Saccharomycetaceae* (which include *Candida, Pichia, Saccharomyces,* and *Debaryomyces*) is likely the most important family, while basidiomycetes, include the genera *Rhodotorula, Cryptococcus, Sporobolomyces,* and *Trichosporon* [6]. The yeasts *Metschnikowia zobelii, Kloeckera apiculata,* and *Debaryomyces* sp. dominate in some marine fish (*Tachurus symmetricus* and *Atherinopis affinis littoralis*) [39], and in these fish species, the yeast concentration was significantly higher inside the fish than in the surrounding sea water, suggesting that the yeast may grow inside the fish intestine [39]. The ascomycetes *Debaryomyces hansenii*, *Candida* sp., and *Saccharomyces cerevisiae*, the basidiomycete *Leucosporidium* sp., and *Rhodotorula* have been frequently isolated as the dominant yeast found in the rainbow trout intestine [6]. Yeast can also be isolated from the waters of fishponds with different abundance and diversity depending on the season of the year. Fishponds from the Záhorie Lowlands in Slovakia, sampled in summer, harbor the most heterogeneous yeast species, with *Aureobasidium*, *Sporobolomyces*, *Candida*, and *Cryptococcus* as the most frequently isolated species [40]. In autumn, the yeast numbers were higher than in

summer, with *Candida, Hyphopichia burtonii*, *Aureobasidium pullulans*, *Hansenula anomala* and *Cryptococcus laurentii* being most frequently identified [40].

It has been reported that yeasts isolated from the intestine of rainbow trout may adhere to and grow in intestinal mucus [41]. Some yeast cells can colonize the intestine of fish after dietary introduction [42], and this ability to colonize may be related to cell surface hydrophobicity [43] and the ability of the strains to grow on mucus [41, 44]. Some experiments have shown that high levels of yeast intestinal colonization can be achieved when a pure culture of yeast is inoculated into fish. Rainbow trout and turbot were inoculated with *Rhodotorula glutinis* and *D. hansenii* HF1, and up to 3.8×10^4, 3.1×10^6, and 2.3×10^9 viable yeast cells/g of intestine or feces were recovered in three separate colonization experiments [45]. It is important to note that the majority of the studies published until 2007, on the yeast species identified from the aquaculture fish gut, were published by the Gatesoupe Lab [6]. Later, studies focused more on the probiotic effects of different yeast strains in aquaculture fish (as described below), and less on the actual yeast species isolated from the fish gut.

Location	Fish intestine	Identified yeast	Identification method	Reference
Estuarine and coastal areas (Biscayne Bay, Florida)	*Haemulon* *Carana* *Anisotremus*	*T. cutaneum* *C. parapsilosis* *C. guilliermondi* *C. tropicalis* *R. rubra* *R. pilimanae* *H. anomala* *D. kloeckeri* *II. valbyensis*	identification of cultivated yeast by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]
Tropical island-(Bimini, The Bahamas, 1960)	*Haemulon* *Stenotomus* *Ocyurus* *Anisotremus* *Lachnolainus*	*R. minuta* *C. parapsolosis* *R. glutinis*	identification of cultivated yeast by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]
Tropical island-(Bimini, The Bahamas, 1961)	*Ilaemulon* *Lutjanus* *Sphyraena* *Seriola* *Balistes* *Malacanthus* *Halichoeres* *Holocentrus* *Carana*	*C. tropicalis* *R. pilimanae* *Torulopsisspp.* *C. parapsilosis*	identification of cultivated yeast by the methods described by Lodder and Kreger-van Rij [23] and Wickerham [24].	[25]

Location	Fish intestine	Identified yeast	Identification method	Reference
	Anisotremus			
La Jolla coast	*Atherinopis affinis littoralis* *Trachurus symmetricus*	*Metschnikowia zobelliii* *Kloeckera apiculata* *Debaryomyces sp.*	identification of cultivated yeast by the methods described by Lodder and Kreger-van Rij [23], Wickerham [24], and van Uden and Farinha [46].	[39]
Clyde estuary North Sea	*Herring* *Haddock* *Whiting* *Skate* *Halibut* *Flounder* *Lemon sole*	*Candida* *Cryptococcus* *Debaryomyces* *Rhodotorula* *Torulopsis* *Trichosporon pullulans*	identification of cultivated yeast by the methods described by Lodder and Kreger-van Rij [23] and Kreger-van Rij [47].	[48]
Sweden farm	*Salmo gairdneri*	*S. cerevisiae* *D. hansenii* *Cryptococcus* *Leucosporidium* *Rhodotorula rubra* *R. glutinis*	Identified by the CBS Yeast Collection	[45]
Swedish west coast	*P. platessa* *P. flesus*	*Rhodotorula*	Identified by the CBS Yeast Collection	[45]
Experimental fish farm at Sizun (France)	*Oncorhynchus mykiss*	*Debaryomyces hansenii* *D. hansenii var. fabryi* *Trichosporon* *Rhodotorula mucilaginosa*	ITS 1, 5.8S rRNA gene, ITS 2, and partial sequencing of 26S rRNA gene	[49]
Experimental fish farm at Sizun (France)	*Oncorhynchus mykiss*	*Debaryomyces hansenii*	ITS 1, 5.8S rRNA gene, ITS 2, and partial sequencing of 26S rRNA gene	[42]
Cabras (Oristano, Sardinia)	*Mugil auratus* *M. chelo* *M. capito* *M. saliens* *M. cephalus*	*Candida* *Metschnikowia* *Sporidiobolus* *Clavispora* *Sporobolomyces*	morphological, physiological and biochemical tests of the isolated yeast strains according to [50]	[51]

Table 2. Yeast identified in the fish intestine

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4. *Debaryomyces hansenii*, an ubiquitous yeast frequently associated with fish and the marine environment

Debaryomyces hansenii is a halotolerant, non-pathogenic ubiquitous yeast capable of growing in a variety of environments, such as the marine environment and the fish gut. *D. hansenii* has been described as one of the most frequently isolated yeast associated with fish (Table 2). This species is prevalent in seawater, which may explain its high incidence in the fish gut. One study reported the presence of sequences affiliated with *D. hansenii* in hydrothermal sediments [35]. A major biotechnological advantage of *D. hansenii* over *Saccharomyces cerevisiae* is that *D. hansenii* is osmotolerant and can grow in media containing up to 4 M NaCl, whereas the growth of *S. cerevisiae* is restricted to media containing less than 1.7 M NaCl. *D. hansenii* has been extensively studied because of its significant enzymatic potential [52]. For example, several enzymes of biological and biotechnological interest have been identified and characterized in this yeast, including inulinase [53], protease [54], superoxide dismutase (SOD) [55], lipase [56], catalase [57] and α-galactosidase [58-62]. Additionally, β-glucosidase from *D. pseudopolymorphus* [63] and phytase from *D. castellii* [64] has also been identified. The ability of *D. hansenii* to synthesize α-galactosidase has been useful in the treatment of soybean products to reduce raffinose oligosaccharides [59], which are recognized as anti-nutritional factors for mammals and fish. Interestingly, *D. hansenii* SOD has been proposed as a therapeutic anti-inflammatory agent in animal models (Wistar rats) [55]. Because of these characteristics, *D. hansenii* is one of the yeast species that has been selected for complete sequencing [65]. The beneficial effects of this yeast species in cultured fish are described below.

5. Methods to analyze the yeast microbiota

In the past, the identification of yeast species was a tedious and labor-intensive process that was generally based on the morphological and physiological properties of the isolated yeasts. To resolve these problems, the identification of cultivated yeasts is now based on DNA sequence analysis, which is a faster and more accurate process. Sequence-based approaches to the study of yeast biodiversity have resulted in a two-fold increase in the number of described species over the past decade, and a 100-fold increase is predicted in the coming decades. A previous work [8] has studied the phylogenetics of the Saccharomycetales by performing DNA sequence analysis based on five loci: 1) the nuclear small subunit (SSU) ribosomal RNA gene, 2) the D1/D2 region of the nuclear large subunit (LSU) 26S rDNA, 3) the elongation factor 1α gene (EF-1α), and 4) the largest and 5) the second largest subunits of the RNA polymerase II gene (RPB1 and RPB2). Based on the availability of the sequence data in the GenBank and AFTOL databases, the LSU rDNA genes were found to be more reliable for yeast identification. Sequencing the 400-650 bp D1/D2 region or a wider LSU region is extremely useful and distinguishes yeast rapidly at a near-species level. The D1/D2 LSU rDNA region has been sequenced for almost all known yeast, both as an identification tool and as a means for estimating phylogenetic relationships among the Saccharomycetales. A previously study [66] published the sequences of the fungal primers ITS1 and ITS4, which amplify the

internal transcribed spacer (ITS) ITS1-5.8S-ITS2 region that has also been used for yeast identification. Some specialized databases are available on the web in order to help with yeast identification. The Centraal bureau voor Schimmelcultures (CBS) database aids BLAST analysis by allowing pairwise identification of LSU and SSU rDNA, ITS [67] and miscellaneous sequences [68].

It is generally accepted that in every ecosystem, there are cultivable organisms as well as viable organisms that cannot be cultivated in the laboratory. Less than 1% of the microbial species from the marine environment can be cultivated [69]. Similarly, only approximately 1% of yeast species has been described thus far [70]. In the last decades, several molecular methods have been developed to study natural samples. These methods allow for the identification of microorganisms without isolation and for the determination of the phylogenetic affiliation of community members, thus revealing the enormous extent of microbial diversity. Methods based on the amplification of fragments coding for the 16S rRNA gene have emerged as a powerful tool for studying the bacterial diversity. Denaturing or temperature gradient gel electrophoresis (PCR-DGGE/TTGE) techniques have been introduced into molecular microbial ecology to determine the genetic diversity of the bacterial communities found in the fish gut [71-76]. These techniques were also applied to characterize the dominant active bacteria in the intestine of different rainbow trout families using RNA that was extracted directly from the samples [77]. One important limitation of PCR-based methods, however, is low sensitivity, which can identify approximately 1% of the total number of species [78]. On the other hand, the use of rRNA gene fingerprinting requires sequencing of the cloned bands to identify the community's members accurately. Sequencing is necessary because the amplicons from different species may migrate to the same positions, or one species may give multiples bands because of multiple gene copies with intra-gene differences [74, 79]. The use of molecular approaches to study yeast communities has been scarce and generally limited to the study of food matrices. Yeast communities have been studied using PCR-DGGE, and amplification of a portion of the 26S rRNA gene of yeast [80-85], while PCR-TTGE has been applied to establish phylogenetic relationships of species of the genus *Saccharomyces* [86].

Recently, high-throughput sequencing methods, such as pyrosequencing, have been shown to be fast and very efficient tools for identifying members of the complex populations. In general, two approaches can be taken: diversity studies based on the sequencing of ribosomal gene (rRNA gene) amplicons, and metagenomic studies where whole-community DNA is subjected to shotgun sequencing [87]. While sequencing ribosomal amplicons is much cheaper because only one gene is being sequenced, the metagenomic approach sequences all of the DNA genes, thus revealing the functions of the microbial community [87]. A useful innovation for these two approaches is to analyze multiple samples at the same time (multiplexing), which can be accomplished using barecoded pyrosequencing or by physically separating the samples in the sequencing plates. In the barecode technique, the sequences in each sample are tagged with a unique barecode using barcoded primers during PCR amplification. The result of these high-throughput sequencing methods is several thousand sequences per sample in just few days, which must necessarily be analyzed using bioinformatics tools. Although the costs associated with these new technologies are less than for the Sanger method (considering the

cost for one sequence), high-throughput sequencing methods remain an expensive approach. To date, these methods have been applied to the study of the microbial diversity of several communities, especially from human and animal guts [88-93]. Diversity analysis targeting the D1/D2 domain of the 26S rRNA gene or the internal transcribed regions (ITSs) allow yeast species to be distinguished [8], and have recently been applied to the study of some fungal communities [94, 95], and to the identification of some clinical yeast isolates [96-98]. These methods appear to be very suitable for studying the yeast biodiversity in the fish gut. This new knowledge, together with the information available in various databases, will allow both the accurate identification of new yeast isolates and the application of molecular strategies to characterize the yeast population in the fish gut.

6. Use of yeast as probiotics in aquaculture: stimulation of the immune and antioxidant systems, gut maturation and fish growth

The natural occurrence of numerous yeast species in the gastrointestinal tract of healthy fish has been well described, and yeast have been shown to constitute an important part of the microbiota of the fish gut [6]. In addition to *S. cerevisiae*, the halotolerant yeast *D. hansenii* has been considered an excellent probiotic candidate in fish aquaculture. Because, the number of experiences reporting the use of *D. hansenii* is increasing, this allows us to know the capacity of this yeast to enhance growth, survival, and gut maturation and to improve of the immune and antioxidant systems in fish larvae and juveniles. Yeast cells can be a hundred times larger than bacterial cells, which may explain the fact that the introduction of a low yeast population (10^4 CFU/g) through feed can induce beneficial effects in the host.

The effects of probiotics differ greatly depending on the microbial species, isolation source, experimental concentration and finally, the rearing conditions of the fish. However, the improvement of the immune response is one of the most encountered side effects in the host because immune system stimulation or immunomodulation are considered important mechanisms supporting probiosis. Yeast have immunostimulatory properties because they possess components such as β-glucan, mannoproteins, chitin (as a minor component) and nucleic acids [99].

Recent studies have shown the beneficial effect of dietary administered *Saccharomyces cerevisiae* in fish. Yeast supplemented diets stimulate growth, feed efficiency, blood biochemistry, survival rate, and non- specific immune responses in *Uronema marinum*-infected olive flounder (*Paralichthys olivaceus*) [100]. A diet supplemented with *S. cerevisiae* treated with beta-mercaptoethanol was better than whole cell yeast and n-3 highly unsaturated fatty acids (HUFA)-enriched yeast as an immune system and growth stimulator in juvenile rainbow trout challenged with *Yersinia ruckeri* [101]. Similarly, the dietary administration of the probiotic *S. cerevisiae* P13 at a minimum level of 10^5 CFU/kg enhanced the growth, innate immune responses and disease resistance of grouper (*Epinephelus coioides*) [102]. Cellular yeast components also stimulate the immune system: an improvement in gut mucus lysozyme activity was observed in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS)

derived from the outer cell wall of a select strain of *S. cerevisiae* (Bio-Mos, Alltech Inc, USA) [103]. Furthermore, channel catfish (*Ictalurus punctatus*) juveniles fed diets supplemented with whole-cell *S. cerevisiae* (Levucell SB20®) or yeast subcomponents such as commercial preparations of β-glucan (MacroGard ® and Betagard A ®) had a significantly higher survival rate after *Edwardsiella ictaluri* challenge than did catfish fed with a controlled diet [104].

As previously described, most published studies have been performed using *S. cerevisiae*; however, promising results have also been obtained with *Debaryomyces hansenii*. A diet supplemented with *D. hansenii* stimulates the immune system of juvenile leopard grouper, *Mycteroperca rosacea*, by increasing IgM and superoxide dismutase (SOD) activity and enhances the resistance of the fish to infection by the dinoflagellate, *Amyloodinium ocellatum* [105]. Additionally, other studies have demonstrated the immune system improvement of *M. rosacea* when the fish were fed for 4 weeks with a compound diet enriched with 1.1% *D. hansenii*. After the 4 weeks, the fish were challenged with the pathogenic bacteria *Aeromonas hydrophila* strain Ah-315, resulting in an increase in IgM levels as well as catalase (CAT) and SOD activities in those fish fed yeast diets. Improvements were also observed at the molecular level, where CAT and HSP70 expression levels were enhanced in *M. rosacea* fed with *D. hansenii*, and challenged with *A. hydrophila* [106].

D. hansenii administration to the gilthead seabream (*Sparus aurata* L.) significantly enhances leukocyte peroxidase and respiratory burst activity by week 4 of feeding with yeast. Yeast feeding causes an up-regulation in the expression of the immune-associated genes Hep, IgM, TCR-ß, NCCRP-1, MHC-IIa, CSF-1R, C3, TNF-α and IL-1ß in the head-kidney: C3 expression was only stimulated in the liver, whereas the expression of TCR-ß, TNF-α and C3 was stimulated in the intestine of *S. aurata* [107].

When *D. hansenii* was administered at 1.1% in a compound diet to *D. labrax* larvae, the yeast stimulated the antioxidant status [108]. The group fed with yeast showed lower glutathione peroxidase (GPX) and SOD activity compared to fish fed the control diet, suggesting a possible involvement of superoxide anion retention in fish larvae, which could represent importance to the host to increase cell or tissue responsiveness to growth- and/or differentiation-enhancing factors [109]. The group fed the control diet showed oxidative stress represented by an increase in GPX activity at 48 days post hatching (dph) and gene expression levels for both GPX and SOD at 23 dph.

The ontogeny of the digestive tract of fish larvae has been the subject of many studies in the last decades with the purpose of increasing production rates by reducing the bottlenecks in larviculture. In this sense, the number of reports concerning the use of yeast to enhance gut maturation and digestive enzyme activity in fish are also increasing. The activity and expression of digestive enzyme-related genes during fish development provides an excellent marker of digestive development in fish larvae. The enzymes secreted from the pancreas (trypsin, lipase, and amylase) as well as those encountered in the intestinal brush border membranes (BBM) (leucine aminopeptidase N, alkaline phosphatase, maltase, γ-glutamyl-transpeptidase, and the cytosolic leucine-alanine peptidase), are the most common indicators of digestive system maturation in fish larvae. The degree of enterocyte maturation is described by increasing ratios of activities of BBM enzymes *vs* cytosolic leucine alanine peptidase; in the case of

pancreatic enzymes, a decrease in amylase activity or expression level with the concomitant increase in trypsin or lipase activities characterizes the maturation of the exocrine pancreas.

Given this previous work, the effect of *D. hansenii*-enriched microparticulated diets were tested in European sea bass larvae. The authors observed an increase in intestinal brush border and pancreatic enzyme activities at 27 dph, indicating an achievement of gut morphology in this larvae stage compared with the control diet lacking yeast [110]. In a second feeding trial, where the introduction of yeast to the microparticulated diet was improved, larvae fed 1% *D. hansenii* matured earlier than fish fed a control diet after day 26, as revealed by lower amylase expression, higher lipase and trypsin expression, and high levels of the BBM enzymes, aminopeptidase N, maltase and alkaline phosphatase [111].

In another study [42], two yeast strains: *Saccharomyces cerevisiae* and *S. boulardii*, were evaluated as probiotics for rainbow trout (*Oncorhynchus mykiss*) fry to compare the cross effects of the two rearing conditions, with the intestinal microbiota and the brush border enzyme activities. Intestinal maturation at 10 dph was observed in trout fed *S. boulardii*, and kept in spring water, and these fish displayed the highest ratios of BBM leucine aminopeptidase N *vs* leucine-alanine peptidase, compared with those fish fed *S. cerevisiae*, and kept in river water.

Overall, yeast has been added directly to the water, administered as an additive in microparticulated diets, and has been used alive to feed live food (rotifers or *Artemia*) as a possible vector to deliver yeast into the gut of fish larvae. Rotifers have been established as the most common live prey to feed larval fish in hatcheries around the world, and baker's yeast (*S. cerevisiae*) is the most common nutrient source for culturing rotifers in addition to algae, emulsified oils or bacteria. Currently, efforts are being made to introduce *D. hansenii* into *Brachionus rotundiformis* to deliver yeast into the intestine of *L. guttatus* to accelerate digestive maturation [112].

In this regard, the use of Levucell® (*S. boulardii*), Bactocell® (*Pediococcus acidilactici*) and live yeast (*S. cerevisiae*) produced no significant effect on trypsin, lipase, and leucine aminopeptidase activities in California halibut larvae, *Paralichtys californicus*, at 46 dph. Contrary to this, an increase in pepsin and chymotrypsin activity was only observed in fish larvae fed Bactocell® at the final endpoint of the experiment (46 days), suggesting a potential use of these probiotics once metamorphosis is completed [113].

In fish aquaculture, the most utilized growth-promoting additives are hormones, antibiotics, ionophores, and salts [114]. The use of probiotics as growth-promoters has been recognized in the last decade with a number of studies related to this topic being published. Probiotics can be used as an alternative to avoid the use of antibiotics for growth promotion, thus eliminating the possibility of generating antibiotic-resistant bacteria in the aquaculture systems. When yeast probiotics have been used in the earliest developmental stages of fish larvae, enhanced growth and survival have been observed. Several yeast species have been documented to enhance growth following artificial colonization, particularly *S. cerevisiae* and *D. hansenii* either alone, or in synergic association with bacteria. One study [115] observed that pollock (*Pollachius pollachius*) larvae grew better when *Artemia nauplii* was first treated with *S. cerevisiae* var. *boulardii* CNCM I-1079 and then with *Pediococcus acidilactici* MA185 M than did larvae fed with one or no probiotic. When *S. cerevisiae* was used alone in feeding trials, it improved feed

efficiency in Israeli carp and Nile tilapia [116, 117]. In addition to growth enhancement, an improvement in the conformation of the larvae was also observed in *D. labrax* fed with *D. hansenii*; a reduction in spinal deformity from 13.6% to 1.1% in fish fed yeast *vs* the control group was observed [111].

The use of probiotics in well-established fish industry is easier of because the existence of many individuals to experiment upon (*S. salar*, *D. labrax*, *S. aurata*, *S. maximus*, *O. mykiss*, etc). Nevertheless, it is advisable to use probiotics in species with the potential for exploitation to optimize the results for growth, survival and health. In this context, several studies have been performed in fish species with emerging aquaculture potential to contribute to the establishment of a continuous production line for experimental purposes. A commercial preparation of live yeast (*S. cerevisiae* and *Lactobacillus coagulans*) was tested on Indian carp fry, *Labeo rohita,* with no conclusive effects on growth [118]. *D. hansenii* has been observed to function as a growth promoter in *Mycteroperca rosacea* juveniles because after 4 weeks of continuous feeding, a weight gain (33%) and condition factor were observed in those fish fed a microparticulated diet enriched with yeast, compared with those fish fed without yeast [106]. In spotted sand bass larvae, *Paralabrax maculatofasciatus*, the highest survival (13.0%) was obtained with *D. hansenii* enriched microparticulated diets, but no effects on growth were observed with the use of probiotics [119].

At present, much of the existing evidence indicates that yeast promotes growth, and survival because of gut maturation, conformation of the larvae, and stimulation of the immune system by a possible involvement of endoluminal yeast-secreted polyamines in the host. As was earlier demonstrated, *D. hansenii* produces more polyamines (putrescine, spermidine and spermine) than *S. cerevisiae* and *S. boulardii* [111]. Polyamines participate in several physiological processes such as cell proliferation and differentiation and appear to have a broad influence on digestive tract maturation. In particular, the roles of dietary spermine and spermidine have been previously described [120]. These molecules enter enterocytes, where they induce a hormonal cascade that affects organs such as the pancreas and liver. Recently, the production of polyamines in 13 strains of *D. hansenii*, isolated from different sources, using high-pressure liquid chromatography (HPLC) has been reported [121]. In this study, they found that the L2, and CBS004 strains isolated from citrus fruit and marine water, respectively, were the main polyamine-secreting yeasts. Later, L2 strain was shown to have a probiotic effect because it enhanced the immune status, and intestinal function of gilthead seabream, *Sparus aurata* [122].

Finally, evidence of polyamine contribution to larviculture performance was reported when the spotted sand bass larvae, *P. maculatofasciatus* fed with *D. hansenii* with un-inhibited ornithine decarboxylase (ODC) activity had precocious digestive maturation compared to those larvae fed ODC-inhibited (with α-difluoromethylornithine (DFMO)) yeast [119]. Ornithine decarboxylase, which catalyzes the formation of putrescine, is the rate-limiting enzyme in the biosynthesis of polyamines in cells.

7. Yeast β-glucans: Structure, mechanisms of action and its application as immunostimulant in aquaculture

The glucose polymer β-glucan is a major structural component of the cell wall of some plants (such as the cereals oat and barley), seaweeds, and the outer cell wall of bacteria, fungi and yeast. Different β-glucans vary in structure, size, branching frequency, structural modifications, conformation and solubility, which may influence their physiological functions. Glucose molecules, in all β-glucan polymers, are linked together by a β-(1→3) linear β-glycosidic chain core, but differ in their length and branching structures. For example, the β-glucans from oat and barley are linear with β-(1→4) linkages, and shorter stretches of β-(1→3) linkages, while the structure of yeast β-glucans is composed of β-(1→3)-D-glucans with β-(1→6)-glycosidic linked branches, which apparently corresponds to the most active form of β-glucan. The relationship between structure and biological activity is controversial, but it appears that large molecular weight β-glucans are the most active compared with small β-glucans below 5,000-10,000 Da that are generally inactive. The solubility of β-glucans also influences their biological activity, with soluble β-glucans appearing to be more active.

The consumption of β-glucans has been associated with beneficial health effects in humans, including anticancer properties [123], metabolic syndrome prevention [124, 125], cholesterol-lowering effects [126], anti-atherogenic properties [127] and skin health promotion [128]. *In vitro* and *in vivo* studies in animals and humans show that the β-glucans derived from fungi and yeasts in particular, have interesting immune modulating properties [129-132]. This immune stimulation can be achieved when β-glucans are administered by a parenteral or an oral (dietary) route.

Despite their structural versatility, β-glucans are highly conserved structural components and belong to a group of physiologically active compounds called biological response modifiers [133]. Because of their large molecular weight, they cannot penetrate the cell membrane and therefore they must interact with cell-surface receptors; it has been shown that β-glucans are recognized by several receptors found on neutrophils, macrophages, and dendritic cells [129, 134]. Additionally, β-glucans belong to the group of non-self-molecules called pathogen-associated molecular patterns (PAMPS), which are recognized by pattern recognition receptors (PRRs) on the cell surface [135]. The principal β-glucans PRRs are dectin-1 and the toll-like receptors (TLRs), but other receptors are suggested to be involved, such as scavenger receptors, complement receptor 3, and lactosylceramide [136, 137]. Dectin-1 specifically recognizes β-(1→3)(1→6) glucans from fungi, plants, and bacteria [138], but it is not reactive toward β-(1→4) glucans or α-mannan [139]. The stimulation of dectin-1 activates the innate immune response, ROS and inflammatory cytokine production [140] through the activation of phospholipase C [141], the PI3K/Akt pathway, MAPK, NFAT, and NF-κB [142]. The interaction of β-glucans with TLRs results in activation of NF-κB and MAPK signaling [143]. Zymosan (β-glucans from the yeast *Saccharomyces cerevisiae*) binds to TLR2 and TLR4 (as well as dectin-1) found on macrophages, leading to an increase in cytokine production, such as TNF-α and IL-12, through the NF-κB pathway [143]. The β-glucan (β-(1→3) (1→6)-D-glucan) from *Aureobasidium pullulans* ADK-34 stimulates intestinal Peyer's patch cells both *in vitro* and *in vivo* as reflected

by an increase in IL-5, IL-6, and IgA [129]. The interaction of β-glucans with specific receptors on macrophages and dendritic cells can induce the production of several cytokines, which in turn activate other immune cells such as B and T cells, thus activating the systemic immune response.

Yeast β-glucans have been applied in aquaculture to modulate the innate immune system of fish to improve their survival until adaptive immune responses are sufficiently developed to mount effective responses against pathogens [144, 145]. If the β-glucans are administered as feed additives, they can exert their primary effects at the intestinal level through the induction of cytokines, which in turn affect the systemic immune response in fish. Different sources of β-glucans have been evaluated, although the most frequent sources are those obtained from the baker's yeast, *Saccharomyces cerevisiae.* Some commercial preparations of β-glucan (MacroGard ®, Betagard A ®, EcoActiva™, Nutriferm ™, BG, Fibosel®, etc) are available on the market that can be used in aquaculture. Many studies have explored the *in vitro* response of the macrophage to β-glucan [146, 147], while other studies have addressed the *in vivo* effect of β-glucans in different fish species (Table 3). The β-glucans from several sources have been administered to fish via the oral or intra-peritoneal route with different effects. Many studies have shown the immune effects of β-glucans specifically on antibody production, expression of immune system genes, survival, resistance to infectious diseases, and improvement in stress resistance. The growth enhancement of fish has also been observed as another beneficial effect of β-glucans (Table 3).

Recently, juvenile channel catfish (*Ictalurus punctatus*) fed diets supplemented with whole-cell *Saccharomyces cerevisiae* (Levucell SB20®) or yeast subcomponents such as commercial preparations of β-glucan (MacroGard ® and Betagard A ®) had a significantly higher survival rate after challenge with *Edwardsiella ictaluri* than did catfish fed with a control diet [104]. Atlantic cod (*Gadus morhua* L.) were fed for 5 weeks with a purified β-glucan product [148], after which the fish were bath-challenged with the bacterial pathogen, *Vibrio anguillarum*. The transcription of selected cytokines (proinflammatory: IL1-β, IL-8, IFNγ; anti-inflammatory: IL-10) in different intestinal segments was analyzed using qPCR, and the β-glucan product was found to have a differential effect on the expression of the cytokine genes. In the anterior intestine and rectum, the β-glucan significantly elevated the expression of IL-1β when challenged with *V. anguillarum*. Moreover, an effect on the anti-inflammatory cytokine IL-10 was also visible in the rectum after the pathogen challenge. The differential responses of cytokines in the intestine of fish upon exposure to *V. anguillarum* suggests that β-glucans impact the ability of Atlantic cod to respond to the pathogen [148].

In another recently study, different concentrations of the yeast β-glucan preparation MacroGard ® (0.1%, 1% or 2%) were orally administered to mirror carp (*Cyprinus carpio* L.) for 8 weeks [149]. Fish fed diets containing 1% and 2% MacroGard® showed significant improvements in weight gain, specific growth rate and feed conversion ratio compared to fish fed both the control and the 0.1% MacroGard® containing diet. At the end of the experiment, the haematocrit value was significantly elevated in fish fed the 2% MacroGard® diet, compared to the control fed fish, with the blood monocyte fraction significantly higher in fish fed the 1% and 2% MacroGard® diets [149].

Zebrafish (*Danio rerio*) have been suggested as a model aquacultured fish, especially for genetic [150], nutritional and comparative growth studies [151]. Furthermore, zebrafish have been suggested as a model for pathogen studies in finfish [152]. Yeast β-glucans have also been evaluated in the zebrafish (*Danio rerio*) model with promising results [153]. In the study, a 5 mg/ml β-glucan preparation derived from *S. cerevisiae* was injected intra-peritoneally into adult zebrafish, leading to a significant reduction in mortality after challenge with *Aeromonas hydrophila*. In zebrafish treated with β-glucan, the ability of kidney cells to kill *A. hydrophila* was enhanced. Moreover, the myelomonocytic cell population in the kidney at 6 h post-challenge with *A. hydrophila* was increased. The β-glucan also appears to modulate the expression of IFN-γ and chemokines in the kidney [153].

Recently, the effect of β-glucan (derived from yeast, Fibosel® (Lallemand) on the growth performance and antioxidant enzyme activity in red snapper (*Lutjanus peru*), before and after exposure to lipopolysaccharides (LPS), was investigated. The fish were fed commercial diets with 0.0%, 0.1% and 0.2% Fibosel® for 6 weeks, after which, LPS was injected intra-peritoneally. The results showed a significant increase in growth performance after 6 weeks of β-glucan feeding; the SOD activity was also significantly higher in diets containing 0.1% β-glucan in weeks 4 and 6 with respect to the control group. At 72 h after injection of LPS, samples showed a significant increase in CAT activity in fish fed diets supplemented with 0.2% β-glucan and SOD activity increased under diets containing 0.1% and 0.2% β-glucan compared to controls [154]. To explain the enhanced growth, the authors suggested that some bacterial populations modify the host's digestive enzyme activity through their ability to produce and liberate exogenous digestive enzymes, as was previously observed [155]. Other authors reported that polysaccharides used as prebiotics can stimulate the growth of beneficial microbiota in fish [156].

β-glucan sources	Administration route	Fish species	Biological effects	Ref.
β-glucan (Aqua-In-Tech, Inc.)	Oral	Nile tilapia (*Oreochromis niloticus*)	no effect	[157]
Betagard A®	oral	Nile tilapia (*Oreochromis niloticus*)	Immune modulation	[158]
S. cerevisiae (Hang Zhou Bio-Technology Co)	oral	Nile tilapia (*Oreochromis niloticus*)	Immune modulation	[159]
MacroGard® Betagard A®	oral	Channel catfish (*Ictalurus punctatus*)	improvement in stress resistance	[160]
MacroGard® Betagard A®	oral	Channel catfish (*Ictalurus punctatus*)	Immune modulation	[104]
Saccharomyces cerevisiae	oral	Large yellow croaker (*Pseudosciaena crocea*)	Immune modulation growth enhancement	[161]
MacroGard® Zymosan	oral	Fathead minnows (*Pimephales promelas*)	Immune modulation	[162]

β-glucan sources	Administration route	Fish species	Biological effects	Ref.
GY (Sigma)* GB (Sigma)**				
Saccharomyces cerevisiae	intra-peritoneal	Atlantic salmon (*Salmo salar* L)	Immune modulation	[163]
Saccharomyces cerevisiae	oral	Atlantic salmon (*Salmo salar* L)	Enhancement of salmon lice resistance	[164]
marine diatom *Chaetoceros mülleri*	oral	Atlantic cod (*Gadus morhua* L.)	Survival and growth enhancement	[165]
β-1,3/1,6 glucan: BG (Biorigin Europe, Oslo, Norway)	oral	Atlantic cod (*Gadus morhua* L.)	Immune modulation	[148]
Barley	intra-peritoneal	Rohu (*Labeo rohita)*	Immune modulation	[166]
β-glucan (Sigma)	oral	Rohu (*Labeo rohita)*	Immune modulation	[167]
β-1,3 glucan (Sigma)	oral	Rohu (*Labeo rohita*)	Immune modulation	[168]
β-1,3 glucan (Sigma)	oral	Rohu (*Labeo rohita*)	Immune modulation	[169]
Yeast cell wall preparation from *Saccharomyces cerevisiae* (Nutriferm ™)	oral	Rohu (*Labeo rohita*)	Immune modulation	[170]
β-1,3 glucan (Sigma)	oral	Asian catfish (*Clarias batrachus*)	Immune modulation	[171, 172]
Glucan (Taito Co.Ltd., Tokyo, Japan)	oral	Rainbow trout (*Oncorhynchus mykiss*)	Decrease in plasmatic cortisol	[173]
MacroGard®	oral	Rainbow trout (*Oncorhynchus mykiss*)	Immune modulation	[174]
MacroGard®	oral	Rainbow trout (*Oncorhynchus mykiss*)	Immune modulation	[175]
Barley	oral	Rainbow trout (*Oncorhynchus mykiss*)	Immune modulation	[176]
β(1,3)-D-glucan (laminaran) from *Laminaria hyperborea*	intra-peritoneal	Rainbow trout (*Oncorhynchus mykiss*)	Immune modulation	[177]
β(1,3)-D-glucan (laminaran) from *Laminaria hyperborea*	inmersion	Rainbow trout (*Oncorhynchus mykiss*)	Immune modulation	[178]

β-glucan sources	Administration route	Fish species	Biological effects	Ref.
Saccharomyces cerevisiae	intra-peritoneal oral	Rainbow trout (*Oncorhynchus mykiss*)	Immune modulation	[179]
Saccharomyces cerevisiae	intra-peritoneal	Carp (*Cyprinus carpio*)	Immune modulation	[180, 181]
Saccharomyces cerevisiae	intra-peritoneal oral	Carp (*Cyprinus carpio*)	Immune modulation	[182]
Saccharomyces cerevisiae	oral	Carp (*Cyprinus carpio*)	Immune modulation	[183]
MacroGard®	oral	Carp (*Cyprinus carpio*)	Immune modulation	[184]
MacroGard®	oral	Carp (*Cyprinus carpio*)	apoptosis modulation	[185]
MacroGard®	oral	Carp (*Cyprinus carpio*)	Growth enhancement	[186]
Saccharomyces cerevisiae	intra-peritoneal	Zebrafish (*Danio rerio*)	Immune modulation	[153]
MacroGard®	oral	Sea bass (*Dicentrarchus labrax*)	Immune modulation	[187]
MacroGard®	oral	Sea bass (*Dicentrarchus labrax*)	Immune modulation	[188]
MacroGard®	oral	European sea bass (*Dicentrarchus labrax*)	Immune modulation	[189]
EcoActiva™	oral	Pink snapper (*Pagrus auratus*)	growth enhancement Immune modulation	[190]
oyster mushroom (*Pleurotus florida*)	intra-peritoneal	Catla (*Catla catla*)	Immune modulation	[191]
Poria cocos	oral	*Ctenopharyngodon idella*	Immune modulation	[192]
Fibosel® (Lallemand) *Saccharomyces cerevisiae*	oral	*Lutjanus peru*	growth enhancement Immune modulation	

*GY: β-1,3-glucan from baker's yeast
**GB: β -1,3-glucan from barley

Table 3. Biological effect of different β-glucans in fish

8. Conclusions

It is interesting to note that even after several decades of investigation, the potential of yeast, especially those of marine origin, has not yet been fully exploited. Yeasts can be part of the gut microbiota of wild and cultivated fish; however, more information derived using molecular approaches, is needed regarding the yeast composition in fish. Although much has been reported on the molecular aspects of yeasts, the exploration of the complete yeast community through the analysis of yeast DNA or RNA is lacking. The application of such methodologies will provide us with an overview of the non-cultivated yeasts, which could play a major role in the fish host. Different enzymes can be synthesized by yeast that have biotechnological potential, but the direct contribution of this potential to fish nutrition must be explored. In contrast, several publications confirm the beneficial probiotic effects of yeast in aquaculture, but the majority of these studies are focused on two species: *S. cerevisiae* and *D. hansenii*. The identification of new yeast species/strains from other cultured fish species is required to explore new beneficial properties to improve fish health and nutrition for a more sustainable aquaculture.

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References

[1] FAO. http://www.fao.org/fishery/aquaculture/en (accessed 5 April 2013)

[2] Rumsey GL, Siwicki AK, Anderson DP, Bowser PR. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Vet Immunol Immunopath 1994;41(3–4) 323-39.

[3] Van den Ing T, Olli JJ, Krogdahl A. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. Aquaculture 1991;94 297-305.

[4] Sealey WM, Barrows FT, Smith CE, Overturf K, LaPatra SE. Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout *Oncorhynchus mykiss* to utilize high levels of soybean meal during grow-out. Aquaculture 2009;293(3-4) 195-203.

[5] Rawls JF, Samuel BS, Gordon JI. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. P Natl Acad Sci USA 2004;101(13) 4596-601.

[6] Gatesoupe FJ. Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture 2007;267(1-4) 20-30.

[7] Kurtzman CP. Molecular taxonomy of the yeasts. Yeast 1994;10(13) 1727-40.

[8] Suh SO, Blackwell M, Kurtzman CP, Lachance MA. Phylogenetics of Saccharomycetales, the ascomycete yeasts. Mycologia 2006;98(6) 1006-17.

[9] Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS yeast research 2002;2(4) 495-517.

[10] Lachance MA, Starmer WT. Ecology and yeasts. In: Kurtzman CP, Fell JW, editors. The Yeasts, a Taxonomic Study. Amsterdam: Elsevier; 1998. p. 21-30.

[11] Raghukumar S. The Role of Fungi in Marine Detrital Processes. In: Ramaiah N, editor. Marine Microbiology: Facets & Opportunities: National Institute of Oceanography, Goa; 2004. p. 91-101.

[12] Rhishipal R, Philip R. Selection of marine yeasts for the generation of single cell protein from prawn-shell waste. Bioresource Technol 1998;65(3) 255-6.

[13] Rocha LL, Cordeiro RDA, Cavalcante RM, do Nascimento RF, Martins SCS, Santaella ST, et al. Isolation and characterization of phenol-degrading yeasts from an oil refinery wastewater in Brazil. Mycopathologia 2007;164(4) 183-8.

[14] Burgaud G, Arzur D, Durand L, Cambon-Bonavita MA, Barbier G. Marine culturable yeasts in deep-sea hydrothermal vents: species richness and association with fauna. Fems Microbiol Ecol 2010;73(1) 121-33.

[15] Chi ZM, Chi Z, Zhang T, Liu GL, Li J, Wang XH. Production, characterization and gene cloning of the extracellular enzymes from the marine-derived yeasts and their potential applications. Biotechnol Adv 2009;27(3) 236-55.

[16] Lee JK, Park KS, Park S, Park H, Song YH, Kang SH, et al. An extracellular ice-binding glycoprotein from an Arctic psychrophilic yeast. Cryobiology 2010;60(2) 222-8.

[17] Li J, Chi ZM, Wang XH, Peng Y, Chi Z. The selection of alkaline protease-producing yeasts from marine environments and evaluation of their bioactive peptide production. Chin J Oceanol Limn 2009;27(4) 753-61.

[18] Peng Y, Chi ZM, Wang XH, Li J. Purification and molecular characterization of exo-beta-1,3-glucanases from the marine yeast *Williopsis saturnus* WC91-2. Appl Microbiol Biot 2009;85(1) 85-94.

[19] Song CL, Chi ZM, Li J, Wang XH. beta-Galactosidase production by the psychrotolerant yeast *Guehomyces pullulans* 17-1 isolated from sea sediment in Antarctica and lactose hydrolysis. Bioproc Biosyst Eng 2010;33(9) 1025-31.

[20] Song CL, Liu GL, Xu JL, Chi ZM. Purification and characterization of extracellular beta-galactosidase from the psychrotolerant yeast *Guehomyces pullulans* 17-1 isolated from sea sediment in Antarctica. Process Biochem 2010;45(6) 954-60.

[21] Kutty SN, Philp R. Marine yeasts - a review. Yeast 2008;25(7) 465-83.

[22] Kandasamy K, Alikunhi N, Subramanian M. Yeasts in marine and estuarine environments. Journal of Yeast and Fungal Research 2012;3(6) 74-82.

[23] Lodder J, Kreger-van rij JW. The Yeasts. A taxonomic study. Amsterdam, Holland.: North Holland Publ. Co.,; 1952.

[24] Wickerham L. Taxonomy of yeasts. U S Dept Agr Tech Bull 1951;1029 1-55.

[25] Roth F, Ahean D, Fell JW, Meyers S, Meyer S. Ecology and taxonomy of yeasts isolated from various marine substrates. Limnology and Oceanography 1962;7(2) 178-85.

[26] Lodder J. The yeasts. A taxonomic study. Amsterdam: North- Holland Publishing Co.; 1970.

[27] Lazarus C, Koburger J. Identification of Yeasts From the Suwannee River Florida Estuary. Applied Microbiology 1974;27(6) 1108-11.

[28] Hernandez-Saavedra NY, Hernandez-Saavedra D, Ochoa JL. Distribution of *Sporobolomyces* (Kluyver-Et-Vanniel) Genus in the Western Coast of Baja-California-Sur, Mexico. Syst Appl Microbiol 1992;15(2) 319-22.

[29] Nagahama T, Hamamoto M, Nakase T, Horikoshi K. *Kluyveromyces nonfermentans* sp nov., a new yeast species isolated from the deep sea. International journal of systematic bacteriology 1999;49 1899-905.

[30] Nagahama T, Hamamoto M, Nakase T, Takami H, Horikoshi K. Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific Ocean. Anton Leeuw Int J G 2001;80(2) 101-10.

[31] Nagahama T, Hamamoto M, Nakase T, Horikoshi K. *Rhodotorula lamellibrachii* sp nov., a new yeast species from a tubeworm collected at the deep-sea floor in Sagami Bay and its phylogenetic analysis. Anton Leeuw Int J G 2001;80(3-4) 317-23.

[32] Nagahama T, Hamamoto M, Nakase T, Horikoshi K. *Rhodotorula benthica* sp nov and *Rhodotorula calyptogenae* sp nov., novel yeast species from animals collected from the deep-sea floor, and *Rhodotorula lysiniphila* sp nov., which is related phylogenetically. International journal of systematic and evolutionary microbiology 2003;53 897-903.

[33] Gadanho M, Sampaio JP. Occurrence and diversity of yeasts in the Mid-Atlantic ridge hydrothermal fields near the Azores Archipelago. Microb Ecol 2005;50(3) 408-17.

[34] Nagahama T, Hamamoto M, Horikoshi K. *Rhodotorula pacifica* sp nov., a novel yeast species from sediment collected on the deep-sea floor of the north-west Pacific Ocean. International journal of systematic and evolutionary microbiology 2006;56 295-9.

[35] Bass D, Howe A, Brown N, Barton H, Demidova M, Michelle H, et al. Yeast forms dominate fungal diversity in the deep oceans. P R Soc B 2007;274(1629) 3069-77.

[36] Nagahama T, Abdel-Wahab MA, Nogi Y, Miyazaki M, Uematsu K, Hamamoto M, et al. *Dipodascus tetrasporeus* sp nov., an ascosporogenous yeast isolated from deep-sea sediments in the Japan Trench. International journal of systematic and evolutionary microbiology 2008;58 1040-6.

[37] Chen YS, Yanagida F, Chen LY. Isolation of marine yeasts from coastal waters of northeastern Taiwan. Aquat Biol 2009;8(1) 55-60.

[38] Kutty SN, Philip R, Damodaran R. Marine yeasts in the slope sediments of Arabian sea and bay of Bengal. European Journal of Experimental Biology 2013;3(3) 311- 27.

[39] van Uden N, Branco C. Distribution and population densities of yeast species in Pacific water, air, animals and kelp off southern California. Limnology and Oceanography 1963;8 323-9.

[40] Slavikova E, Vadkertiova R. Yeasts and yeast-like organisms isolated from fish-pond waters. Acta microbiologica Polonica 1995;44(2) 181-9.

[41] Andlid T, Vazquez-Juarez R, Gustafsson L. Yeasts isolated from the intestine of rainbow trout adhere to and grow in intestinal mucus. Mol Mar Biol Biotech 1998;7(2) 115-26.

[42] Wache Y, Auffray F, Gatesoupe FJ, Zambonino J, Gayet V, Labbe L, et al. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss,* fry. Aquaculture 2006;258(1-4) 470-8.

[43] Vázquez-Juárez R, Andlid T, Gustafsson L. Cell surface hydrophobicity and its relation to adhesion of yeasts isolated from fish gut. Colloids and Surfaces B: Biointerfaces 1994;2(1-3) 199-209.

[44] Vazquez-Juarez R, Andlid T, Gustafsson L. Adhesion of yeast isolated from fish gut to crude intestinal mucus of rainbow trout, *Salmo gairdneri*. Mol Mar Biol Biotechnol 1997;6(1) 64-71.

[45] Andlid T, Juarez RV, Gustafsson L. Yeast Colonizing the Intestine of Rainbow-Trout (*Salmo-Gairdneri*) and Turbot (*Scophtalmus-Maximus*). Microb Ecol 1995;30(3) 321-34.

[46] van Uden N, Farinha M. On the significance of temperature relations and vitamin deficiency patterns in the delimitation of yeast species. Port Acta Biol, Sér B 1958;6 161-78.

[47] Kreger-van rij JW. A Taxonomic study of the Yeast genera *Endomycopsis*, *Pichia* and *Debaryomyces.* . Kerkwerve, Netherlands: Backhuys Biological Books 1964.

[48] Bruce J, Morris EO. Psychrophilic yeasts isolated from marine fish. Antonie van Leeuwenhoek 1973;39(1) 331-9.

[49] Aubin J, Gatesoupe FJ, Labbe L, Lebrun L. Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research 2005;36(8) 758-67.

[50] Barnett JA, Payne RW, Yarrow D. Yeast: Characteristics and Identification. 2nd ed. Cambridge: Cambridge Univ. Press.; 1990.

[51] Laconi S, Pompei R. Study and characterization of intestinal yeasts of mullet (*Mugil* spp.) for potential probiotic use. J Food Agric Environ 2007;5(3-4) 475-80.

[52] Breuer U, Harms H. *Debaryomyces hansenii* - an extremophilic yeast with biotechnological potential. Yeast 2006;23(6) 415-37.

[53] Gao LM, Chi ZM, Sheng J, Wang L, Li J, Gong F. Inulinase-producing marine yeasts: Evaluation of their diversity and inulin hydrolysis by their crude enzymes. Microb Ecol 2007;54(4) 722-9.

[54] Bolumar T, Sanz Y, Aristoy MC, Toldra F. Purification and characterisation of proteases a and D from *Debaryomyces hansenii.* Int J Food Microbiol 2008;124(2) 135-41.

[55] Garcia-Gonzalez A, Lotz M, Ochoa JL. Anti-inflammatory activity of superoxide dismutase obtained from *Debaryomyces hansenii* on type II collagen induced arthritis in rats. Rev Invest Clin 2009;61(3) 212-20.

[56] Takac S, Sengel BS. Extracellular lipolytic enzyme activity of a newly isolated *Debaryomyces hansenii.* Preparative biochemistry & biotechnology 2010;40(1) 28-37.

[57] Segal-Kischinevzky C, Rodarte-Murguia B, Valdes-Lopez V, Mendoza-Hernandez G, Gonzalez A, Alba-Lois L. The Euryhaline Yeast *Debaryomyces hansenii* has Two Cata-

lase Genes Encoding Enzymes with Differential Activity Profile. Curr Microbiol 2011;62(3) 933-43.

[58] Viana PA, de Rezende ST, Alves AD, Manfrini RM, Alves RJ, Bemquerer MP, et al. Activity of *Debaryomyces hansenii* UFV-1 alpha-galactosidases against alpha-D-galactopyranoside derivatives. Carbohyd Res 2011;346(5) 602-5.

[59] Viana PA, de Rezende ST, Falkoski DL, Leite TD, Jose IC, Moreira MA, et al. Hydrolysis of oligosaccharides in soybean products by *Debaryomyces hansenii* UFV-1 alpha-galactosidases. Food Chem 2007;103(2) 331-7.

[60] Viana PA, de Rezende ST, Marques VM, Trevizano LM, Passos FML, Oliveira MGA, et al. Extracellular alpha-galactosidase from *Debaryomyces hansenii* UFV-1 and its use in the hydrolysis of raffinose oligosaccharides. Journal of Agricultural and Food Chemistry 2006;54(6) 2385-91.

[61] Viana PA, de Rezende ST, Passos FML, Machado SG, Maitan GP, Coelho VTD, et al. alpha-Galactosidases production by *Debaryomyces hansenii* UFV-1. Food Sci Biotechnol 2011;20(3) 601-6.

[62] Viana PA, de Rezende ST, Passos FML, Oliveira JS, Teixeira KN, Santos AMC, et al. *Debaryomyces hansenii* UFV-1 Intracellular alpha-Galactosidase Characterization and Comparative Studies with the Extracellular Enzyme. Journal of Agricultural and Food Chemistry 2009;57(6) 2515-22.

[63] Barbosa AM, Giese EC, Dekker RFH, Borsato D, Perez AIB, Iranzo JFU. Extracellular beta-glucosidase production by the yeast *Debaryomyces pseudopolymorphus* UCLM-NS7A: optimization using response surface methodology. New Biotechnol 2010;27(4) 374-81.

[64] Ragon M, Aumelas A, Chemardin P, Galvez S, Moulin G, Boze H. Complete hydrolysis of myo-inositol hexakisphosphate by a novel phytase from *Debaryomyces castellii* CBS 2923. Appl Microbiol Biot 2008;78(1) 47-53.

[65] Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, et al. Genome evolution in yeasts. Nature 2004;430(6995) 35-44.

[66] White TJ, Bruns T, Lee S, Taylor S. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MAG, P.H.; Sninsky, J.J.; White, T.J. , editor. PCR Protocols. San Diego, USA: Academic Press Inc; 1990. p. 315-22.

[67] The Centraal bureau voor Schimmelcultures (CBS) database. http://www.cbs.knaw.nl/collections/ BioloMICSSequences.aspx (accessed 5 April 2013)

[68] The Centraal bureau voor Schimmelcultures (CBS) database. http://www.cbs.knaw.nl/collections/BiolomicsID.aspx?IdentScenario=Yeasts%20species%20online%20ID (accessed 5 April 2013)

[69] Amann RI, Ludwig W, Schleifer KH. Phylogenetic Identification and in-Situ Detection of Individual Microbial-Cells without Cultivation. Microbiol Rev 1995;59(1) 143-69.

[70] Urubschurov V, Janczyk P. Biodiversity of Yeasts in the Gastrointestinal Ecosystem with Emphasis on Its Importance for the Host. In: Grillo O, Venora G, editors. The Dynamical Processes of Biodiversity - Case Studies of Evolution and Spatial Distribution: INTECH; 2011.

[71] Hovda MB, Lunestad BT, Fontanillas R, Rosnes JT. Molecular characterisation of the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 2007;272 581-8.

[72] Kim DH, Brunt J, Austin B. Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). J Appl Microbiol 2007;102(6) 1654-64.

[73] Navarrete P, Espejo RT, Romero J. Molecular analysis of microbiota along the digestive tract of juvenile Atlantic salmon (*Salmo salar* L.). Microb Ecol 2009;57(3) 550-61.

[74] Navarrete P, Magne F, Mardones P, Riveros M, Opazo R, Suau A, et al. Molecular analysis of intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). Fems Microbiol Ecol 2010;71(1) 148-56.

[75] Pond MJ, Stone DM, Alderman DJ. Comparison of conventional and molecular techniques to investigate the intestinal microflora of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2006;261(1) 194-203.

[76] Romero J, Navarrete P. 16S rDNA-based analysis of dominant bacterial populations associated with early life stages of coho salmon (*Oncorhynchus kisutch*). Microb Ecol 2006;51(4) 422-30.

[77] Navarrete P, Magne F, Araneda C, Fuentes P, Barros L, Opazo R, et al. PCR-TTGE analysis of 16S rRNA from rainbow trout (*Oncorhynchus mykiss*) gut microbiota reveals host-specific communities of active bacteria. Plos One 2012;7(2).

[78] Muyzer G, Dewaal EC, Uitterlinden AG. Profiling of Complex Microbial-Populations by Denaturing Gradient Gel-Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes-Coding for 16s Ribosomal-Rna. Appl Environ Microb 1993;59(3) 695-700.

[79] Lachance MA, Daniel HM, Meyer W, Prasad GS, Gautam SP, Boundy-Mills K. The D1/D2 domain of the large-subunit rDNA of the yeast species *Clavispora lusitaniae* is unusually polymorphic. FEMS yeast research 2003;4(3) 253-8.

[80] Cocolin L, Aggio D, Manzano M, Cantoni C, Comi G. An application of PCR-DGGE analysis to profile the yeast populations in raw milk. Int Dairy J 2002;12(5) 407-11.

[81] Cocolin L, Manzano M, Rebecca S, Comi G. Monitoring of yeast population changes during a continuous wine fermentation by molecular methods. Am J Enol Viticult 2002;53(1) 24-7.

[82] Gatto V, Torriani S. Microbial population changes during sourdough fermentation monitored by DGGE analysis of 16S and 26S rRNA gene fragments. Ann Microbiol 2004;54(1) 31-42.

[83] Molnar O, Wuczkowski M, Prillinger H. Yeast biodiversity in the guts of several pests on maize; comparison of three methods: classical isolation, cloning and DGGE. Mycol Prog 2008;7(2) 111-23.

[84] Prakitchaiwattana CJ, Fleet GH, Heard GM. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. FEMS yeast research 2004;4(8) 865-77.

[85] Valmorri S, Tofalo R, Settanni L, Corsetti A, Suzzi G. Yeast microbiota associated with spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the Abruzzo region (Italy). Anton Leeuw Int J G 2010;97(2) 119-29.

[86] Ubeda JF, Fernandez-Gonzales M, Briones AI. Application of PCR-TTGE and PCR-RFLP for Intraspecific and Interspecific Characterization of the Genus *Saccharomyces* Using Actin Gene (ACT1) Primers. Curr Microbiol 2009;58(1) 58-63.

[87] Hamady M, Knight R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. Genome research 2009;19(7) 1141-52.

[88] Beloshapka AN, Dowd SE, Suchodolski JS, Steiner JM, Duclos L, Swanson KS. Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing. Fems Microbiol Ecol 2013;84(3) 532-41.

[89] Delgado S, Cabrera-Rubio R, Mira A, Suarez A, Mayo B. Microbiological Survey of the Human Gastric Ecosystem Using Culturing and Pyrosequencing Methods. Microb Ecol 2013;65(3) 763-72.

[90] Marzorati M, Maignien L, Verhelst A, Luta G, Sinnott R, Kerckhof FM, et al. Barcoded pyrosequencing analysis of the microbial community in a simulator of the human gastrointestinal tract showed a colon region-specific microbiota modulation for two plant-derived polysaccharide blends. Anton Leeuw Int J G 2013;103(2) 409-20.

[91] Oikonomou G, Teixeira AGV, Foditsch C, Bicalho ML, Machado VS, Bicalho RC. Fecal Microbial Diversity in Pre-Weaned Dairy Calves as Described by Pyrosequencing of Metagenomic 16S rDNA. Associations of *Faecalibacterium* Species with Health and Growth. Plos One 2013;8(4).

[92] Roos S, Dicksved J, Tarasco V, Locatelli E, Ricceri F, Grandin U, et al. 454 Pyrosequencing Analysis on Faecal Samples from a Randomized DBPC Trial of Colicky Infants Treated with *Lactobacillus reuteri* DSM 17938. Plos One 2013;8(2).

[93] Zened A, Combes S, Cauquil L, Mariette J, Klopp C, Bouchez O, et al. Microbial ecology of the rumen evaluated by 454 GS FLX pyrosequencing is affected by starch and oil supplementation of diets. Fems Microbiol Ecol 2013;83(2) 504-14.

[94] Heinrichs G, Hubner I, Schmidt CK, de Hoog GS, Haase G. Analysis of Black Fungal Biofilms Occurring at Domestic Water Taps (I): Compositional Analysis Using Tag-Encoded FLX Amplicon Pyrosequencing. Mycopathologia 2013;175(5-6) 387-97.

[95] Monard C, Gantner S, Stenlid J. Utilizing ITS1 and ITS2 to study environmental fungal diversity using pyrosequencing. Fems Microbiol Ecol 2013;84(1) 165-75.

[96] Pannanusorn S, Elings MA, Romling U, Fernandez V. Pyrosequencing of a hypervariable region in the internal transcribed spacer 2 to identify clinical yeast isolates. Mycoses 2012;55(2) 172-80.

[97] Heinrichs G, de Hoog GS, Haase G. Barcode Identifiers as a Practical Tool for Reliable Species Assignment of Medically Important Black Yeast Species. J Clin Microbiol 2012;50(9) 3023-30.

[98] Kim JY, Hahn HJ, Choe YB, Lee YW, Ahn KJ, Moon KC. Molecular Biological Identification of *Malassezia* Yeasts Using Pyrosequencing. Ann Dermatol 2013;25(1) 73-9.

[99] Ortuno J, Cuesta A, Rodriguez A, Esteban MA, Meseguer J. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.). Vet Immunol Immunopathol 2002;85(1-2) 41-50.

[100] Harikrishnan R, Kim MC, Kim JS, Balasundaram C, Heo MS. Immunomodulatory effect of probiotics enriched diets on Uronema marinum infected olive flounder. Fish Shellfish Immunol 2011;30(3) 964-71.

[101] Tukmechi A, Rahmati Andani HR, Manaffar R, Sheikhzadeh N. Dietary administration of beta-mercapto-ethanol treated *Saccharomyces cerevisiae* enhanced the growth, innate immune response and disease resistance of the rainbow trout, *Oncorhynchus mykiss*. Fish Shellfish Immunol 2011;30(3) 923-8.

[102] Chiu CH, Cheng CH, Gua WR, Guu YK, Cheng W. Dietary administration of the probiotic, *Saccharomyces cerevisiae* P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. Fish Shellfish Immunol 2010;29(6) 1053-9.

[103] Torrecillas S, Makol A, Benitez-Santana T, Caballero MJ, Montero D, Sweetman J, et al. Reduced gut bacterial translocation in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). Fish Shellfish Immunol 2011;30(2) 674-81.

[104] Welker TL, Lim C, Yildirim-Aksoy M, Klesius PH. Effect of short-term feeding duration of diets containing commercial whole-cell yeast or yeast subcomponents on im-

mune function and disease resistance in channel catfish, *Ictalurus punctatus*. Journal of animal physiology and animal nutrition 2012;96(2) 159-71.

[105] Reyes-Becerril M, Tovar-Ramirez D, Ascencio-Valle F, Civera-Cerecedo R, Gracia-Lopez V, Barbosa-Solomieu V. Effects of dietary live yeast *Debaryomyces hansenii* on the immune and antioxidant system in juvenile leopard grouper *Mycteroperca rosacea* exposed to stress. Aquaculture 2008;280(1-4) 39-44.

[106] Reyes-Becerril M, Tovar-Ramirez D, Ascencio-Valle F, Civera-Cerecedo R, Gracia-Lopez V, Barbosa-Solomieu V, et al. Effects of dietary supplementation with probiotic live yeast *Debaryomyces hansenii* on the immune and antioxidant systems of leopard grouper *Mycteroperca rosacea* infected with *Aeromonas hydrophila*. Aquaculture Research 2011;42(11) 1676-86.

[107] Reyes-Becerril M, Salinas I, Cuesta A, Meseguer J, Tovar-Ramirez D, Ascencio-Valle F, et al. Oral delivery of live yeast *Debaryomyces hansenii* modulates the main innate immune parameters and the expression of immune-relevant genes in the gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol 2008;25(6) 731-9.

[108] Tovar-Ramirez D, Mazurais D, Gatesoupe JF, Quazuguel P, Cahu CL, Zambonino-Infante JL. Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 2010;300(1-4) 142-7.

[109] Buetler TM, Krauskopf A, Ruegg UT. Role of superoxide as a signaling molecule. News Physiol Sci 2004;19 120-3.

[110] Tovar D, Zambonino J, Cahu C, Gatesoupe FJ, Vazquez-Juarez R, Lesel R. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 2002;204(1-2) 113-23.

[111] Tovar-Ramirez D, Infante JZ, Cahu C, Gatesoupe FJ, Vazquez-Juarez R. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. Aquaculture 2004;234(1-4) 415-27.

[112] Tovar-Ramirez D. El efecto probiótico de la levadura *Debaryomyces hansenii* CBS 8339 sobre el desarrollo larvario del pargo Lunarejo *Lutjanus guttatus*. La Paz, BCS, México: Personal Communication; 2013.

[113] Zacarias-Soto M, Lazo JP, Viana MT. Effect of three probiotics administered through live feed on digestive enzyme activity in California halibut, *Paralichthys californicus*, larvae. J World Aquacult Soc 2011;42(3) 321-31.

[114] Klaenhammer TR, Kullen MJ. Selection and design of probiotics. Int J Food Microbiol 1999;50(1-2) 45-57.

[115] Gatesoupe FJ. Probiotic and formaldehyde treatments of *Artemia nauplii* as food for larval pollack, *Pollachius pollachius*. Aquaculture 2002;212(1-4) 347-60.

[116] Lara-Flores M, Olvera-Novoa MA, Guzman-Mendez BE, Lopez-Madrid W. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 2003;216(1-4) 193-201.

[117] Noh SH, Han K, Won TH, Choi YJ. Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. Korean Journal of Animal Science 1994;36(480-486).

[118] Mohanty SN, Swain SK, Tripathi SD. Growth and survival of rohu spawn fed on liver based diet. Journal of Inland Fisheries Society of India 1993;25(2) 41-5.

[119] Guzmán-Villanueva L, Tovar-Ramírez D, Civera-Cerecedo R, editors. Effect of wild and ornithine decarboxylase deficient *Debaryomyces hansenii,* on *Paralabrax maculatofasciatus* larvae development. Caribbean and Latin American Aquaculture; 2007 6–9 November; San Juan Puerto Rico.

[120] Dufour C, Dandrifosse G, Forget P, Vermesse F, Romain N, Lepoint P. Spermine and spermidine induce intestinal maturation in the rat. Gastroenterology 1988;95(1) 112-6.

[121] Reyes-Becerril M, Ángeles Esteban MA, Tovar-Ramírez D, Ascencio-Valle F. Polyamine determination in different strains of the yeast *Debaryomyces hansenii* by high pressure liquid chromatography. . Food Chem 2011;127 1862-5.

[122] Reyes-Becerril M, Ascencio-Valle F, Meseguer J, Tapia-Paniagua ST, Moriñigo MA, Esteban MÁ. *Debaryomyces hansenii* L2-enriched diet enhances the immunity status, gene expression and intestine functionality in gilthead seabream (*Sparus aurata* L.). Aquaculture Research 2012;43 1107-18.

[123] Aleem E. beta -Glucans and their Applications in Cancer Therapy: Focus on human studies. Anti-cancer agents in medicinal chemistry 2013;13(5) 709-19.

[124] Cloetens L, Ulmius M, Johansson-Persson A, Akesson B, Onning G. Role of dietary beta-glucans in the prevention of the metabolic syndrome. Nutrition reviews 2012;70(8) 444-58.

[125] Tosh SM. Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. Eur J Clin Nutr 2013;67(4) 310-7.

[126] Othman RA, Moghadasian MH, Jones PJH. Cholesterol-lowering effects of oat beta-glucan. Nutrition reviews 2011;69(6) 299-309.

[127] Andersson KE, Hellstrand P. Dietary oats and modulation of atherogenic pathways. Molecular nutrition & food research 2012;56(7) 1003-13.

[128] Du B, Bian Z, Xu B. Skin Health Promotion Effects of Natural Beta-Glucan Derived from Cereals and Microorganisms: A Review. Phytotherapy research : PTR 2013.

[129] Tanioka A, Tanabe K, Hosono A, Kawakami H, Kaminogawa S, Tsubaki K, et al. Enhancement of intestinal immune function in mice by β-D-glucan from *Aureobasidium pullulans* ADK-34. Scand J Immunol 2013;78(1) 61-8.

[130] Ganner A, Schatzmayr G. Capability of yeast derivatives to adhere enteropathogenic bacteria and to modulate cells of the innate immune system. Appl Microbiol Biot 2012;95(2) 289-97.

[131] Muramatsu D, Iwai A, Aoki S, Uchiyama H, Kawata K, Nakayama Y, et al. beta-glucan derived from *Aureobasidium pullulans* is effective for the prevention of influenza in mice. Plos One 2012;7(7).

[132] Volman JJ, Ramakers JD, Plat J. Dietary modulation of immune function by beta-glucans. Physiol Behav 2008;94(2) 276-84.

[133] Novak M, Vetvicka V. beta-glucans, history, and the present: Immunomodulatory aspects and mechanisms of action. J Immunotoxicol 2008;5(1) 47-57.

[134] Goodridge HS, Wolf AJ, Underhill DM. beta-glucan recognition by the innate immune system. Immunol Rev 2009;230 38-50.

[135] Brown GD, Gordon S. Immune recognition of fungal beta-glucans. Cell Microbiol 2005;7(4) 471-9.

[136] Dalmo RA, Bogwald J. beta-glucans as conductors of immune symphonies. Fish Shellfish Immun 2008;25(4) 384-96.

[137] Zimmerman JW, Lindermuth J, Fish PA, Palace GP, Stevenson TT, DeMong DE. A novel carbohydrate-glycosphingolipid interaction between a beta-(1-3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. J Biol Chem 1998;273(34) 22014-20.

[138] Palma AS, Feizi T, Zhang YB, Stoll MS. Ligands for the beta-glucan receptor, Dectin-1, assigned using "designer" microarrays of oligosaccharide probes (neoglycolipids) generated from glucan polysaccharides. (vol 281, pg 5771, 2006). J Biol Chem 2006;281(34) 24999-.

[139] Brown GD, Taylor PR, Reid DM, Willment JA, Williams DL, Martinez-Pomares L, et al. Dectin-1 is a major beta-glucan receptor on macrophages. J Exp Med 2002;196(3) 407-12.

[140] Grunebach F, Weck MM, Reichert J, Brossart P. Molecular and functional characterization of human Dectin-1. Exp Hematol 2002;30(11) 1309-15.

[141] Xu S, Huo J, Lee KG, Kurosaki T, Lam KP. Phospholipase C gamma 2 Is Critical for Dectin-1-mediated Ca(2+) Flux and Cytokine Production in Dendritic Cells. J Biol Chem 2009;284(11) 7038-46.

[142] Shah VB, Ozment-Skelton TR, Williams DL, Keshvaraa L. Vav1 and PI3K are required for phagocytosis of beta-glucan and subsequent superoxide generation by microglia. Mol Immunol 2009;46(8-9) 1845-53.

[143] Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by dectin-1 and toll-like receptor 2. J Exp Med 2003;197(9) 1107-17.

[144] Bricknell I, Dalmo RA. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immun 2005;19(5) 457-72.

[145] Meena DK, Das P, Kumar S, Mandal SC, Prusty AK, Singh SK, et al. Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol Biochem 2013;39(3) 431-57.

[146] Jorgensen JB, Sharp GJE, Secombes CJ, Robertsen B. Effect of a Yeast-Cell-Wall Glucan on the Bactericidal Activity of Rainbow-Trout Macrophages. Fish Shellfish Immun 1993;3(4) 267-77.

[147] Paulsen SM, Engstad RE, Robertsen B. Enhanced lysozyme production in Atlantic salmon (*Salmo salar* L.) macrophages treated with yeast beta-glucan and bacterial lipopolysaccharide. Fish Shellfish Immun 2001;11(1) 23-37.

[148] Lokesh J, Fernandes JMO, Korsnes K, Bergh O, Brinchmann MF, Kiron V. Transcriptional regulation of cytokines in the intestine of Atlantic cod fed yeast derived mannan oligosaccharide or beta-Glucan and challenged with *Vibrio anguillarum*. Fish Shellfish Immun 2012;33(3) 626-31.

[149] Kühlwein H, Merrifield D, Rawling M, Foey A, Davies S. Effects of dietary β-(1,3)(1,6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L.). Journal of Animal Physiology and Animal Nutrition (Berl) 2013;doi: 10.1111/jpn.12078.

[150] Dahm R, Geisler R. Learning from small fry: The zebrafish as a genetic model organism for aquaculture fish species. Mar Biotechnol 2006;8(4) 329-45.

[151] Ulloa PE, Iturra P, Neira R, Araneda C. Zebrafish as a model organism for nutrition and growth: towards comparative studies of nutritional genomics applied to aquacultured fishes. Rev Fish Biol Fisher 2011;21(4) 649-66.

[152] Sullivan C, Kim CH. Zebrafish as a model for infectious disease and immune function. Fish Shellfish Immun 2008;25(4) 341-50.

[153] Rodriguez I, Chamorro R, Novoa B, Figueras A. beta-Glucan administration enhances disease resistance and some innate immune responses in zebrafish (*Danio rerio*). Fish Shellfish Immun 2009;27(2) 369-73.

[154] Guzmán-Villanueva LT, Tovar-Ramírez D, Ascencio-Valle F, Macías-Rodríguez ME, Esteban MA, Gisbert E, editors. Effects of β-Glucan 1,3/1,6 on antioxidant enzyme ac-

tivity in *Lutjanus peru* exposed to lipopolysaccharides. . Aquaculture 2012; 2012 1-5 September; Prague, Czech Republic: World Aquaculture Society.

[155] Dimitroglou A, Merrifield DL, Carnevali O, Picchietti S, Avella M, Daniels C, et al. Microbial manipulations to improve fish health and production - A Mediterranean perspective. Fish Shellfish Immun 2011;30(1) 1-16.

[156] Ringo E, Olsen RE. The effect of diet on aerobic bacterial flora associated with intestine of Arctic charr (Salvelinus alpinus L.). J Appl Microbiol 1999;86(1) 22-8.

[157] Whittington R, Lim C, Klesius PH. Effect of dietary beta-glucan levels on the growth response and efficacy of *Streptococcus iniae* vaccine in Nile tilapia, *Oreochromis niloticus*. Aquaculture 2005;248(1-4) 217-25.

[158] R. S, C. L, M. Y-A, Welker TL, Klesius PH. Effect of yeast oligosaccharide diet supplements on growth and disease resistance in juvenile Nile tilpia (*Oreochromis niloticus*). Journal of applied aquaculture 2009;21 61-71.

[159] El-Boshy ME, El-Ashram AM, AbdelHamid FM, Gadalla HA. Immunomodulatory effect of dietary *Saccharomyces cerevisiae*, beta-glucan and laminaran in mercuric chloride treated Nile tilapia (*Oreochromis niloticus*) and experimentally infected with *Aeromonas hydrophila*. Fish Shellfish Immun 2010;28(5-6) 802-8.

[160] Welker TL, Lim C, Yildirim-Aksoy M, Shelby R, Klesius PH. Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. J World Aquacult Soc 2007;38(1) 24-35.

[161] Ai QH, Mai KS, Zhang L, Tan BP, Zhang WB, Xu W, et al. Effects of dietary beta-1,3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*. Fish Shellfish Immun 2007;22(4) 394-402.

[162] Palic D, Andreasen C, Herolt D, Menzel B, Roth J. Immunomodulatory effects of b-glucan on neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820). Dev Comp Immunol 2006;30 817-30.

[163] Rorstad G, Aasjord PM, Robertsen B. Adjuvant Effect of a Yeast Glucan in Vaccines against Furunculosis in Atlantic Salmon (*Salmo salar* L). Fish Shellfish Immun 1993;3(3) 179-90.

[164] Refstie S, Baeverfjord G, Seim RR, Elvebo O. Effects of dietary yeast cell wall beta-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. Aquaculture 2010;305(1-4) 109-16.

[165] Skjermo J, Storseth TR, Hansen K, Handa A, Oie G. Evaluation of beta-(1 -> 3, 1 -> 6)-glucans and high-M alginate used as immunostimulatory dietary supplement during first feeding and weaning of Atlantic cod (*Gadus morhua* L.). Aquaculture 2006;261(3) 1088-101.

[166] Misra CK, Das BK, Mukherjee SC, Pattnaik P. Effect of multiple injections of beta-glucan on non-specific immune response and disease resistance in Labeo rohita fingerlings. Fish Shellfish Immunol 2006;20(3) 305-19.

[167] Misra CK, Das BK, Mukherjee SC, Pattnaik P. Effect of long term administration of dietary beta-glucan on immunity, growth and survival of Labeo rohita fingerlings. Aquaculture 2006;255(1-4) 82-94.

[168] Sahoo PK, Mukherjee SC. Effect of dietary beta-1,3 glucan on immune responses and disease resistance of healthy and aflatoxin B-1-induced immunocompromised rohu (*Labeo rohita* Hamilton). Fish Shellfish Immun 2001;11(8) 683-95.

[169] Sahoo PK, Mukherjee SC. The effect of dietary immunomodulation upon *Edwardsiella tarda* vaccination in healthy and immunocompromised Indian major carp (*Labeo rohita*). Fish Shellfish Immun 2002;12(1) 1-16.

[170] Pal D, Joardar SN, Roy B. Immunostimulatory effects of a yeast (*Saccharomyces cerevisiae*) cell wall feed supplement on rohu (*Labeo rohita*), an Indian major carp. Isr J Aquacult-Bamid 2007;59(3) 175-81.

[171] Kumari J, Sahoo PK. Dietary immunostimulants influence specific immune response and resistance of healthy and immunocompromised Asian catfish *Clarias batrachus* to *Aeromonas hydrophila* infection. Dis Aquat Organ 2006;70(1-2) 63-70.

[172] Kumari J, Sahoo PK. Dietary beta-1,3 glucan potentiates innate immunity and disease resistance of Asian catfish, *Clarias batrachus* (L.). J Fish Dis 2006;29(2) 95-101.

[173] Jeney G, Galeotti M, Volpatti D, Jeney Z, Anderson DP. Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. Aquaculture 1997;154(1) 1-15.

[174] Verlhac V, Obach A, Gabaudan J, Schuep W, Hole R. Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immun 1998;8(6) 409-24.

[175] Siwicki A, Kazuñ K, Glabski E, Terech-Majewska E, Baranowski P, Trapkowska S. The effect of beta-1.3/1.6 – glucan in diets on the effectiveness of anti-*Yersinia ruckeri* vaccine – an experimental study in rainbow trout (*Oncorhynchus mykiss*). Polish journal of food and nutrition sciences 2004;13(54) 59-61.

[176] Sealey WM, Barrows FT, Hang A, Johansen KA, Overturf K, LaPatra SE, et al. Evaluation of the ability of barley genotypes containing different amounts of beta-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*. Anim Feed Sci Tech 2008;141(1-2) 115-28.

[177] Lovoll M, Fischer U, Mathisen GS, Bogwald J, Ototake M, Dalmo RA. The C3 subtypes are differentially regulated after immunostimulation in rainbow trout, but head kidney macrophages do not contribute to C3 transcription. Vet Immunol Immunopathol 2007;117(3-4) 284-95.

[178] Zhang Z, Swain T, Bogwald J, Dalmo RA, Kumari J. Bath immunostimulation of rainbow trout (*Oncorhynchus mykiss*) fry induces enhancement of inflammatory cytokine transcripts, while repeated bath induce no changes. Fish Shellfish Immunol 2009;26(5) 677-84.

[179] Kunttu HMT, Valtonen ET, Suomalainen LR, Vielma J, Jokinen IE. The efficacy of two immunostimulants against *Flavobacterium columnare* infection in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immun 2009;26(6) 850-7.

[180] Selvaraj V, Sampath K, Sekar V. Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. Fish Shellfish Immun 2005;19(4) 293-306.

[181] Selvaraj V, Sampath K, Sekar V. Use of glucan from *Saccharomyces cerevisiae* as an immunostimulant in carp: Impact on hematology, phagocyte function, and infection with *Aeromonas hydrophila*. Isr J Aquacult-Bamid 2005;57(1) 39-48.

[182] Selvaraj V, Sampath K, Vaithilingam S. Adjuvant and immunostimulatory effects of b-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with *Aeromonas hydrophila*. Veterinary Immunology and Immunopathology 2006;114 15-24.

[183] Lin S, Pan Y, Luo L, Luo L. Effects of dietary beta-1,3-glucan, chitosan or raffinose on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi). Fish Shellfish Immunol 2011;31(6) 788-94.

[184] Falco A, Frost P, Miest J, Pionnier N, Irnazarow I, Hoole D. Reduced inflammatory response to *Aeromonas salmonicida* infection in common carp (*Cyprinus carpio* L.) fed with beta-glucan supplements. Fish Shellfish Immunol 2012;32(6) 1051-7.

[185] Miest JJ, Falco A, Pionnier NP, Frost P, Irnazarow I, Williams GT, et al. The influence of dietary beta-glucan, PAMP exposure and Aeromonas salmonicida on apoptosis modulation in common carp (*Cyprinus carpio*). Fish Shellfish Immunol 2012;33(4) 846-56.

[186] Kuhlwein H, Merrifield DL, Rawling MD, Foey AD, Davies SJ. Effects of dietary beta-(1,3)(1,6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L.). Journal of animal physiology and animal nutrition 2013.

[187] Bagni M, Archetti L, Amadori M, Marino G. Effect of long-term oral administration of an immunostimulant diet on innate immunity in sea bass (*Dicentrarchus labrax*). J Vet Med B 2000;47(10) 745-51.

[188] Bagni M, Romano N, Finoia MG, Abelli L, Scapigliati G, Tiscar PG, et al. Short- and long-term effects of a dietary yeast beta-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol 2005;18(4) 311-25.

[189] Bonaldo A, Thompson KD, Manfrin A, Adams A, Murano E, Mordenti AL, et al. The influence of dietary beta-glucans on the adaptive and innate immune responses of European sea bass (*Dicentrarchus labrax)* vaccinated against vibriosis. Ital J Anim Sci 2007;6(2) 151-64.

[190] CooK MT, Hayball PJ, Hutchinson W, Nowak BF, Hayball JD. Administration of a commercial immunostimulant preparation, EcoActiva (TM) as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter. Fish Shellfish Immun 2003;14(4) 333-45.

[191] Kamilya D, Maiti TK, Joardar SN, Mal BC. Adjuvant effect of mushroom glucan and bovine lactoferrin upon Aeromonas hydrophila vaccination in catla, *Catla catla* (Hamilton). J Fish Dis 2006;29(6) 331-7.

[192] Y. K, Q. Z. Effect of b-glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. Fish & Shellfish Immunology 2009;27 336-40.