Rev Fish Biol Fisheries (2014) 24:1005–1032
DOI 10.1007/s11160-014-9361-y

REVIEWS

Application of vaccines and dietary supplements in aquaculture: possibilities and challenges

Einar Ringø · Rolf Erik Olsen · Ingvill Jensen · Jaime Romero · Hélène L. Lauzon

Received: 5 November 2013 / Accepted: 28 June 2014 / Published online: 10 July 2014

Abstract The development of vaccines has proven essential for the development of a successful finfish aquaculture industry by preventing the occurrence of diseases like furunculosis and vibriosis in industrialised finfish farming. Further developments, like DNA vaccines, will aid in controlling even more diseases in the future. There are however many diseases where it is difficult to produce effective vaccines. Furthermore, many disease outbreaks may occur due to impaired animal welfare. Identifying factors associated with disease and optimizing health and welfare through biotechnological developments is likely to be an important research area in the future. The fact that dietary manipulation can affect fish gut microbiota thus improving disease resistance is well known from mammalian science, and is slowly gaining ground in finfish research. Both prebiotic and probiotic approaches have been used in fish, with particular focus on lactic acid bacteria. Positive effects include enhanced growth and feed efficiency, improved immunity and disease resistance. The synbiotic concept (using a combination of probiotics and prebiotics) is particularly promising and is gaining increased interest within the research community. Immunostimulants may also improve disease resistence via increase humoral and cellular immune responses. The most promising immunostimulants at present are β-glucans, alginate and Ergosan. Additionally, medical plant extracts and their products are receiving increased attention as immune modulators, but further studies are needed. There are also great expectations or the future usage of microalgae to control microbiota and optimize fish health.

Keywords Aquaculture · Vaccines · Dietary supplements · Fish health

E. Ringø (✉) · I. Jensen
Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, 9037 Tromsø, Norway
e-mail: Einar.Ringo@uit.no

R. E. Olsen
Institute of Marine Research, Matre Research Station, Matredal, Norway

J. Romero
Laboratorio de Biotecnología, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, El Líbano 5524, Macul, Santiago, Chile

H. L. Lauzon
Matís ohf., Icelandic Food and Biotech R&D, Reykjavík, Iceland

Present Address:
H. L. Lauzon
Primex ehf, Siglufjordur, Iceland

Introduction

Aquaculture traces its roots to the ancient water-oriented civilizations of the East, where fish served as a main part of people's diets and the beginning of

aquaculture can be dated to the period 2000–1000 BC in China where mainly common carp (*Cyprinus carpio*) were utilized. European aquaculture can be dated back to ancient Rome and Gaul (modern France), where oyster cultivation thrived. Like the Chinese, ancient Romans bred fish in ponds. In the United Kingdom, the first hatcheries for rainbow trout (*Oncorhynchus mykiss* Walbaum) and Atlantic salmon (*Salmo salar* L.) were established in the 1850s; but *modern* Atlantic salmon farming in Norway did not start until 1971, with larval Atlantic cod (*Gadus morhua* L.) production commencing in 1998.

It has been predicted that by 2050, the total population on the planet will be 9 billion and aquaculture will have an important role in catering to the increased demand for food (Godfray et al. 2010). Global aquaculture production reached 62.7 million tonnes in 2011 with an estimated production of 66.5 million tonnes in 2012 (FAO 2012). In comparison, global capture fisheries are estimated to be stable around 95 million tonnes, and are not likely to increase in the future (FAO 2012).

All animal production systems have challenges associated with disease and the best way to solve these is often through effective management practices, i.e. management of stock, soil, water, nutrition and environment. A number of approaches have been applied to address this problem, including sanitary prophylaxis, disinfection, and chemotherapy, with particular emphasis on the use of antibiotics. The application of antibiotics and other chemicals to aquaculture is quite expensive, undesirable due to contamination to the surrounding environment, and might lead to antibiotic resistance (Cabello 2006; Romero et al. 2012). According to Heuer et al. (2009) few countries monitor the use of antibiotics in aquaculture and large variations seem to occur between different countries. Smith (2008) estimate that antibiotic consumption ranges from 1 g per tonne production in Norway to 700 g per tonnes in Vietnam. The decreased use of antibiotics in indistrialised fisnfish farming is partly due to widespread use of vaccination against specific diseases. However, there are practical difficulties and undesirable consequences associated with some of these approaches. In spite of the relatively large amount of research performed, few DNA vaccines are commercialized and it has been suggested that DNA vaccines are *third generation vaccines*. If the gastrointestinal (GI) tract is involved in infection there are several alternative strategies to control pathogenic bacteria from adherence and colonization of the intestine; the probiotic, prebiotic and synbiotic concepts, as well as the use of immunostimulants and plant extracts.

From a global perspective, it is recognzed that pressure on natural marine resources should be lowered. For the preservation and optimal use of wild fish stocks and for the healthy development of aquaculture, research on alternative protein and oil sources is therefore essential (FAO 2003) and has gained momentum over the past decades. The main driving force is to meet the protein, amino acid and fatty acid requirements of farmed fish without relying too heavily on fish mean (FM) and fish oil (FO). As there will be a limitation in global supplies of FM and FO in the near future, sustainable alternatives have been explored (Gatlin III et al. 2007). Soybean meal (SBM) and soybean oil (SBO) are considered suitable alternatives for the partial replacement of FM and FO and are extensively utilzed in commercial aquafeeds. Given the predictable increase in the demand for aquafeed resources, the risk of deficits in these ingredients is real. Thus, the changes from FM and FO to soybean products present several metabolic and health challenges for the farmed fish. When using high dietary levels of plant derived materials, particularly those derived from soybean, it is important to consider the impacts on gut microbiota and gut histology (Merrifield et al. 2011a) as the GI tract can be one of the important infection routes for some pathogens in fish (Groff and LaPatra 2000; Birkbeck and Ringø 2005; Ringø et al. 2007, 2010).

Another aspect that has received attention is microalgae and their biotechnological potential as increasing knowledge regarding antibacterial activity of different extracts of microalgae has been reported (e.g. Day and Austin 1990; Alonso et al. 2012; Goecke et al. 2012). Even though some information is available on the use of microalgae in aquaculture, growth performance, feed utilization, immune system, gut morphology, gut microbiota and disease resistance of fish (Tulli et al. 2011; Cerezuela et al. 2012a, b, c) these topics merit further investigations.

This review provides and overview of vaccines and dietary supplements in aquaculture together with a

critical evaluation of the results obtained so far. Finally, directions for further research are proposed.

Use of antibiotics

Due to intensive farming practices, infectious diseases are a major problem in finfish and shellfish aquaculture, causing heavy loss to farmers. In the 1970s and 1980s oxolinic acid, oxytetracycline (OTC), furazolidone, potential sulphonamides (sulphadiazine and trimethoprim) and amoxicillin were the most commonly used antibiotics in fish farming. However, the indiscriminate use of antibiotics in disease control has led to selective pressure of antibiotic resistance in bacteria, a property that may be readily transferred to other bacteria (Cabello 2006; Romero et al. 2012). Furthermore, use of antibiotics to control pathogenic bacteria can also reduce the numbers of non-pathogenic bacteria in the gut. On the other hand, it may be perceived that there is a problem associated with the release of antibiotic into the environment and the occurrence of antibiotic resistance bacteria in marine sediments near fish farms. Some research groups have addressed this topic and shown significant changes in the benthic bacterial community near fish farms with possible links to antibiotic susceptibility (Kerry et al. 1995; Chelossi et al. 2003).

More recently molecular tools such as PCR have been used in antibiotic resistance studies, with water and sediment from fish farms screened and *tet*R genes detected at significantly higher frequencies in water from farms with recent OTC use compared with water from farms without recent OTC use (Seyfried et al. 2010). However, OTC use was not correlated with the prevalence of *tet*R genes in sediment samples. A similar study using qPCR reported greater copy numbers of *tet*A, *tet*C, *tet*H, and *tet*M at the farms compared to pristine sites (Tamminen et al. 2011). However, no resistant genes were found in samples collected 200 m away from any of the farms. Furthermore, the analysis of tetracycline indicated that none of the samples contained therapeutic concentrations at any of the sampling times, suggesting that the prevalence of tetracycline-resistance genes may be caused by the persistence of these genes in the absence of selection pressure. An increase in antibiotic-resistance genes in the absence of the antibiotic itself has also been attributed to co-selection with other antibiotics.

Bacterial vaccines

Compared with human vaccine history, fish vaccine development has a very short history starting in the 1970s with the first licensed fish vaccine made commercially available in 1976 (Evelyn 1997). In fish vaccination, three main delivery approaches are used: injection, oral delivery and immersion; bath and spray vaccination. Vaccination plays an important role in large-scale commercial fish farming and has been a key reason for the success of salmon cultivation. In addition to Atlantic salmon and rainbow trout, commercial vaccines are available for channel catfish (*Ictalurus punctatus*), European sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), Japanese amberjack (*Seriola quinquerdiata*), tilapia (*Oreochromis niloticus*), Atlantic cod, barramundi (*Lates calcarifer*), tilapia (*Tilapia* spp.), turbot (*Scophthalmus maximus* L.), yellowtail (*Seriola quinqueradiata*), purplish and gold-striped amberjack (*Seriola dumereli*) and striped jack (*Pseudocaranx dentex*). The range of bacterial infections for which vaccines are commercially available now comprises classical vibriosis (*Vibrio* (*Listonella*) *anguillarum*), cold-water vibriosis (*Aliivibrio* (*Vibrio*) *salmonicida*), *Vibrio ordalii*, furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*), yersiniosis (*Yersinia ruckeri*), pasteurellosis (*Photobacterium damselae* subsp. *piscicida*), edwardsiellosis (*Edwardsiella ictaluri*), winter ulcer (*Moritella viscosa*), and streptococcosis/lactococcosis (*Streptococcus iniae*/*Lactococcus garvieae*). Furthermore, experimental vaccines are used against infections caused by *Vibrio harveyi* and *Photobacterium damselae* subsp. *damselae* in barramundi, piscirickettsiosis and bacterial kidney disease in salmonids, as well as infection with *Flexibacter maritimus* in turbot. However, vaccination has both advantages and drawbacks and readers with special interest are referred to the reviews of Sommerset et al. (2005), Plant and LaPatra (2011) and Clarke et al. (2013).

A brief overview of the developments in fish vaccinology is presented in Table 1. In general, empirically developed vaccines based on inactivated bacterial pathogens have proven to be very efficacious in fish. Substantial efficacy data is available for new

Table 1 A brief overview of the developments in fish vaccinology

Year	Developments	References
2010	First PLGA-immunization by injection; intraperitoneal delivery	Plant and LaPatra (2011)
2008	First reported delivery (oral) of PLGA-encapsulated DNA to fish; study with Japanese flounder	
2005	First licensed DNA vaccine for fish; Apex-IHN® for protection against IHNV	Garver et al. (2005)
1997	First use of an encapsulated vaccine; oral administration of *Vibrio anguillarum*	Joosten et al. (1997)
1996	First use of PLGA particles in fish; oral intubation of Atlantic salmon with PLGA particles containing human gamma globulin	O'Donnel et al. (1996)
	First DNA vaccination of fish; rainbow trout injected intra- muscularly with a plasmid coding an IHNV antigen	Anderson et al. (1996)
1995	First commercial viral vaccine; Norvax® Protect-IPN was licensed in Norway	Frost and Ness (1997)
1981	First adjuvant vaccine for injection and protection against *Aeromonas salmonicida* was licensed	
1976	First licensed fish vaccine; orally administrated killed *Yersinia ruckeri* to protect against enteric redmouth disease	
1951	The (possibly) first report on viral immunization; intraperitoneal injection of carp with formalin-killed virus (likely spring viraemia virus)	
1942	First report of successful vaccination; oral administration of chloroform-killed *A. salmonicida* induced protection in cutthroat trout against furunculosis after challenge by injection or cohabitation	Duff (1942)
	Intraperitoneal injection of killed or attenuated bacteria induced protection against *Aeromonas hydrophila* upon challenge	
1938	Induction of protective immunity in fish after injection with killed *Aeromonas punctate*	
1935	Heat-killed *V. anguillarum* induced a specific and temperature related agglutinin response after injection in eels	

After Hølvold (2012). Unless otherwise stated the data has been gathered from Van Muiswinkel (2008) and Plant and LaPatra (2011)

fish vaccines and advanced technology has been implemented. However, before such vaccines can be successfully commercialized, several hurdles have to be overcome regarding the production of cheap but effective antigens and adjuvants, while bearing in mind environmental and associated regulatory concerns (e.g., those that limit the use of live vaccines). Pharmaceutical companies have performed a considerable amount of research on fish vaccines; however, limited information is available in scientific publications. In addition, salmonids dominate both the literature and commercial focus, despite their relatively small contribution to the total volume of farmed fish in the world.

Salmonids are usually immunized with multivalent vaccines by intraperitoneal injection. In marine fish species vaccination is generally performed by immersion, but use of injection vaccination is increasing, particularly in the Mediterranean region. Only limited use of orally administered fish vaccines is reported. In general, vaccines against bacterial diseases provide good protection (Plant and LaPatra 2011). The best protection is obtained with injectable, adjuvanted vaccines (Brudeseth et al. 2013). However, injection-site adverse reactions often occur when such products are used (Mutoloki et al. 2006; Brudeseth et al. 2013).

DNA vaccines

To limit the impact of infectious diseases a continuous effort to improve vaccine strategies for fish is required. One of the vaccine strategies that has been tested recently is DNA vaccination. A DNA vaccine is composed of the DNA sequence encoding a protective antigen inserted into a small circular piece of DNA, a plasmid expression vector. A strong viral promoter is present in the plasmid to drive the in vivo expression of the antigen. The plasmid can easily be amplified and purified from bacterial cultures and subsequently used for vaccination. It is possible to

make plasmids encode more than one antigen and also to incorporate sequences for immunostimulatory purposes (adjuvants).

DNA vaccines have several advantages over more traditional vaccines. The vaccine antigen is produced inside the cells of the host, which ensures correct protein folding. Also, this intracellular protein production mimics a natural infection with an intracellular pathogen and both the humoral and cellular arms of the immune system are activated. The production of DNA vaccines is rather easy and does not require purification of protein or of the pathogen, as with subunit or whole pathogen vaccines. Compared to attenuated vaccines there is no risk for reversion to virulence as only one gene from the pathogen is present in the DNA vaccine.

DNA vaccination has been experimentally tested in various fish species against mainly viral pathogens, but also against bacteria and parasites (Tonheim et al. 2008; Gomez-Casado et al. 2011; Liu et al. 2011; von Gersdorff Jorgensen et al. 2012). DNA vaccines to the rhabdoviruses infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicemia virus (VHSV) in rainbow trout have shown very good protective effects. These DNA vaccines are based on the gene encoding the viral surface glycoprotein (G protein). The G protein is responsible for viral cell attachment and the neutralizing antibody response is directed against this protein. DNA vaccines, based on other rhabdoviral proteins, do not provide the same levels of protection (Corbeil et al. 1999). The protective mechanisms provided by the G gene vaccines are based on a rapid and transient innate immune response involving activation of the antiviral interferon system followed by long-term specific immunity (Kim et al. 2000; Lorenzen et al. 2002). Long-term protection may last up to two years after vaccination (Kurath et al. 2006). To optimize efficacy of the G gene vaccines the effects of fish size, vaccine dose and administration routes have been investigated (Corbeil et al. 2000). Intramuscular injection of the vaccines gives good protection. No oil-adjuvants are needed, which for other fish vaccines are known to cause serious side effects. A DNA vaccine based on the IHNV G gene is in commercial use for Atlantic salmon in Canada (Apex-IHN, Novartis) (Salonius et al. 2007; Alonso and Leong 2013). However, in the US and Europe this vaccine has not been approved for commercial use due to safety concerns. Recently an oral DNA vaccine with PLGA (Poly (D,L-lactic-co-glycolic acid)) nanoparticles containing the IHNV G gene plasmid was tested (Adomako et al. 2012). The prevalence of fish expressing the G gene after receiving the feed coated with the vaccine was very low and only a minor increase in survival was recorded after virus challenge. However, the data suggests that it might be possible to deliver a DNA vaccine orally, although major improvements of the technology are required.

For fish pathogenic viruses other than rhabdoviruses moderate to low protective effects have been observed after DNA vaccination (Tonheim et al. 2008; Gomez-Casado et al. 2011). In two recent studies the protective effects of different types of vaccines against infectious pancreatic necrosis virus (IPNV) and salmonid alphavirus (SAV) were compared. In both cases the vaccine based on inactivated whole virus provided better protection after challenge than DNA vaccination (Munang'andu et al. 2012; Xu et al. 2012).

Although the G gene vaccines to VHSV and IHNV have proven to be very efficient, it is only in Canada that such a vaccine has been licensed for commercial use (Salonius et al. 2007). There are uncertainties as to how long plasmid DNA can remain intact in fish tissues and whether there is a risk for integration of plasmid DNA into the genome (Gillund et al. 2008; Tonheim et al. 2008). Different approaches have been used to try to develop plasmid DNA that is considered safer and more acceptable to use as vaccines. Plasmids where viral regulatory sequences have been replaced by regulatory sequences from fish have been developed (Martinez-Lopez et al. 2013). Also to reduce possible homologous recombination between all-fish plasmids with the fish genome, core and enhancer sequences from fish origin have been combined with those of cytomegalovirus (CMV) to design alternative hybrid promoters (Martinez-Lopez et al. 2012). To limit the long-term persistence of plasmid DNA in cells after vaccination a suicidal DNA vaccine construct was developed. After inducing protective immunity the cells harboring the plasmid are killed by apoptosis (Alonso et al. 2011).

The probiotic concept

Probiotics, generally defined as live microorganisms with different beneficial characteristics, are increasingly becoming accepted as an alternative

Table 2 Probiotic selection criteria

Criterion
Essential
It must not be pathogenic, not only with regards to the host species but also with regards to aquatic animals in general and human consumers
It must be free of plasmid-encoded antibiotic resistance genes
It must be resistant to bile salts and low pH
Favourable
It should be able to adhere to and/or grow well within intestinal mucus
It should be able to colonise the intestinal epithelial surface
It should be registered for use as a feed additive
It should display advantageous growth characteristics (e.g. short lag period, a short doubling time and/or growth at host rearing temperatures)
It should exhibit antagonistic properties towards one or more key pathogens
It should produce relevant extracellular digestive enzymes (e.g. chitinase if chitin rich ingredients are to be incorporated into the diet or cellulase if the diet is rich in plant ingredients) and/or vitamins
It should be indigenous to the host or the rearing environment
It should remain viable under normal storage conditions and be robust enough to survive industrial processes

After Merrifield et al. (2010a)

prophylactic treatment for humans and animals to either treat pathogen-related diseases or to be used in preventive treatments. Probiotic research has mainly focused on the host's GI tract, while applications to skin or gill surfaces have been less investigated.

Several reviews have published varied opinions on what are considered to be important characteristics for the selection of probionts for applications in aquaculture (e.g. Gatesoupe 1999; Gram and Ringø 2005; Balcázar et al. 2006; Gómez and Balcázar 2008; Lamari et al. 2013; Lauzon et al. 2014a). Merrifield et al. (2010a) collated such characteristics and extended them to produce the following comprehensive list of criteria (Table 2).

Even though fish microbiologists have gained some knowledge about adherence of probiotic bacteria in the GI tract of fish during the last two decades, there is a long way to go compared to the information available from non-aquaculture studies. For example, in a study using crude mucus from small intestine of a 23-day-old healthy piglet, Macías-Rodríguez et al. (2009) demonstrated that adhesion of the potential probiotic *Lactobacillus fermentum* originally isolated from faeces of a piglet involved two adhesion-associated proteins with a relative molecular weight of 29 and 32 kDa that are attached non-covalently to the cell surface. In a study with *Lactobacillus rhamnosus* a piliated bacterium, von Ossowski et al. (2010) reported that 2 pilin subunits (SpaB and SpaC) in the SpaCBA pilus fiber are involved in binding to intestinal mucus. Moreover, Huang et al. (2013) evaluated the relationship between adhesive ability of probiotic bacteria and soluable acid residues in the human colonic mucin (sHCM). Based on their results using a Biacore binding assay the authors concluded that there was a strong relationship between probiotic adhesion and acid residues of sHCM. As no fish studies have been carried out on cell surface components of marine probiotic bacteria responsible for mucosal adhesion we recommend that this topic merits further investigations especially related to the discussion of whether colonzation of probiotic bacteria to intestinal mucus is a favorable or essential criterion.

In their search for good probiotics to use in aquaculture some authors have hinted on the use of lactic acid bacteria (LAB) isolated from sources other than aquatic animals (El-Haroun et al. 2006; Bagheri et al. 2008; Salinas et al. 2008a; Merrifield et al. 2010a; Salma et al. 2011; Zhou et al. 2012; Ren et al. 2013). This selection criterion is mainly based on their proven efficiency and safety in humans and livestock (Azad and Al-Marzouk 2008). However, efficacy in aquatic environments and safety to new hosts must be demonstrated. Several of the reported probiotic studies conducted in vivo evaluated allochthonous LAB strains (Lauzon and Ringø 2012). It is interesting to consider the application spectrum of allochthonous LAB, their adhesion capacity and/or colonization as well as the reproducibility of beneficial effects towards different hosts.

The use of allochthonous LAB strains in aquaculture has been shown to provide beneficial effects in various aquatic animals, and mainly consists of lactobacilli species, and to a lesser extent carnobacteria, enterococci, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* and *Pediococcus acidilactici* strains. Most of these rearing trials involved the use of monospecies (Table 3), but multispecies applications have also been successful and even complementary (Lauzon and Ringø 2012). Comparing the beneficial

Table 3 Application of LAB as probiotics in aquaculture by monospecies treatments and main beneficial effects observed

Allochthonous LAB	Aquatic animal	Application vector	Treatment dose	Main beneficial effects observed	Reference
Lactobacillus					
Lb. casei (Yakult)	Porthole livebearer	*Artemia* nauplii	log 6/ml (11 wks)	Stress resistance, immune response	Hernandez et al. (2010)
Lb. casei ssp. *casei* CECT4043	Rotifer	RW	log 9/ml	Growth rate	Planas et al. (2004)
	Artemia nauplii	RW	log 8/ml (24 h, 15 °C)	*Vibrio* control	Villamil et al. (2002)
Lb. brevis CECT815	*Artemia* nauplii	RW	log 8/ml (24 h, 15 °C)	*Vibrio* control	Villamil et al. (2003)
Lb. acidophilus (Lactobacil)	Infected[1] carp	Feed	log 8/g (4 weeks)	Disease control, immune response	Harikrishnan et al. (2010c)
	Infected[2] olive flounder	Feed	log 8/g (4 weeks)	Disease control, immune response	Harikrishnan et al. (2010a)
Lb. acidophilus	Carp	Feed	log 7 to 8/g (45 days)	Growth, feed and protein efficiency, survival	Ramakrishnan et al. (2008)
Lb. acidophilus	Infected[3] tilapia	Feed	log 7/g (1–2 mo)	Growth, disease control, immune response	Aly et al. (2008)
Lb. paraplantarum	Abalone	Feed	log 9–10/g (3 weeks)	Colonization, some enzymatic activity	Iehata et al. (2009)
Lb. plantarum	Infected[4] grouper	Feed	log 3-5-7/g (4 weeks)	Colonization, growth, feed efficiency, innate response, disease control	Son et al. (2009)
Lb. rhamnosus IMC 501	Clownfish	Live prey/ LP + RW	log 6/ml, twice daily for 30 d	Colonization, viability, growth, development, less deformities	Avella et al. (2010)
Lb. rhamnosus JCM 1136	Rainbow trout	Feed LI, FD, IN	log 11/g (30 d)	Colonization, immune response	Panigrahi et al. (2005)
	Rainbow trout	Feed	log 9–11/g (30 days)	Colonization, immune response	Panigrahi et al. (2004)
Lb. rhamnosus ATCC 53103	Rainbow trout	Feed	log 9/g (45 d)	Immune response	Panigrahi et al. (2007)
	Infected[5] tilapia	Feed	log 8–10/g (2 weeks)	Disease control, immune response	Pirarat et al. (2006)
	Rainbow trout	Feed	log 4-6-8-10–11/g (2 weeks)	Colonization, immune response	Nikoskelainen et al. (2003)
	Infected[6] rainbow trout	Feed (FD)	log 9–12/g (51 days)	Disease control	Nikoskelainen et al. (2001)
Lb. sakei BK19	Infected[7] kelp grouper	Feed	log 8/g (4 wks)	Disease control, immune response	Harikrishnan et al. (2010b)
Lb. sakei CLFP202	Infected[8] rainbow trout	Feed	log 6/g (2 wks)	Colonization, disease control, immune response	Balcázar et al. (2007b)
Lb. sakei	Brown trout	Feed	log 6/g (2 wks)	Colonization, immune response	Balcázar et al. (2007a)

Table 3 continued

Allochthonous LAB	Aquatic animal	Application vector	Treatment dose	Main beneficial effects observed	Reference
Carnobacterium					
C. divergens (LABS)	Infected[9] cod	Feed	log 8/g (3 wks)	Colonization, some disease control, growth	Gildberg and Mikkelsen (1998)
Carnobacterium sp.	Infected[10] rainbow trout	Feed	log 7 to 8/g (14 d)	Colonization, disease control	Robertson et al. (2000)
Lactococcus					
Lac. lactis ssp. *lactis* CLFP100	Infected[8] rainbow trout	Feed	log 6/g (2 wks)	Colonization, disease control, immune response	Balcázar et al. (2007b)
Lac. lactis ssp. *lactis*	Brown trout	Feed	log 6/g (2 wks)	Colonization, immune response	Balcázar et al. (2007a)
Lac. lactis ssp. *lactis* CECT539	Rotifer	RW	log 9/ml	Growth rate	Planas et al. (2004)
	Turbot	Force-feeding	log 5/day (7 days)	Immune response	Villamil et al. (2002)
Enterococcus					
E. thailandicus	Atlantic cod	Feed	log 7/g (55 days)	Growth, survival, feed efficiency, some colonization, *Vibrio* control	Lauzon et al. (2010c)
E. mundtii	Abalone	Feed	log 9–10/g (3 weeks)	Enzymatic activity	Iehata et al. (2009)
E. faecium (Lactosan GmbH)	Rainbow trout	Feed	log 8/g (10 weeks)	Colonization	Merrifield et al. (2010b)
E. faecium ZJ4	Tilapia	RW	log 7–8/ml (every 4 days, for 40 days)	Growth, immune response	Wang et al. (2008)
E. faecium (Japan)	Rainbow trout	Feed (FD)	log 9/g (45 days)	Immune response	Panigrahi et al. (2007)
Leuconostoc					
Leu. mesenteroides CLFP196	Infected[8] rainbow trout	Feed	log 6/g (2 weeks)	Colonization, disease control, immune response	Balcázar et al. (2007b)
Leu. mesenteroides	Brown trout	Feed	log 6/g (2 weeks)	Colonization, immune response	Balcázar et al. (2007a)
Pediococcus					
Ped. acidilactici MA18/5 M (Bactocell)	Sea bass	Feed	log 6–7/g (~30 days)	Microbiota modulation, growth, less deformities	Lamari et al. (2013)
	Rainbow trout	Feed (LI,FD)	log 7–8/g (10 wks)	Colonization, some immune response	Merrifield et al. (2011a)
	Rainbow trout	Feed	log 7/g (5 wks)	Colonization, gut morphology/development	Merrifield et al. (2010a)
	Red tilapia	Feed	log 7/g (32 d)	Colonization, survival, microbiota control, immune response	Ferguson et al. (2010)

Table 3 continued

Allochthonous LAB	Aquatic animal	Application vector	Treatment dose	Main beneficial effects observed	Reference
	Infected[11] shrimp	Feed	log 7/g (1 mo)	Lower infection and mortality, higher antioxidant defense, lower oxidative stress level	Castex et al. (2010)
	Shrimp	Feed	log 7/g (22 days)	Antioxidant defense, survival, *Vibrio* control	Castex et al. (2009)
	Shrimp	Feed	log 7/g (10 weeks)	Survival, biomass, lower food conversion ratio, enzymatic activity, microbiota control	Castex et al. (2008)
	Rainbow trout	Feed	log 6/g (20 days; 5 mo)	Colonization, microbiota control, low deformities	Aubin et al. (2005)
	Pollack	Disinfected *Artemia*	log 7/ml (16 days)	Colonization, growth	Gatesoupe (2002)
Ped. acidilactici					
NRRL B-5627	Turbot	Rotifer (1 or 24 h), RW	log 8/ml once 3 dph	Colonization	Villamil et al. (2010)

Modified and updated from Lauzon and Ringø (2012)

RW rearing water, *LP* live prey (rotifer and *Artemia*), *LI* live, vegetative cells, *FD* freeze-dried, *IN* inactivated, *d* days, *wks* weeks, *mo* months

[1] Infected by intramuscular (i.m.) injection of *Aeromonas hydrophila* (9×10^4 cfu/fish) 6 days before probiotic feeding treatment

[2] Obtained naturally infected by lymphocystis disease virus (LCDV) from a private hatchery

[3] Infected i.p. by *A. hydrophila, Pseudomonas fluorescens and Streptococcus iniae* (log 8/fish) after 1-mo or 2-mo feeding treatment

[4] Infected i.m. by *Streptococcus* sp. (5.6×10^7 cfu/g fish) or i.p. by a grouper iridovirus (1.7×10^5 TCID50/g fish) after 4-w feeding treatment

[5] Infected i.p. by *Edwardsiella tarda* E381 (log 9 cfu/fish) after 2-w feeding treatment

[6] Infected by cohabitation (15 % of fish i.p. injected with *A. salmonicida* ssp. *salmonicida*, log 6 cfu/fish) after 16-d feeding treatment

[7] Infected intraperitoneally (i.p.) at day 0 with *Strep. iniae* or *Strep. parauberis* separately or mixed ($\sim$log 6 cells/fish)

[8] Infected by cohabitation (15 % of fish i.p. injected with *A. salmonicida* ssp. *salmonicida* CLFP501, 1.7×10^5 cells/fish) after 2-w feeding treatment

[9] Infected by batch challenge with *Vibrio anguillarum* LFI 1243 (log 7/ml rearing water for 1 h) after 3-w feeding treatment

[10] Infected by cohabitation (16.7 % of fish i.p. injected with *A. salmonicida* Hooke or *Y. ruckeri* PR110, log 6 cfu/fish) after 14-d feeding treatment

[11] Infected by immersion for 2 h with *Vibrio nigripulchritudo* SFn1 (log 5 cfu/ml) and measurements undertaken during the first 72 h

effects in gilthead sea bream observed during the application of closely related strains (Diaz-Rosales et al. 2006) compared with more distant ones (Salinas et al. 2005) enhanced immunomodulation was detected. In this regard, a question has risen whether the systematic relationship of multispecies probiotics is an influencing factor (Dimitroglou et al. 2011).

Adhesion capacity and/or colonization of allochthonous LAB after fish treatment is not always verified or successfully confirmed. Detection of lactobacilli in the gut of fish a few days post-treatment has been reported (Nikoskelainen et al. 2003; Panigrahi et al. 2005; Iehata et al. 2009; Son et al. 2009), for carnobacteria (Robertson et al. 2000; Irianto and Austin 2002), enterococci (Lauzon et al. 2010a, b) and *Ped. acidilactici* (Villamil et al. 2010). Analysis of mucosal samples obtained from treated fish has also demonstrated the ability of allochthonous LAB to colonize the gut of rainbow trout (Merrifield et al. 2010b, 2011b). Indeed, LAB have in general a good ability to adhere to different cell types (Rinkinen et al. 2003; Lauzon et al. 2008). It is noteworthy that competition of allochthonous LAB with autochthonous bacteria added at high levels in rearing trials have shown that autochthonous bacteria isolated from larvae will more rapidly colonize the gut at an early developmental stage (Ringø 1999). In contrast autochthonous bacteria from adult fish seem to colonize the gut only after fish metamorphosis, allowing allochthonous LAB to colonize at earlier stages but displacing them at a later stage (Carnevali et al. 2004). The possible influence of the fish developmental stage on probiont colonization may explain the decreasing colonization of probiotic strains observed from larval to juvenile cod stages (Lauzon et al. 2010a, b, c). These findings should be considered in the selection of probionts for multispecies probiotics.

Another important matter to reflect on during the selection of autochthonous LAB relates to reproducibility of the beneficial effects produced by a LAB species towards different hosts. Due to different experimental design and parameters analyzed during probiotic application, very few trials can be compared. Nevertheless, disease control has often resulted in the presence of lactobacilli species to combat different fish pathogens; *Aeromonas hydrophila* in carp (Harikrishnan et al. 2010b), LCDV virus in olive flounder (Harikrishnan et al. 2010a), *Streptococcus* spp. in groupers (Son et al. 2009; Harikrishnan et al. 2010c), *Edwardsiella tarda* in tilapia (Pirarat et al. 2006), and *A. salmonicida* subsp. *salmonicida* in rainbow trout (Nikoskelainen et al. 2001; Balcázar et al. 2007a). *C. divergens* from salmon provided short term protection against *V.* (*L.*) *anguillarum* during rearing of cod juveniles (Gildberg and Mikkelsen 1998), while another *Carnobacterium* strain reduced the effect in rainbow trout (Robertson et al. 2000). *Lac. lactis* subsp. *lactis* and *Leu. mesenteroides* also controlled the *A. salmonicida* subsp. *salmonicida* infection in rainbow trout (Balcázar et al. 2007a). Finally, *Ped. acidilactici* also showed promising results in combatting a pathogenic *Vibrio* in shrimp (Castex et al. 2010).

In most of these studies, the immune response was enhanced by the probiotics applied. LAB species affecting immunomodulation include *Lb. rhamnosus* in rainbow trout (Nikoskelainen et al. 2003; Panigrahi et al. 2004, 2005, 2007) and tilapia (Pirarat et al. 2006); *Lb. sakei* in kelp grouper (Harikrishnan et al. 2010b), rainbow trout (Balcázar et al. 2007b) and brown trout (Balcázar et al. 2007a); *Lb. delbrüeckii* subsp. *lactis* in gilthead sea bream (Salinas et al. 2005; 2008b) and Atlantic salmon (Salinas et al. 2008a); *Lac. lactis* subsp. *lactis* in rainbow trout (Balcázar et al. 2007b), brown trout (Balcázar et al. 2007a) and turbot (Villamil et al. 2002); *Enterococcus faecium* in tilapia (Wang et al. 2008) and rainbow trout (Panigrahi et al. 2007); and *Ped. acidilactici* (Bactocell®) in red tilapia (Ferguson et al. 2010) and rainbow trout (Merrifield et al. 2011a). Enhanced growth is commonly reported during probiotic treatments, where various strains affect different aquatic species (Table 3).

Finally, an important characteristic of probiotics is their safety to the host. Integrity of gut mucosa supports the safety of probiotic administration, which has been demonstrated upon use of *Lb. delbrüeckii* subsp. *lactis* (Salinas et al. 2008a, b), *Lb. plantarum* and *Lb. fructivorans* (Picchietti et al. 2007). In contrast to these results, Salma et al. (2011) noticed severe cell damage when distal intestine of beluga (*Huso huso*) was exposed to *Lb. plantarum* originally isolated from traditional Sabalan Iranian cheese prepared from raw sheep milk. Based on the latter results, we therefore recommend the use of light—and electron microscopy investigations of the intestine when evaluating the potential of probiotic bacteria in fish.

During the last two decades several comprehensive reviews have reflected on the promising use of probiotics in aquaculture. The use of probiotics has

also opened a new era of health management strategies in aquaculture; immunity. Readers with special interest in probiotics and immunity in fish are referred to the comprehensive review of Nayak (2010).

The prebiotic concept

The use of probiotics is generally difficult in the feed production industry because of the low viability of the bacteria after pelleting and storage, as well as problems with feed handling and preparation. In addition, there is the possibility of probiotics entering into the environment. As an alternative, prebiotics have been assessed in an attempt to overcome these issues. Rather than introducing probiotic bacteria, the aim of prebiotics is to stimulate selected beneficial indigenous microbiota populations. In order for a food ingredient to be classified as a prebiotics, Gibson and Roberfroid (1995) suggested that prebiotics should; (1) be neither hydrolyzed nor absorbed in the upper part of the GI tract, (2) be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated, (3) consequently, be able to alter the colonic flora in favor of a healthier composition and (4) induce luminal or systemic effects that are beneficial to the host health.

The initial research with prebiotics dates back to the end of the 1970s when Japanese scientists showed that bifidobacteria selectively fermented several carbohydrates (especially fructooligosaccharides; FOS). Prebiotics consist mainly of oligosaccharides; mannan oligosaccharides (MOS), fructooligosaccharides (FOS, including short chain-fructooligosaccharides; sc-FOS), glucooligosaccharides (GOS) and *trans*-galactooligosaccharides (TOS; galactooligosaccharides are also included). According to Lauzon et al. (2014b) inulin, a fructan polysaccharide, also has documented prebiotic qualities.

Readers with special interest in the use of prebiotics in aquaculture are referred to the reviews of Merrifield et al. (2010a), Ringø et al. (2010, 2014), Ganguly et al. (2013), Daniels and Hoseinifar (2014) and Torrecillas et al. (2014), and the recent research papers of Lokesh et al. (2012), Zhang et al. (2012a, b), Anguiano et al. (2013), Liu et al. (2013), Hoseinifar et al. (2013), Torrecillas et al. (2013), Wu et al. (2013a) and Zadeh et al. (2014).

The synbiotic concept

Synbiotic refers to nutritional supplements combining a mixture of probiotics and prebiotics in a form of synergism. The idea is that prebiotics will improve the survival of the live microbial supplements in the GI tract of the host (Gibson and Roberfroid 1995). Since the first fish study on synbiotics was published in 2009 (Rodriguez-Estrada et al. 2009) there has been a growing interest in the use of synbiotics in aquaculture (Cerezuela et al. 2011). However, since this review was published several synbiotic studies have emerged (Table 4). The focus of these studies have spanned from growth performance, feed utilization, digestive enzyme activities, body composition, immunological responses, haematological/serum biochemical parameters, disease resistance, survival rate and gut microbiota of synbiotic fed finfish, shellfish and echinoderms. To avoid duplication, fish studies reviewed by Cerezuela et al. (2011) are not discussed in this sub-section and readers with special interest are referred to the original review.

The effect of Biomin IMBO (*Enterococcus faecium* and FOS; 0.5, 1, 1.5 g kg^{-1}) on rainbow trout's specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), survival and disease resistance towards *Saprolegnia parasitica* was evaluated by Firouzbakhsh et al. (2012). All inclusion levels significantly improved SGR, FCE, survival and resistance against *S. parasitica* while FCR and condition factor (CF) were decreased. Similar improvements on growth, and in some cases survival, have been observed with the application of commercial synbiotic Biomin IMBO to kutum (*Rutilus frisii* Nordmann, 1840) (Haghighi et al. 2010), angelfish (*Pterophyllum scalare*) and zebrafish (*Danio rerio*) (Nekoubin et al. 2012b).

Tapia-Paniagua et al. (2011) evaluated modulation of the intestinal allochthonous microbiota of gilthead sea bream ($\sim$ 80 g) by administration of *Debaryomyces hansenii* in combination with inulin. Experimental fish were fed either a commercial diet (control diet), or diet supplemented with *D. hansenii* strain L2 (10^6 CFU g^{-1}) plus 3 % inulin (experimental diet II) for 4 weeks. After 2 and 4 weeks of feeding, samples of the whole intestine were aseptically removed for allochthonous microbiota analysis using PCR-DGGE and sequence analysis. Additionally, the expression of 12 selected genes related to the immune response

Table 4 Synbiotic applications in finfish

Fish species	Probiotic	Prebiotic	Results	References
Rainbow trout	*E. faecalis*	MOS, PHB	↑ Body weight, SGR, haematocrit, phagocytic index and mucus production	Rodriguez-Estrada et al. (2009)*
			↓ Mortality, FCR	
	E. faecium	FOS	↑ Body weight, weight gain, SGR, FCE, SR, crude protein and serum protein	Mehrabi et al. (2012)*
	E. faecium	FOS	↑ SGR, FCE and disease resistance against *Saprolegnia parasitica*	Firouzbakhsh et al. (2012)
			↓ FCR and CF	
Atlantic salmon	*Ped. acidilactici*	scFOS	↑ Intestinal immune response, serum lysozyme Activity and intestinal morphology	Abid et al. (2013)
			→ Growth, SGR and FCR	
Cobia	*B. subtilis*	Chitosan	↑ SGR, lysozyme, ACP, phagocytosis and respiratory burst	Geng et al. (2011)*
			↓ Mortality	
Gilthead sea bream	*D. hansenii*	Inulin	↑ Intestinal microbiota, peroxidise activity and gene expression	Tapia-Paniagua et al. (2011)
			→ Immune parameters	
	B. subtilis	Inulin	↑ Some immune related gene response	Cerezuela et al. (2012d)
			↓ Disease resistance against *P. damselae*	
			Modulate gut microbiota	
	B. subtilis	Inulin	Effect on gut histology	Cerezuela et al. (2013a)
			↑ Gene expression of β-actin and occludin	
	B. subtilis	Inulin	Expression of the other genes were up-regulated but not significantly affected	Cerezuela et al. (2013b)
Japanese flounder (*Paralichtys olivaceus*)	*B. clausii*	MOS, FOS	↑ Body weight, weight gain, crude protein, lipid, lysozyme, protease and amylase activities	Ye et al. (2011)*
			↓ FCR, TG and LDL-C	
Yellow croaker (*Larimichthys crocea*)	*B. subtilis*	FOS	↑ SGR, FER, lysozyme and SOD	Ai et al. (2011)*
			↓ mortality	
Kutum	*E. faecium*	FOS	↑ Body weight, SGR, FCR, PER	Haghighi et al. (2010)
Angelfish	*E. faecium*	FOS	↑ SGR and FCE	Nekoubin et al. (2012a)
			→ Hatching rate	
Zebrafish	*E. faecium*	FOS	↑ SGR and FCE	Nekoubin et al. (2012b)
			↓ FCR and CF	

Table 4 continued

Fish species	Probiotic	Prebiotic	Results	References
Koi carp	*B. coagulans*	COS	↑ SGR, total leukocyte counts, respiratory burst, phagocytic capacity, lysozyme activity, SOD and resistance against *A. veronii* ↓ FCR	Lin et al. (2012)
Hybrid surubims	*W. cibaria*	Inulin	↑ Level of midgut LAB, erythrocytes, total IgM by synbiotic treatment → Blood glucose, serum protein or lysozyme levels By synbiotic treatment ↓ *Pseudomonas* spp. and *Vibrio* spp. and circulating neutrophils in the synbiotic treatment	Mouriño et al. (2012)

* Studies discussed in the review of Cerezuela et al. (2011)

Bacterial abbreviations: *B. Bacillus*, *E. Enterococcus*, *Ped. Pediococcus*, *W. Weissella*

Prebiotic abbreviations: *MOS* mannan oligosaccharide, *PHB* polyhydroxybutyrate acid; *FOS* fructooligosaccharide, *IMO* isomaltooligosaccharide, *COS* Chitosan oligosaccharides

Abbreviations parameters investigated: *WGR* weight gain rate, *GP* growth performance, *BW* body weight, *BEG* body weight gain, *SGR* specific growth rate, *CF* condition factor, *SR* survival rate, *FCR* feed conversion ratio, *FU* feed utilization, *FER* feed efficient ratio, *BC* body composition; *TG* triglycerides, *PER* protein efficiency ratio, *LDL-C* low-density lipoprotein cholesterol, *IR* immunological response, *IRGR* immune related gene response, *PC* phagocytosis, *HP* haematological/serum biochemical parameters, *SOD* superoxide dismutase, *DR* disease resistance, *DR* against *P. damselae* subsp. *piscicida*, *DEA* digestive enzyme activities, *GM* gut microbiota, *GH* gut histology, *RP* reproductive parameters

Symbols represent an increase (↑), no effect (→) or decrease (↓) in the parameter of the synbiotic relative to the control

(IgM, MHCIα, MHCIIα, C3, IL-1β, TLR9A, TNFα, CSF-1R, NCCRP-1, Hep, TCRβ and CD8) from the skin, intestine, liver and HK tissue was analyzed by real-time PCR. Samples of blood and HK were obtained for the determination of humoral and cellular immune parameters. The results revealed that fish fed the experimental diet had lower intestinal microbial species richness and greater similarity indices compared with fish fed the control diet for 4 weeks, but *Pseudomonas* spp. dominated the intestinal microbiota in both experimental groups. Peroxidase activity was the only haematological parameter that was significantly increased in fish fed the synbiotic diet. RT-PCR revealed that several immune-related genes were up-regulated in the skin and intestine after 2 weeks of feeding. The maximum intestinal transcript levels for the major histocompatibility complex (MHC) genes MHCI and MHCII were significantly up-regulated. After 4 weeks of feeding, relatively lower gene transcript levels were recorded in the skin and intestine, but higher levels of complement 3, the pro-inflammatory cytokine TNFα and colony stimulating factor 1 receptor (CSF-1R), a receptor for a cytokine which controls macrophages production, differentiation and function, were observed in the intestine. In addition, at week 4 a greater effect was observed in the HK than week 2. This was especially prominent in the up-regulation of C3, the pro-inflammatory cytokine IL-1β, CSF-1R and non-specific cytotoxic cell receptor protein 1 (NCCRP-1; a surface protein which functions in target cell recognition and cytotoxicity) and the potential for improved disease resistance. Indeed, Lin et al. (2012) reported elevated peripheral total leucocyte counts, respiratory burst–, lysozyme—and superoxide activities, which afforded increased protection against *Aeromonas veronii* infection in koi fed a synbiotic application of *Bacillus coagulans* and COS. In addition, the inclusion of the synbiotic significantly improved SGR and FCR.

In three recent studies using gilthead sea bream, Cerezuela and colleagues evaluated the effect of *Bacillus subtilis* and inulin on immune-related gene expression and disease resistance against *P. damselae* subsp. *piscicida* (Cerezuela et al. 2012d), gut microbiota and gut histology (Cerezuela et al. 2013a), as well as the expression of different genes in the anterior intestine (Cerezuela et al. (2013b). Synbiotic administration significantly increased complement activity following four weeks of feeding, but not after two weeks of feeding. Respiratory burst activity was not affected. Serum IgM level was significantly higher after 2 weeks of feeding but not after four weeks. The expression of immune-related genes in HK of fish fed synbiotic for two weeks displayed no significant effect. Surprisingly, the cumulative mortality after challenge with *P. damselae* subsp. *piscicida* (i.p) was significant higher in the synbiotic group compared to the control group. In the study of Cerezuela et al. (2013a), the synbiotic group revealed signs of damage in the anterior intestine; similar to that reported in Arctic charr (*Salvelinus alpinus* L.) fed inulin (Olsen et al. 2001). Synbiotic administration also significantly increased villi height and intestinal diameter, but reduced the number of goblet cells and microvilli height. Gut microbiota, evaluated by DGGE, revealed that number of OTUs in fish fed synbiotic was significantly lower (6.0 ± 0.0) than that of the control fish (17.3 ± 0.9). Cerezuela et al. (2013b) investigated the effect of synbiotic administration on intestinal gene expression in gilthead sea bream, and revealed that only β-actin and occludin were significantly affected by synbiotic supplementation. The conclusions of these studies are that the synbiotic application of *B. subtilis* and inulin increases some immune parameters, but has a negative effect on gut morphology and gut microbiota, with a lesser effect on intestinal gene expression in the anterior intestine and a negative effect on disease resistance towards *P. damselae* subsp. *piscicida*. Further investigations are warranted to ascertain if benefits can be achieved with optimized inclusion levels.

Immunostimulants

The use of immunostimulants offers a unique approach for fish culturists to control disease losses in their facilities. Numerous polysaccharides from a variety of sources have the ability to stimulate the immune system, and thus behave as immunostimulants (Raa 1996; Vadstein 1997; Sakai 1999; Bricknell and Dalmo 2005; Soltanian et al. 2009; Ringø et al. 2012; Meena et al. 2013). The biological effects of immunostimulants are highly dependent on the receptors on the target cells recognizing them as potential high-risk molecules thus triggering various defense pathways. Thus, it is also important to increase knowledge of whether receptor specificity and the

inflammatory processes are induced with each potential immunostimulant. However, many mammalian receptors reported to bind immunostimulants such as NLR (NOD-like receptors) have yet to be reported in fish. Nevertheless, assuming that fish and mammalian cells share many similar receptors, one may predict the biological outcome of immunostimulants in fish.

β-glucan

Immunostimulants have been used as feed additives for several years in aquaculture, and yeast β-glucan may be the one with the longest track record. In nature, β-glucans are widespread and have been characterized in microorganisms, algae, fungi and plants (Volman et al. 2008). The chemical structure of β-glucan varies with respect to molecular weight and degree of branching. For example, β-glucan from yeast contains a particular carbohydrate consisting of glucose and mannose residues and is a major constituent in the cell membrane. In aquaculture, glucans have been successfully used to enhance the resistance of finfish and crustaceans against bacterial and viral infections. Readers are referred to the reviews of Soltanian et al. (2009), Ringø et al. (2012) and Meena et al. (2013) for detailed overview of studies on glucans as immunostimulants in aquaculture.

The second major by-product from the brewing industry is baker's yeast (*Saccharomyces cerevisiae*) which contains various immunostimulating compounds such as β-glucans (the cell walls are constructed almost entirely from β-1,3-D-glucan, β-1,6-D-glucan, mannoproteins and chitin bound together by covalent linkages), nucleic acids and oligosaccharides (Ferreira et al. 2010). Bakers yeast has the capacity to enhance growth and increase both humoral (myeloperoxidase and antibody titer) and cellular (phagocytosis, respiratory burst and cytotoxicity) immune responses, and to increase or confer resistance against pathogenic bacteria in various fish species (Soltanian et al. 2009; Ringø et al. 2012).

MacroGard®

According to Biorigin, MacroGard® is a source of highly purified, exposed, and preserved β 1,3/1,6 glucans produced from a specially-selected strain of the yeast *Saccharomyces cerevisiae* (http://www.biorigin.net). It is an environmentally sound alternative to antibiotics and the compound has been in use worldwide for almost 25 years as an immune modulating agent in animal husbandry and aquaculture (e.g. Sealey et al. 2008; Soltanian et al. 2009; Ringø et al. 2012; Meena et al. 2013).

Alginate

The adaptive immune system is poorly developed in the early developmental stages of fish, and in this respect, alginate has been proposed as a potential immune stimulator candidate. Alginate is a polysaccharide composed of β-1,4-D-mannuronic acid (M) and C5-epimer α-L-glucuronic acid (G) (Remminghorst and Rehm 2006).

Commercially available alginates have M-content ranging between 30 and 70 %. Alginates with up to 80 % M-content have also been shown to be potent stimulators of immune cells such as human monocytes (Skjåk-Brak et al. 2000). High-M alginate has also been used as an immunostimulant for enhancement of innate immune resistance in fish larvae and fry (Vadstein 1997; Skjermo and Vadstein 1999; Vollstad et al. 2006; Ringø et al. 2012).

Ergosan

This is an algal based product that contains 1 % alginic acid extracted from *Laminaria digitata*. To the author's knowledge, the first study on Ergosan in aquaculture was reported by Miles et al. (2001) on striped snakehead (*Channa striata*). Ergosan was injected intraperitoneally and improved the ability of macrophages to inhibit growth and the ability of serum to inhibit growth and germination of *Aphanomyces invadans*.

In order to present an acceptable overview of the information available on Ergosan, general information is presented here.

A single intraperitoneal (i.p.) injection of 1 mg of Ergosan significantly stimulated the non-specific immune system of rainbow trout, augmented the proportion of neutrophils in the peritoneal wall, increased the degree of phagocytosis, respiratory burst activity and expression of interleukin-1β (IL-1β), interleukin-8 (IL-8) and one of the two known isoforms of tumor necrosis factor-alpha (TNF-α) in peritoneal leucocytes one day post-injection (Peddie et al. 2002). However, humoral immune parameters

were less responsive to intraperitoneal alginate administration with complement stimulation only evident in the 1 mg-treated group at 2 days post-injection.

An evaluation of the effect of Ergosan (5 g kg^{-1}) in prevention of columnaris disease (*Flavobacterium columnare*) reported that supplementation with Ergosan had no effect on cumulative mortality of 1.2 g rainbow trout, but a small non-significant improvement was noticed when 5 g fish were used (Suomalainen et al. 2009).

Sheikhzadeh et al. (2010) evaluated the effect of Ergosan (6 and 20 mg kg^{-1}) on semen quality (spermatocrit, sperm concentrations, sperm motility and seminal plasma compositions) of rainbow trout (~2,300 g). In fish receiving 20 mg Ergosan kg^{-1}; a significant increase of spermatocrit and sperm count and Ca^{2+} compared to the control group was observed. The aspartate aminotransferase and lactate dehydrogenase significantly decreased in both Ergosan groups, while no effect on sperm motility, K^+, K^+/Na^+ ratio, total protein, glucose and triglycerides compared to the control group were observed. Ergosan exerts positive effects in male trout broodstock, but further studies are warranted with regards to mechanisms (Sheikhzadeh et al. 2010).

Ergosan (5 g kg^{-1}) also significantly elevated SGR and feed intake (136.8 vs. 111 g $fish^{-1}$), but reduced FCR (1.43 vs. 2.0) in rainbow trout (~110 g) (Heidarieh et al. 2012). Furthermore, lipase activity and leukocyte and erythrocyte counts also increased in Ergosan fed fish, but trypsin and amylase activities were not affected. Gut morphology evaluation of pyloric caeca and proximal intestine by light microscopy displayed normal appearance in both dietary groups, but a higher percentage of goblet cells (mucus producing cells) were seen in pyloric caeca and proximal intestine of the Ergosan fed fish.

Dietary Ergosan (5 g kg^{-1}) significantly increased growth performance, lysozyme, protease, alkaline phosphatase and esterase activities in rainbow trout (≈110 g) compared to the control group where skin mucus agglutination of enterocytes was not observed (Sheikhzadeh et al. 2012). However, agglutination was observed in Ergosan fed fish. Moreover, the antibacterial activity of skin mucus towards *Yersinia ruckeri* was significantly higher in Ergosan fed fish after 50 days.

Merrifield et al. (2011c) investigated the effect of 5 g Ergosan kg^{-1} on growth performance, intestinal microbiota and gut histology of tilapia; for 9 weeks. Dietary Ergosan did not affect growth performance and intestinal microbiota (allochthonous and autochthonous, and species diversity and richness). No signs of cell or tissue damage, evaluated by light and electron microscopy, were seen in the Ergosan group compared to the control group. Trends towards elevated survival and body protein content, and a lower microvilli density in the posterior intestine were also reported. As dietary Ergosan did not affect the gut health status, a critical question arises. Does Ergosan reach the intestine and is it fermented in the stomach? This topic merits further investigation.

In a study evaluating the immunomodulatory activity of Ergosan (0.5 % supplementation) in sea bass, significant elevation in serum complement activity was reported after 15 days treatment, while significant increases were noticed in serum lysozyme, gill and liver heat shock protein (HSP) after 30 days (Bagni et al. 2005). However at the end of the experiment (45 days), no significant differences were noticed along with no effect on growth performance and FCR. A dramatic decrease in both innate and acquired immune parameters during the winter season was observed, but a partial recovery was noticed when the rearing temperature increased.

A 60-day study on beluga juveniles (~42 g) investigating the effect of different inclusion level of Ergosan (0, 2, 4 and 6 g kg^{-1}) revealed a significant elevation in growth rate, FCR and body protein when beluga were fed at the two highest inclusion levels (Jalali et al. 2009). Generally, supplementation of Ergosan did not alter haematological parameters, except for lymphocyte count and survival rate was not different among the dietary treatments. In a more recent study, Heidarieh et al. (2011) evaluated whether Ergosan, 5 g kg^{-1} affected growth performance, immunocompetent cell population and plasma lysozyme content of beluga (~110 g). A significant increase was noticed in growth performance, lymphocyte count and lysozyme activity in plasma of fish fed Ergosan compared to the control group.

An evaluation of the effect of Ergosan on immune stimulation of white shrimp reported no obvious differences of haemolymph proteins and total haemocyte counts (~10^5 cells ml^{-1}) (Montero-Rocha et al. 2006). However, a detailed analysis of the haemotocyte population showed significant changes in the relative levels of hyaline, semi-granular- and granular

haemocytes. In vitro antibacterial activity of haemolymph towards two shrimp pathogens, *V. harveyi* and *Vibrio parahaemolyticus* revealed enhanced activity of the Ergosan treated shrimps. It is worth noting that the enhancement was greatest against *V. parahaemolyticus*. Furthermore, a significant improvement in growth and length of the shrimp was seen when they were fed Ergosan. Although Ergosan revealed positive effects to physiological and immunological parameters, further studies are recommended to elucidate optimum timing and concentration to ensure maximum benefit (Montero-Rocha et al. 2006).

In order to evaluate whether immunostimulants may act in synergy with pro– and prebiotics additional research is required. Probiotics appear to modulate immunity of the host by improving the barrier properties of mucosa and modulating production of cytokines (protein mediators produced by immune cells) and contribute to cell growth, differentiation and defense mechanisms of the host (Nayak 2010). Viable live probionts are better than the non-viable heat-killed probionts in inducing higher immune responses in rainbow trout, especially enhancing head kidney leucocyte phagocytosis, serum complement activity etc. In recent years, several in vivo and in vitro studies have investigated the interaction between dietary probiotics and immunocompetence in humans as well as in fish and aquatic animals (Gómez and Balcázar 2008; Dimitroglou et al. 2011; Ganguly et al. 2010; Nayak 2010). By increasing the host's adaptive and innate immune mechanisms, LAB can protect the host against infection by enteric pathogens and tumor development. Immunological and other mechanisms behind the probiotic action may include; simulation of antibody secreting cell response (Kaila et al. 1992), enhancement of phagocytosis of pathogens (Panigrahi et al. 2004; 2005), modification/enhancement of cytokine production/natural complement activity (Panigrahi et al. 2007; Salinas et al. 2008b) and improvement of the host innate or acquired immune responses, direct effect on other microorganisms in the digestive tract, adhesion sites, microbial action or response stemming from microbial products, host products or food components (Oelschlaeger 2010). Consequently, probiotic bacteria may influence both adaptive and innate immune responses, and may reverse the increased intestinal permeability induced by antigens, but no information is available about long-term effects.

Nucleotide-supplemented diets are not strictly immunostimulants by definition but provide a dietary supplement that allows improved resistance to a pathogen insult. Readers with special interest on the use of nucleotide-supplementations in finfish and shellfish aquaculture are referred to the reviews of Li and Gatlin (2006) and Ringø et al. (2012).

Plant extracts

Some immunostimulants cannot be used because of various disadvantages, such as high production cost or limited effectiveness upon administration. Accordingly, numerous investigations have evaluated the effect of plant products on innate and adaptive immune response and their ability to control fish and shellfish diseases. To avoid duplication, studies on the topic; effect of plant products on disease resistance, innate and adaptive immune response of fish and shellfish reviewed by Dügenci et al. (2003), Galina et al. (2009), Harikrishnan et al. (2011) and Ringø et al. (2012) are not discussed in this sub-section and readers with special interest are referred to the original reviews. Recent research on the use of plant products in aquaculture is displayed in Table 5.

Nootash et al. (2013) investigated oral administration of green tea (*Camellia sinensis*) on expression of immune relevant genes and biochemical parameters in rainbow trout ($\sim$23.5 g) concluding that dietary supplementation, especially at an inclusion level of 100 mg kg^{-1}, enhanced the antioxidant system and augmented the investigated immune parameters including immune-related gene expression. However, further investigations into different gene expressions, gut morphology, gut microbiota and challenge studies are waranted.

Chakrabarti and Srivastava (2012) evaluated the effect of prickly chaff-flower (*Achyranthes aspera*) on rohu (*Labeo rohita*) larvae and concluded that administration of 5 g kg^{-1} prevented tissue damage and provided protection against oxidative stress. Furthermore, prickly chaff-flower improved disease resistance against *A. hydrophila* when injected intraperitoneally (i.p.).

The effects of ginger (*Zingiber officinale* Roscoe) on growth performance, haematological and biochemical parameters, immune response and disease response against *V. harveyi* of Asian sea bass (*Lates calcarifer*

Table 5 Recent use of plant extract in aquaculture

Fish species/ weight (g)	Plant extracts	Adm.	Doses	Exposure	Results	References
Rainbow trout (23.5 ± 2.6)	Green tea (*Camellia sinensis*)	Diet	0, 20, 100, 500 mg kg^{-1}	35 days	↑ SOD (100 mg kg^{-1}); SBA, TP, I-1β-T in spleen (all doses) ↑ Immune system (100 mg kg^{-1})	Nootash et al. (2013)
Rohu larva (1 ± 0.01 mg)	Prickly chaff-flower (*Achyranthes aspera*)	Diet	0, 1, 2.5, 5 g kg^{-1}	70 days	↑ DR against *A. hydrophila*, ↑ TTP (2.5 and 5 g kg^{-1}), Ly and NOS (5 g kg^{-1}) ↓ GOT, GPT, TBRSA (5 g kg^{-1})	Chakrabarti and Srivastava (2012)
Asian sea bass (18 ± 1)	Ginger (*Zingiber officinale*)	Diet	0, 1, 2, 3, 5, 10 g kg^{-1}	15 days	↑ DR against *V. harveyi* ↑WG, FCR, RBC, WBC, Pa, RBS, Ly, Ba, An ↓ Blood Glu, L, TG, Cho	Talpur et al. (2013)
Crucian carp (58.3 ± 4.6)	Polypore mushroom (*Coriolus versicolor*)	Diet	0, 0.25, 0.5, 1, 2, 4 g kg^{-1}	56 days	↑ DR against *A. hydrophila* ↑ RBC, WBC, Hb, TP, ALP (0.5 and 1 g kg^{-1}) ↓ ESR, ALT, AST, Glu, Cho, TG, BUN (0.5 and 1 g kg^{-1})	Wu et al. (2013b)
Tilapia (45 ± 5)	*Sophora flavescens*	Diet	0, 0.25, 0.5, 1, 2, 4 g kg^{-1}	25 days	↑ DR against *S. agalactiae* ↑ Ly, An, HCo, My, ROS, RNS	Wu et al. (2013c)

Readers with special interest in papers published prior to 2012 are referred to the reviews of Dügenci et al. (2003), Galina et al. (2009), Harikrishnan et al. (2011) and Ringø et al. (2012)

SOD superoxide dismutase, *SBA* serum bactericidal activity, *TP* total protein, *I-1β-T* interleukin-1 β transcription, *DR* disease resistance, *TTP* total tissue protein, *Ly* lysozyme, *NOS* nitric oxide synthase, *GOT* glutamic oxaloacetic transaminase, *GPT* glutamate pyruvate transaminase, *TBARS* thiobarbituric acid reactive substance, *WG* weight gain, *FCR* feed conversion ratio, *RBC* number of erythrocytes, *WBC* leucocytes, *Pa* phagocytosis, *RBS* respiratory burst, *Ba* bactericidal, *An* antiprotease, *Glu* glucose, *L* lipid, *TG* triglyceride, *Cho* cholesterol, *Hb* haemoglobin, *ALP* alanine phosphatase, *ESR* erythrocyte sedimentation rate, *ALT* alanine amino transferase, *AST* aspartate amino transferase, *BUN* blood urea nitrogen, *HCo* haemolytic complement, *My* myeloperoxidase, *ROS* reactive oxygen species, *RNS* reactive nitrogen species

Symbols represent an increase/enhanced (↑), no effect (→) or decrease (↓) in the parameter of the plant extract relative to the control

Bloch) were investigated by Talpur et al. (2013). Growth performance was improved and blood parameters; glucose, lipid, triglyceride and cholesterol levels, were lower by dietary ginger. Moreover, ginger strengthened the non-specific immunity and protection against *V. harveyi*.

Wu et al. (2013b) investigated the effect of polypore mushroom (*Coriolus versicolor*) polysaccharides (CVP) on haematological, biochemical parameters as well as disease resistance against *A. hydrophila*; injected i.p. in allogynogenetic crucian carp. At an inclusion level of 0.5 and 1 g CVP kg^{-1} affected haematological and biochemical parameters, in contrast to a low inclusion level (0.25 g kg^{-1}; no effect) and high inclusion (2 and 4 g kg^{-1}; negative effect). Furthermore, fish fed 1 g CVP kg^{-1} prevented the experimental infection by *A. hydrophila*.

Wu et al. (2013) tested the effect of *Sophora flavescens* on the non-specific humoral responses (lysozyme, antiprotease and complement) and cellular immune responses (reactive oxygen species and nitrogen species and myeloperoxidase) and disease protection against i.p. injection of *Streptococcus agalactiae* in tilapia. Supplementation of *S. flavescens*, at all inclusion levels, significantly enhanced non-specific humoral responses and myeloperoxidase activity. Cumulative mortality in the challenge experiment was significantly reduced in all groups fed *S. flavescens*, but inclusion level at 1 g gave the best protection. Based on their results, the authors

suggested that *S. flavescens* is a promising immunostimulant in tilapia aquaculture.

Macro- and microalgae and biotechnology potential

The generas *Gracilaria* (red algae; *Rhodophyta*) and *Ulva* (sea lettuces a group of edible green algae), have fast growth and low-cost production (Viera et al. 2005). In addition to their successful use in bioremediation of aquaculture effluent (Marinho-Soriano et al. 2012), they may also be used as feed additives in aquaculture, replacing FM. Positive properties of *Gracilaria* and *Ulva* are that they are biocompatible, biodegradable and safe for the environment and human health (Viera et al. 2005). However, prior to use as feed additives it is of importance to evaluate their effect on fish health; gut microbiota, gut morphology, immune stimulation and disease resistance.

Mass-cultured microalgae are the main component of the first tropic level in the aquatic food chain and they are the source of indispensable nutrients for larval and juvenile bivalves, and for the larvae of some crustacean and multiple fish species in mariculture (Brown et al. 1997). During the last decade, microalgae production and its use in aquaculture has been extended and optimized in hatcheries. Most algae species in aquaculture have been selected on the basis of their mass-cultured potential, cellular size and overall nutritional value (Brown et al. 1997; Alonso et al. 2012). The most frequently used microalgae species in aquaculture are; *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *Chaetoceros calcitrans*, *Isochrysis galbana*, *Tetraselmis suecica* and *Chlorella* spp. (Coutteau 1996). These species can be produced industrially (Spolaore et al. 2006) or at a small scale in batches or in continuous in hatchery installations (Jorquera et al. 2010), where the combination of different microalgae species is optimized to provide a well-balanced diet and improve larval development (Benemann 1992).

Although the progress has been slow in the genetic engineering of microalgae, Walker et al. (2005) demonstrated the potential for genetic modification of *Phaeodactylum* spp. as well as the application of transgenic microalgae in aquaculture (Sayre et al. 2001). These investigations open the possibility of genetic transformation of microalgae, but the topic merits further investigation.

The wide variety of species and the morphological similarity between some algae species, make it necessary to use a combination of biochemical, physiological and morphological characters to correctly understand the taxonomic classifications. Molecular characterization with 18S rRNA and 16S rRNA has been used in the classification of 18 species of microalgae used in aquaculture (Alonso et al. 2012). Even though the molecular markers used in this study allowed optimal classification to genus level, the authors concluded that that other conserved markers should be evaluated in further studies.

Increasing knowledge regarding the antibacterial activity of different extracts of microalgae has evolved as new sources of specific antibacterial compounds have been reported. To the author's knowledge, the first studies using microalgae in this respect were carried out by Austin and Day (1990) and Austin et al. (1992). Heterotrophically grown, spray-dried *T. suecica* used as feed for penaeids was observed to rapidly inhibit growth of prawn pathogenic strains of *Vibrio* (Austin and Day 1990) and when used as a feed additive for Atlantic salmon, the algal cells led to a reduction in the level of bacterial diseases (Austin et al. 1992). In a more recent study, pressurized lipid extracts from *Dunaliella salina* had an antimicrobial effect against several microorganisms of importance for the food industry (*Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*) (Herrero et al. 2006). When discussing marine bioactives it is also of importance to note that extracts from marine phytoplankton and macroalgae exhibit antibacterial activities (del Pilar Sánchez-Saavedra et al. 2010; Goecke et al. 2012).

Information is available on the use of *Chlorella minutissima* and *Tetraselmis chuii* bioencapsulated in *Artemia* during weaning of Senegalese sole (*Solea senegalensis* Kaup; Makridis et al. 2009) and the effect of *T. suecica* on growth, feed utilization and fillet composition of European sea bass (Tulli et al. 2012). Recently, several papers have investigated the effect of microalgae inclusion in gilthead seabream (*Sparus aurata* L.) diets on; the immune system (Cerezuela et al. 2012a), immune system and disease resistance (Cerezuela et al. 2012b), intestinal ultrastructure and gut microbiota (Cerezuela et al. 2012c), and in combination with synbiotics (inulin and *Bacillus*

subtilis) on intestinal gene expression (Cerezuela et al. 2013b).

It is well known that quorum sensing, bacterial cell-to-cell communication with small signal molecules such acyl-homoserine lactones, regulates the virulence of many pathogenic bacteria. In a study with 19 micro-algal strains, Natrah et al. (2011) investigated the effect of the acyl-homoserine lactones, and reported that extracts of the most promising micro-algal strain; *Chlorella saccharophilia* inhibited quorum sensing regulated gene expression in all three reporter strains, *Chromobacterium violaceum*, *Escherichia coli* and *V. harveyi*, tested. These results are of high interest for future aquaculture and the topic merits further investigation.

The concept of functional food as a method to protect or improve consumer health was introduced in Japan at the beginning of the 1980s, based on several studies demonstrating the connection between diet and possible health effect (e.g. Salminen et al. 1998; Saulnier et al. 2009; Lordan et al. 2011). Ibañez and Cifuentes (2013) discussed the benefits of using algae as natural sources of functional ingredients. Even though there are beneficial effects for one or more functions of the human organism, the authors suggested that more research is needed for a comprehensive screening of bioactive metabolites produced by different marine organisms and that biomass production, recovery of bioactives and further processing must be optimized. These arguments are also valid for the aquaculture industry and deserve further attention.

Conclusions and further perspectives

The present study addressed key issues of importance in finfish and shellfish aquaculture. However, there are several related issues that also deserve attention. We therefore recommend readers to have a closer look at the review papers of Defoirdt et al. (2011; alternative to antibiotics for the control of bacterial disease in aquaculture), Crab et al. (2012; biofloc technology in aquaculture), Raina et al. (2009; quorum sensing), Galloway et al. (2012; inhibitors of quorum sensing in Gram-negative bacteria), Kalia (2013; quorum sensing inhibitors), Beaz-Hidalgo and Figueras (2013; *Aeromonas*—secretion systems, iron acquisition and quorum sensing mechanisms) and Cabrita et al. (2010; cryopreservation of fish sperm). Furthermore, the research papers of Dr. Martins's group (Tacchi et al. 2011, 2012; transcriptomic responses to functional feeds and FM substitution), professor Zhou's group (Chen et al. 2010; Cao et al. 2012; *N*-acetyl homoserine lactones as sisnal molecule), seaweeds as potential ingredient in aquafeed (Henry 2012; Saez et al. 2013), seafood biopreservation by LAB (Ghanbari et al. 2013) and the untapped source of novel compounds in the marine environment and their potential as novel drugs, personal care products and antimicrobial peptides (Kim et al. 2008; Pan et al. 2008; Wijffels 2008; Sperstad 2009; Schumacher et al. 2011) also focus on important issues that merit further investigation.

Recently, rapid genetic sequencing methods have become available, and these could be important tools to elucidate the diversity of antibiotic-resistance genes present in the fish gut and aquaculture environments. These approaches should allow the diversity of antibiotic resistance genes in the gut to be analysed, even when antibiotics are not used, and allow appropriate therapies to be proposed based on the presence of any resistant genes.

DNA vaccines are promising candidates for future disease control in aquaculture. DNA vaccines against Rhabdoviruses have proven to be highly efficacious which has resulted in commercialisation of a vaccine against IHNV. For other pathogens these vaccines have shown variable effects and investigations to improve vaccine potency are being undertaken. Aspects related to the safety of DNA vaccines have to be addressed to make the use of these vaccines more acceptable.

During the last two decades several comprehensive reviews have reflected on the promising use of probiotics in aquaculture. This paper emphasizes the wide application spectrum of allochthonous LAB, which should stimulate further developments in the field. Research in aquaculture probiotics is still at its infancy, and emphasis should be towards topics dealing with probiotic adhesion and mechanisms, among others. Importantly, host safety must be considered as well as the early application of probiotics, which has been shown to provide enhancement of beneficial effects. Even though numerous studies have investigated the effect of immunostimulants on the immune system of finfish and crustaceans the issue still merits further investigation as innate immune response is biologically linked to gut health. There is also a need to emphasise the effect of

immunostimulants on adherece and colonization of potential probiotics to the intestinal muscus, ligand-receptor interaction, involved signal transduction pathways, and expression of pro-inflamatory and anti-inflamatory cytokines. Although our understanding of microalgae and their biotechnology potential has grown during the last decade, additional knowledge is needed especially on their antibacterial potential and their potential as functional dietary ingredients.

Furthermore, with the increased inclusion of plant-based feedstuffs in diets, the intake of antinutritional factors (ANFs) will increase. The effects of different ANFs on digestive physiology and ultimately on metabolism will change utilization of specific nutrients (Francis et al. 2001; Krogdahl et al. 2010). This will change the dietary levels of specific nutrients needed to meet nutritional requirements. Such adjustments require extensive research in addition to the research needed to adjust recommended nutrient requirements for today's farmed fish. Furthermore, the gut microbiota, which may be influenced by various dietary nutrients, non-nutrients and ANFs, is also of importance for the host's gut and general health (Bauer et al. 2006). In their review devoted to important ANFs, Krogdahl et al. (2010) speculated that the intestinal microbiota may modify the ANFs and hence influence their interactions and biological effects. However, to the authors' knowledge no information is available on this topic in relation to finfish and shellfish and merits further investigation.

Acknowledgments The editor improved the manuscript by critical comment and suggestions.

References

Abid A, Davies SJ, Waines P, Emery M, Castex M, Gioacchini G, Carnevali O, Bickerdike R, Romero J, Merrifield DL (2013) Dietary synbiotic application modulates Atlantic salmon (*Salmo salar*) intestinal microbial communities and intestinal immunity. Fish Shellfish Immunol 35:1948–1956

Adomako M, St-Hilaire SY, Zheng Y, Eley J, Marcum RD, Sealey W, Donahower BC, LaPatra S, Sheridan PP (2012) Oral DNA vaccination of rainbow trout, Oncorhynchus mykiss (Walbaum), against infectious haematopoietic necrosis virus using PLGA [Poly(D, L-Lactic-Co-Glycolic Acid)] nanoparticles. J Fish Dis 35:203–214

Ai Q, Xu H, Mai K, Xu W, Wang J, Zhang W (2011) Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile yellow croaker, *Larimichthys crocea*. Aquaculture 317:155–161

Alonso M, Leong JA (2013) Licensed DNA vaccines against infectious hematopoietic necrosis virus (IHNV). Rec Pat DNA Gene Sequenc 7:62–65

Alonso M, Chiou PP, Leong JA (2011) Development of a suicidal DNA vaccine for infectious hematopoietic necrosis virus (IHNV). Fish Shellfish Immunol 30:815–823

Alonso M, Lago FC, Vieites JM, Espiñeira M (2012) Molecular characterization of microalgae used in aquaculture with biotechnology potential. Aquacult Int 20:847–857

Aly SM, Ahmed YAG, Ghareeb AAA, Mohamed MF (2008) Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunol 25:128–136

Anderson ED, Mourich DV, Fahrenkrud SC, LaPatra S, Shepherd J, Leong JA (1996) Genetic immunization of rainbow trout (*Oncorhynchus mykiss*) against infectious hematopoietic virus. Mol Mar Biol Biotechnol 5:114–122

Aubin J, Gatesoupe FJ, Labbe L, Lebrun L (2005) Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquacult Res 36:758–767

Austin B, Day JD (1990) Inhibition of prawn pathogenic *Vibrio* spp. by a commercial spray-died preparation of *Tetraselmis suecica*. Aquaculture 90:389–392

Austin B, Baudet E, Stobie M (1992) Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. J Fish Dis 15:55–61

Avella MA, Olivotto I, Silvi S, Place AR, Carnevali O (2010) Effect of dietary probiotics on clownfish: a molecular approach to define how lactic acid bacteria modulate development in a marine fish. Am J Physiol Regul Integra Comp Physiol 298:R359–R371

Azad IS, Al-Marzouk A (2008) Autochthonous aquaculture probiotics—a critical analysis. Res J Biotechnol Sp Iss SI:171–177

Bagheri T, Hedayati SA, Yavari V, Alizade M, Farzanfar A (2008) Growth, survival and gut microbial load of rainbow trout (*Oncorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turkish J Fish Aquatic Sci 8:43–48

Bagni M, Romano N, Finoia MG, Abelli L, Scapigliati G, Tiscar PG, Sarti M, Marino G (2005) Short- and long-term effects of a dietary yeast B-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol 18: 311–325

Balcázar JL, de Blas I, Ruiz-Zazuela I, Cunningham D, Vendrell D, Muzquiz JL (2006) The role of probiotics in aquaculture. Vet Microbiol 114:173–186

Balcázar JL, de Blas I, Ruiz-Zarzuela I, Vendrell D, Girones O, Muzquiz JL (2007a) Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (*Oncorhynchus mykiss*). FEMS Immunol Med Microbiol 51:185–193

Balcázar JL, De Blas I, Ruiz-Zarzuela I, Vendrell D, Calvo AC, Marquez I, Girones O, Muzquiz JL (2007b) Changes in intestinal microbiota and humoral immune response

following probiotic administration in brown trout (*Salmo trutta*). Br J Nutr 97:522–527
Bauer E, Williams BA, Smidt H, Mosenthin R, Verstegen MWA (2006) Influence of dietary components on development of the microbiota in single-stomached species. Nutr Res Rev 19:63–78
Beaz-Hidalgo R, Figueras MJ (2013) *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. J Fish Dis 36:371–388
Benemann JR (1992) Microalgae aquaculture feeds. J Appl Phycol 4:233–245
Birkbeck TH, Ringø E (2005) Pathogenesis and the gastrointestinal tract of growing fish. In: Holzapfel W, Naughton P (eds) Microbial ecology in growing animals. Elsevier, Edinburgh, pp 208–234
Bricknell I, Dalmo RA (2005) The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol 19:457–472
Brown MR, Jeffrey SW, Volkman JK, Dunstran GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151:315–331
Brudeseth KR, Wiulsrød R, Fredriksen BN, Lindmo K, Løløing K-E, Bordevik M, Steine N, Gravningen K (2013) Status and future perspectives of vaccines for industrialised finfish farming. Fish Shellfish Immunol 35:1759–1768
Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8:1137–1144
Cabrita E, Sarasquete C, Martínez-Páramo S, Robles V, Beirão J, Pérez-Cerezales S, Herráez MP (2010) Cryopreservation of fish sperm: applications and perspectives. J Appl Ichthyl 26:623–635
Cao Y, He S, Zhou Z, Zhang M, Mao W, Zhang H, Yao B (2012) Orally administred termostable *N*-acyl homoserine lactonase from *Bacillus* sp. strain AI96 attenuates *Aeromonas hydrophila* infection in zebrafish. Appl Environ Microbiol 78:1899–1908
Carnevali O, Zamponi MC, Sulpizio R, Rollo A, Nardi M, Orpianesi C, Silvi S, Caggiano M, Polzonetti AM, Cresci A (2004) Administration of probiotic strain to improve sea bream wellness during development. Aquacult Int 12:377–386
Castex M, Lemaire P, Wabete N, Chim L (2010) Effect of probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress of *Litopenaus stylirostris* under *Vibrio nigripulchritudo* challenge. Fish Shellfish Immunol 28:622–631
Cerezuela R, Meseguer J, Esteban MÁ (2011) Current knowledge in synbiotic use for fish aquaculture. A review. J Aquacult Res Develop S1:008. doi:10.4172/2155-9546.S1-008
Cerezuela R, Guardiola FA, Meseguer J, Esteban MÁ (2012a) Enrichment of gilthead seabream (*Sparus aurata* L.) diet with microalgae: effects on the immune system. Fish Physiol Biochem 38:1729–1739
Cerezuela R, Guardiola FA, González P, Meseguer J, Esteban MÁ (2012b) Effects of dietary *Bacillus subtilis*, *Tetraselmis chuii*, and *Phaeodactylum tricornutum*, singularly or in combination, on the immune response and disease resistance system of sea bream (*Sparus aurata* L.). Fish Shellfish Immunol 33:342–349
Cerezuela R, Fumanal M, Tapia-Paniagua ST, Meseguer J, Moriñigo MÁ, Esteban MÁ (2012c) Histological alterations and microbial ecology of the intestine in gilthead seabream (*Sparus aurata* L.) fed dietary probiotics and microalgae. Cell Tiss Res 350:477–489
Cerezuela R, Guardiola FA, Meseguer J, Esteban MÁ (2012d) Increases in immune parameters by inulin and *Bacillus subtilis* dietary administration to gilthead seabream (*Sparus aurata* L.) did not correlate with disease resistance to *Photobacterium damselae*. Fish Shellfish Immunol 32:1032–1040
Cerezuela R, Fumanal M, Tapia-Paniagua ST, Meseguer J, Moriñigo MÁ, Esteban MÁ (2013a) Changes in intestinal morphology and microbiota caused by dietary administration of inulin and *Bacillus subtilis* in gilthead seabream (*Sparus aurata* L.) specimes. Fish Shellfish Immunol 34:1063–1070
Cerezuela R, Meseguer J, Esteban MÁ (2013b) Effects of dietary inulin, *Bacillus subtilis* and microalgae on intestinal gene expression in gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol 34:843–848
Chakrabarti R, Srivastava PK (2012) Effect of dietary supplementation with *Achyranthes aspera* seed on larval rohu *Labeo rohita* challenged with *Aeromonas hydrophila*. J Aquatic Anim Health 24:213–218
Chelossi E, Vezzulli L, Milano A, Branzoni M, Fabiano M, Riccardi G, Abanat IM (2003) Antibiotic resistance of benthic bacteria in fish-farms and control sediments of the Western Mediterranean. Aquaculture 219:83–97
Chen R, Zhou Z, Cao Y, Bai Y, Yao B (2010) High yield expression of an AHL-lactonase from *Bacillus* sp. B546 in *Pichia pastoris* and its application to reduce *Aeromonas hydrophila* mortality in aquaculture. Mic Cell Fac 9:39. doi:10.1186/1475-2859-9-39
Clarke JL, Waheed MT, Lössl AG, Martinussen I, Daniell H (2013) How can plant genetic engineering contribute to cost-effective fish vaccine delelopment for promoting sustainable aquaculture? Plant Mol Biol 83:33–40
Corbeil S, LaPatra SE, Anderson ED, Jones J, Vincent B, Hsu YL, Kurath G (1999) Evaluation of the protective immunogenicity of the N, P, M, NV and G proteins of infectious hematopoietic necrosis virus in rainbow trout *Oncorhynchus mykiss* using DNA vaccines. Dis Aquat Org 39:29–36
Corbeil S, Kurath G, LaPatra SE (2000) Fish DNA vaccine against infectious hematopoietic necrosis virus: efficacy of various routes of immunisation. Fish Shellfish Immunol 10:711–723
Coutteau P (1996) Manual on the production and use of live food for aquaculture. FAO Fisheries, Technical Paper
Crab R, Defoirdt T, Bossier P, Verstraete W (2012) Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture 356–357:351–356
Daniels C, Hoseinifar S (2014) Prebiotics in crustaceans. In: Merrifield D, Ringø E (eds) Aquaculture nutrition: gut health, probiotics and prebiotics. Wiley-Blackwell Publishing, Oxford, UK (in press)
Defoirdt T, Sorgeloos P, Bossier P (2011) Alternative to antibiotics for the control of bacterial disease in aquaculture. Curr Op Microbiol 14:251–258
del Pilar Sánchez-Saavedra M, Licea-Navarro A, Bernáldez-Sarabia J (2010) Evaluation of the antibacterial activity of

different species of phytoplankton. Rev Biol Mar Oceano 45:531–536

Díaz-Rosales P, Salinas I, Rodríguez A, Cuesta A, Chabrillón M, Balebona MC, Morinigo MA, Esteban MA, Meseguer J (2006) Gilthead seabream (*Sparus aurata* L.) innate immune response after dietary administration of head-inactivated potential probiotics. Fish Shellfish Immunol 20:482–492

Dimitroglou A, Merrifield DL, Carnevali O, Picchietti S, Avella M, Daniels C, Guroy D, Davies SJ (2011) Microbial manipulations to improve fish health and production—a Mediterranean perspective. Fish Shellfish Immunol 30: 1–16

Duff DCB (1942) The oral immunization of trout against *Bacterium salmonicida*. J Immunol 44:87–94

Dügenci SK, Arda N, Candan A (2003) Some medicinal plants as immunostimulants for fish. J Ethnopharmacol 88: 99–106

El-Haroun ER, Goda A, Chowdhury MAK (2006) Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquacult Res 37:1473–1480

Evelyn TPT (1997) A historical review of fish vaccinology. In: Gudding R, Lillehaug A, Midslyng PJ, Brown F (eds) Developments in biological standardization: Fish Vaccinology, vol. 90. International Association of Biological Standardization. Karger, Basel, Switserland, pp 3–12

FAO (2003) Review of the state of world aquaculture. FAO Fisheries Circular, 886 p

FAO (2012) Fisheries Global Information System (FAO-FIGIS) - Web site. Fisheries & Aquaculture Global Capture Production. http:/www.fao.org

Ferguson RMW, Merrifield DL, Harper GM, Rawling MD, Mustafa S, Picchietti S, Balcázar L, Davies SJ (2010) The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). J Appl Microbiol 109:851–862

Ferreira IMPLVO, Pinho O, Vieira E, Tavarela JG (2010) Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. Trend Food Sci Technol 21:77–84

Firouzbakhsh F, Mehrabi Z, Heydari M, Khalesi MJ, Tajick MA (2012) Protective effects of a synbiotic against experimental *Saprolegnia parasitica* infection in rainbow trout (*Oncorhynchus mykiss*). Aquacult Res early view. doi:10.1111/j.1365-2109.2012.03261.x

Francis G, Makkar HPS, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197–227

Frost P, Ness A (1997) Vaccination of Atlantic salmon with recombinant VP2 of infectious pancreatic necrosis virus (IPNV), added to a multivalent vaccine suppresses viral replication following IPNV challenge. Fish Shellfish Immunol 7:609–619

Galina J, Yin G, Ardo G, Jenny Z (2009) The use of immunostimulating herbs in fish. An overview of research. Fish Physiol Biochem 35:669–676

Galloway WRJD, Hodgkinson JT, Bowden S, Welch M, Spring DR (2012) Applications of small molecule activators and inhibitors of quorum sensing in Gram-negative bacteria. Trend Microbiol 20:449–458

Ganguly S, Paul I, Mukhopadhayay SK (2010) Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: a review. Israeli J Aquacult Bamidgeh 62:130–138

Ganguly S, Dora KC, Sarkar S, Chowdhury S (2013) Supplementation of prebiotics in fish feed: a review. Rev Fish Biol Fisheries 23:195–199

Garver KA, LaPatra SE, Kurath G (2005) Efficacy of an infectious hematopoietic necrosis (IHN) virus DNA vaccine in chinook *Oncorhynchus tshawytscha* and sockeye *O. nerka* salmon. Dis Aquat Org 64:13–22

Gatesoupe F-J (1999) The use of probiotics in aquaculture. Aquaculture 180:147–165

Gatesoupe FJ (2002) Probiotic and formaldehyde treatments of *Artemia* nauplii as food for larval pollack, *Pollachius pollachius*. Aquaculture 212:347–360

Gatlin DM III, Barrows F, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl Å, Nelson R, Overturf K, Rust M, Sealey W, Skonberg D, Souza EJ, Stone G, Wilson R, Wurtele E (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquacult Res 38:551–579

Geng X, Dong X-H, Tan B-P, Yang Q-H, Chi S-Y, Liu H-Y, Liu X-Q (2011) Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. Fish Shellfish Immunol 31:400–406

Ghanbari M, Jami M, Domig KJ, Kneifel W (2013) Seafood biopreservation by lactic acid bacteria—a review. Food Sci Technol 54:315–324

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human clonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1405

Gildberg A, Mikkelsen H (1998) Effects of supplementing the feed to Atlantic cod (*Gadus morhua*) fry with lactic acid bacteria and immuno-stimulating peptides during a challenge trial with *Vibrio anguillarum*. Aquaculture 167:103–113

Gillund F, Dalmo R, Tonheim TC, Seternes T, Myhr AI (2008) DNA vaccination in aquaculture—expert judgments of impacts on environment and fish health. Aquaculture 284:25–34

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson SR, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Goecke F, Labes A, Wiese J, Imhoff JF (2012) Dual effect of macroalgal extracts on growth of bacteria in Western Baltic Sea. Rev Biol Mar Oceano 47:75–86

Gómez GD, Balcázar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. FEMS Immunol Med Microbiol 52:145–154

Gomez-Casado E, Estepa A, Coll JM (2011) A comparative review on European-farmed finfish RNA viruses and their vaccines. Vaccine 29:2657–2671

Gram L, Ringø E (2005) Prospects of fish probiotics. In: Holzapfel W, Naughton P (eds) Microbial ecology in growing animals. Elsevier, Edinburgh, pp 379–417

Groff J, LaPatra S (2000) Infectious diseases impacting the commercial culture of salmonids. J Appl Aquacult 10:17–90

Haghighi DT, Fallahi M, Abdolahtabr Y (2010) The effect of different levels of Biomin® Imbo synbiotic on growth and, survival of *Rutilus frisii kutum* fry. J Fish 4:1–14

Harikrishnan R, Balasundaram C, Heo MS (2010a) Effect of probiotics enriched diet on *Paralichthys olivaceus* infected with lymphocystis disease virus (LCDV). Fish Shellfish Immunol 29:868–874

Harikrishnan R, Balasundaram C, Heo MS (2010b) Potential use of probiotic- and triherbal extract-enriched diets to control *Aeromonas hydrophila* infection in carp. Dis Aquatic Org 92:41–49

Harikrishnan R, Balasundaram C, Heo MS (2010c) *Lactobacillus sakei* BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, *Epinephelus bruneus*. Fish Shellfish Immunol 29:1037–1043

Harikrishnan R, Balasundaram C, Heo MS (2011) Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture 317:1–15

Heidarieh M, Soltani M, Tamimi AH, Toluei MH (2011) Comparative effect of raw fiber (Vitacel) and alginic acid (Ergosan) on growth performance, immunocompetent cell population and plasma lysozyme content of giant sturgeon (*Huso huso*). Turkisk J Fish Aquatic Sci 11:445–450

Heidarieh M, Mirvaghefi AR, Akbari M, Farahmand H, Sheikhzadeh N, Shahbazfar AA, Behgar M (2012) Effect of dietary Ergosan on growth performance, digestive enzymes, intestinal histology, hematological parameters and body composition of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 38:1169–1174

Henry EC (2012) The use of algae in fish feeds as alternatives to fishmeal. Int Aquafeed 2012:8p

Hernandez LHH, Barrera TC, Mejia JC, Mejia GC, Del Carmen M, Dosta M, Andrade RD, Sotres JAM (2010) Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilopsis gracilis* (Poecilidae). Aquacult Nutr 16:407–411

Herrero M, Ibañez E, Cifuentes A, Reglero G, Santoyo S (2006) *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. J Food Prot 69:2471–2477

Heuer OE, Kruse H, Grave K, Colligon P, Karunasagar I, Angulo FJ (2009) Human health consequences of use of antimicrobial agents in aquaculture. Clin Inf Dis 49:1248–1253

Hølvold LB (2012) PLGA (poly(D,L-lactic-co-glycolic) acid particles as DNA carriers in Atlantic salmon (*Salmo salar* L). Ph.D. thesis. Norwegian College of Fishery Science, Faculty of Bioscience, Fisheries and Economics, University of Tromsø, Norway

Hoseinifar SH, Khalili M, Rostami HK, Esteban MÁ (2013) Dietary galactooligosaccharide affects intestinal microbiota, stress resistance, and performance of Caspian roach (*Rutilus rutilus*) fry. Fish Shellfish Immunol 35:1416–1420

Huang I-N, Okawara T, Watanabe M, Kawai Y, Kitazawa S, Ohnuma S, Shibata C, Horii A, Kimura K, Taketomo N, Xiao J-Z, Iwatsuki K, Saito T (2013) New screening methods for probiotics with adhesion properties to sialic acid and sulphate residues inhuman colonic mucin using Biacore assay. J Appl Microbiol 114:854–860

Ibañez E, Cifuentes A (2013) Benefits of using algae as natural sources of functional ingredients. J Sci Food Agric 93:703–709

Iehata S, Inagaki T, Okunishi S, Nakano M, Tanaka R, Maeda H (2009) Colonization and probiotic effects of lactic acid bacteria in the gut of the abalone *Haliotis gigantea*. Fish Sci 75:1285–1293

Irianto A, Austin B (2002) Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 25:333–342

Jalali MA, Ahmadifar E, Sudagar M, Takami GA (2009) Growth efficiency, body composition, survival and haematological changes in great sturgeon (*Huso huso* Linnaeus, 1758) juveniles fed diets supplemented with different levels of Ergosan. Aquacult Res 40:804–809

Joosten PHM, Tiemersma E, Threels A, Caumartin-Dhieuxal C, Rombout JHWM (1997) Oral vaccination of fish against *Vibrio anguillarum* using alginate microparticles. Fish Shellfish Immunol 7:471–485

Jorquera O, Kiperstok A, Sales EA, Embirucu M, Ghirard ML (2010) Comparative energy life-cycle analysis of microalgal biomass production in open ponds and photobioreactors. Biores Technol 1001:1406–1413

Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H (1992) Enhanchement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Ped Res 32:141–144

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245

Kerry J, Hiney M, Coyne R, NicGabhainn S, Gilroy D, Cazabon D, Smith P (1995) Fish feed as a source of oxytetracycline-resistant bacteria in the sediments under fish farms. Aquaculture 131:101–113

Kim CH, Johnson MC, Drennan JD, Simon BE, Thomann E, Leong JA (2000) DNA vaccines encoding viral glycoproteins induce nonspecific immunity and Mx protein synthesis in fish. J Virol 74:7048–7054

Kim S-K, Ravichandran YD, Khan SB, Kim YT (2008) Prospective of the cosmeceuticals derived from marine organisms. Biotechnol Bioprocess Engin 13:511–523

Krogdahl Å, Penn M, Thorsen J, Refstie S, Bakke AM (2010) Important antinutrient in plant feedstuff for aquaculture: an update on recent findings regarding responses in salmonids. Aquacult Res 41:333–344

Kurath G, Garver KA, Corbeil S, Elliott DG, Anderson ED, LaPatra SE (2006) Protective immunity and lack of histopathological damage two years after DNA vaccination against infectious hematopoietic necrosis virus in trout. Vaccine 24:345–354

Lamari F, Castex M, Larcher T, Ledevin M, Mazurais D, Bakhrouf A, Gatesoupe F-J (2013) Comparison of the effects of the dietary addition of two lactic acid bacteria on the development and conformation of sea bass larvae, *Dicentrarchus labrax*, and the influence on associated microbiota. Aquaculture 376–379:137–145

Lauzon HL, Ringø E (2012) Prevalence and application of lactic acid bacteria in Aquatic Environments. In: Lahtinen S, Ouwehand AC, Salminen S, von Wright A (eds) Lactic

acid bacteria: microbiological and functional aspects, 4th edn. CRC Press, Boca Raton, pp 593–631

Lauzon HL, Gudmundsdottir S, Pedersen MH, Budde BB, Gudmundsdottir BK (2008) Isolation of putative probionts from cod rearing environment. Vet Microbiol 132:328–339

Lauzon HL, Gudmundsdottir S, Steinarsson A, Oddgeirsson M, Martinsdottir E, Gudmundsdottir BK (2010a) Impact of probiotic intervention on microbial load and performance of Atlantic cod (*Gadus morhua* L.) juveniles. Aquaculture 310:139–144

Lauzon HL, Gudmundsdottir S, Steinarsson A, Oddgeirsson M, Petursdottir SK, Reynisson E, Bjornsdottir R, Gudmundsdottir BK (2010b) Effects of bacterial treatment at early stages of Atlantic cod (*Gadus morhua* L.) on larval survival and development. J Appl Microbiol 108:624–632

Lauzon HL, Magnadottir B, Gudmundsdottir BK, Steinarsson A, Arnason IO, Gudmundsdottir S (2010c) Application of prospective probionts at early stages of Atlantic cod (*Gadus morhua* L.) rearing. Aquacult Res 41:e576–e586

Lauzon HL, Pérez-Sánchez T, Merrifield DL, Ringø E, Balcázar JL (2014a) Probiotic applications in coldwater fish species. In: Merrifield D, Ringø E (eds) aquaculture nutrition: gut health, probiotics and prebiotics. Wiley-Blackwell Publishing, Oxford (in press)

Lauzon HL, Dimitroglou A, Merrifield DL, Ringø E, Davies SJ (2014b) Probiotics and prebiotics—concepts, definitions and history. In: Merrifield D, Ringø E, (eds) Aquaculture nutrition: gut health, probiotics and prebiotics. Wiley-Blackwell Publishing, Oxford (in press)

Li P, Gatlin DM (2006) Nucleotide nutrition in fish: current knowledge and future applications. Aquaculture 251:141–152

Lin S, Mao S, Guan Y, Luo L, Luo L, Pan Y (2012) Effects of dietary chitosan oligosaccharides and *Bacillus coagulans* on the growth, innate immunity and resistance of kopi (*Cyprinus carpio koi*). Aquaculture 342–343: 36–41

Liu R, Chen J, Li K, Zhang X (2011) Identification and evaluation as a DNA vaccine candidate of a virulence-associated serine protease from a pathogenic *Vibrio parahaemolyticus* isolate. Fish Shellfish Immunol 30:1241–1248

Liu B, Xu L, Ge X, Xie J, Xu P, Zhou Q, Pan L, Zhang YY (2013) Effects of mannan oligosaccharide on the physiological responses, HSP70 gene expression and disease resistance of Allogynogenetic crucian carp (*Carassius auratus gibelio*) under *Aeromonas hydrophila* infection. Fish Shellfish Immunol 34:1395–1403

Lokesh J, Fernandes JMO, Korsnes K, Bergh Ø, Brinchmann MF, Kiron V (2012) Transcriptional regulation of cytokines in the intestine of Atlantic cod fed yeast derived mannan oligosaccharide or β-glucan and challenged with *Vibrio anguillarum*. Fish Shellfish Immunol 33:629–631

Lordan S, Ross RP, Stanton C (2011) Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar Drugs 9:1056–1100

Lorenzen N, Lorenzen E, Einer-Jensen K, LaPatra SE (2002) Immunity induced shortly after DNA vaccination of rainbow trout against rhabdoviruses protects against heterologous virus but not against bacterial pathogens. Develop Comp Immunol 26:173–179

Macías-Rodríguez ME, Zagorec M, Ascencio F, Vázquez-Juárez R, Rojas M (2009) *Lactobacillus fermentum* BCS87 expresses mucus and mucin-binding proteins on the cell surface. J Appl Microbiol 107:1866–1874

Makridis P, Moreira C, Alves Costa R, Rodrigues P, Dinis MT (2009) Use of microalgae bioencapsulated in *Artemia* during the weaning of Senegalese sole (*Solea senegalensis* Kaup). Aquaculture 292:153–157

Marinho-Soriano E, Azevedo CAA, Trigueiro TG, Pereira DC, Carneiro MAA, Camara MR (2012) Bioremediation of aquaculture wastewater using macroalgae and *Artemia*. Int Biodeter Biodegrad 65:253–257

Martinez-Lopez A, Chinchilla B, Encinas P, Gomez-Casado E, Estepa A, Coll JM (2012) Replacement of the human cytomegalovirus promoter with fish enhancer and core elements to control the expression of the G gene of viral haemorrhagic septicemia virus (VHSV). J Biotechnol 164:171–178

Martinez-Lopez A, Encinas P, Garcia-Valtanen P, Gomez-Casado E, Coll JM, Estepa A (2013) Improving the safety of viral DNA vaccines: development of vectors containing both 5' and 3' homologous regulatory sequences from non-viral origin. Appl Microbiol Biotechnol 97:3007–3016

Meena DK, Das P, Kumar S, Mandal SC, Prusty AK, Singh SK, Akhtar MS, Behera BK, Kumar K, Pal AK, Mukherjee SC (2013) Beta-glucan: an ideal immunostimulants in aquaculture (a review). Fish Physiol Biochem 39:431–457

Mehrabi Z, Firouzbakhsh F, Jafarpour A (2012) Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Anim Physiol Anim Nutr 96:474–481

Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker RR, Bøgwald J, Castex M, Ringø E (2010a) The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302:1–18

Merrifield DL, Bradley G, Baker RTM, Davies SJ (2010b) Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment. Aquacult Nutr 16:496–503

Merrifield DL, Olsen RE, Myklebust R, Ringø E (2011a) Dietary effect of soybean (*Glycine max*) products on gut histology and microbiota of fish. In: El-Shemy H (ed) Soybean and nutrition. Rijeka, Croatia, InTech, pp 231–250. ISBN 978-953-307-536-5

Merrifield DL, Bradley G, Harper GM, Baker RTM, Munn CB, Davies SJ (2011b) Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquacult Nutr 17:73–79

Merrifield DL, Harper GM, Mustafa S, Carnevali O, Picchietti S, Davies SJ (2011c) Effect of dietary alginic acid on juvenile tilapia (*Oreochromis niloticus*) intestinal microbial balance, intestinal histology and growth performance. Cell Tiss Res 344:135–146

Miles DJC, Polchana J, Lilley JH, Kanchanakhan S, Thompson KD (2001) Immunostimulation of striped snakehead *Channa striata* against epizootic ulcerative syndrome. Aquaculture 195:1–15

Montero-Rocha A, McIntosh D, Sánchez-Merino I (2006) Immunostimulation of white shrimp (*Litopenaeus*

vannamei) following dietary administration of Ergosan. J Invertebrate Pathol 91:188–194

Munang'andu HM, Fredriksen BN, Mutoloki S, Brudeseth B, Kuo TY, Marjara IS, Dalmo RA, Evensen O (2012) Comparison of vaccine efficacy for different antigen delivery systems for infectious pancreatic necrosis virus vaccines in Atlantic salmon (*Salmo salar* L.) in a cohabitation challenge model. Vaccine 30:4007–4016

Mutoloki S, Reite OB, Brudeseth B, Tverdal A, Evensen Ø (2006) A comparative immunopathological study of infection site reactions in salmonids following intraperitoneal injection with oil-adjuvanted vaccines. Vaccine 24:578–588

Natrah FMI, Kenmegne MM, Wiyoto W, Sorgeloos P, Bossier P, Defoirdt T (2011) Effects of micro-algae commonly used in aquaculture on acyl-homoserine lactone quorum sensing. Aquaculture 317:53–57

Nayak SK (2010) Probiotics and immunity: a fish perspective. Fish Shellfish Immunol 29:2–14

Nekoubin H, Hatefi S, Javahery S, Sudagar M (2012a) Effects of synbiotic (*Biomin Imbo*) on growth performance, survival rate, reproductive parameters of angelfish (*Pterophyllum scalare*). Walailak J Sci Technol 9:327–332

Nekoubin H, Gharedashi E, Imanpour MR, Asgharimoghadam A (2012b) The influence of synbiotic (Biomin Imbo) on growth factors and survival rate of zebrafish (*Danio rerio*) larvae via supplementation with biomar. Global Vet 8:503–506

Nikoskelainen S, Ouwehand A, Salminen S, Bylund G (2001) Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. Aquaculture 198:229–236

Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius EM (2003) Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish Shellfish Immunol 15:443–452

Nootash S, Sheikhzadeh N, Baradaran B, Oushani AK, Moghadam MRM, Nofouzi K, Monfaredan A, Aghebati L, Zare F, Shabanzadeh S (2013) Green tea (*Camellia sinensis*) administration induces expression of immune relevant genes and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 35:1916–1923

O'Donnel GB, Reilly P, Davidson GA, Ellis AE (1996) The uptake of human gamma globulin incorporated into poly (D, L-lactide-co-glycolide) microparticles following oral intubation in Atlantic salmon, Salmo salar L. Fish Shellfish Immunol 6:507–520

Oelschlaeger TA (2010) Mechanisms of probiotic actions—a review. Int J Med Microbiol 300:57–62

Olsen RE, Myklebust R, Kryvi H, Mayhew TM, Ringø E (2001) Damaging effect of dietary inulin to intestinal enterocytes in Arctic charr (*Salvelinus alpinus* L.). Aquacult Res 32:931–934

Pan J-H, Gareth Jones EB, She Z-G, Pang J-Y, Lin Y-C (2008) Review of bioactive compounds from fungi in the South China Sea. Bot Mar 51:179–190

Panigrahi A, Kiron V, Kobayashi T, Puangkaew J, Satoh S, Sugita H (2004) Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Vet Immunol Immunopathol 102:379–388

Panigrahi A, Kiron V, Puangkaew J, Kobayashi T, Satoh S, Sugita H (2005) The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. Aquaculture 243:241–254

Panigrahi A, Kiron V, Satoh S, Hirono I, Kobayshi T, Sugita H, Puangkaew J, Aoki T (2007) Immune modulation and expression of cytokine genes in rainbow trout *Oncorhynchus mykiss* upon probiotic feeding. Develop Comp Immunol 31:372–382

Peddie S, Zou J, Secombes CJ (2002) Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. Vet Immunol Immunopathol 86:101–113

Picchietti S, Mazzini M, Taddei AR, Renna R, Fausto AM, Mulero V, Carnevali O, Cresci A, Abelli L (2007) Effects of administration of probiotic strains on GALT of larval gilthead seabream: immunohistochemical and ultrastructural studies. Fish Shellfish Immunol 22:57–67

Pirarat N, Kobayashi T, Katagiri T, Maita M, Endo M (2006) Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*). Vet Immunol Immunopathol 113:339–347

Planas M, Vázquez JA, Marques J, Pérez-Lomba R, González MP, Murado M (2004) Enhancement of rotifer (*Brachionus plicatilis*) growth by using terrestrial lactic acid bacteria. Aquaculture 240:313–329

Plant KP, LaPatra SE (2011) Advances in fish vaccine delivery. Dev Comp Immunol 35:1256–1262

Raa J (1996) The use of immunostimulatory substances in fish and shellfish farming. Rev Fish Sci 4:229–288

Raina S, De Vizio D, Odell M, Clements M, Vanhulle S, Keshavarz T (2009) Microbial quorum sensing: a tool or target for antimicrobial therapy? Biotechnol Appl Biochem 54:65–84

Ramakrishnan CM, Manohar MA, Dhanaraj M, Arockiaraj AJ, Seetharaman S, Arunsingh SV (2008) Effects of probiotics and spirulina on survival and growth of juvenile common carp (*Cyprinus carpio*). Israel J Aquacult Bam 60:128–133

Remminghorst U, Rehm B (2006) Bacterial alginates: from biosynthesis to applications. Biotechnol Lett 28:1701–1712

Ren P, Xu L, Yang Y, He S, Liu W, Ringø E, Zhou Z (2013) *Lactobacillus plantarum* subsp. *plantarum* JCM 1149 *vs*. *Aeromonas hydrophila* NJ-1 in the anterior intestine and posterior intestine of hybrid tilapia *Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂: an ex vivo study. Fish Shellfish Immunol 35:146–153

Ringø E (1999) Does *Carnobacterium divergens* isolated from Atlantic salmon (*Salmo salar* L.) colonise the gut of early developing turbot (*Scophthalmus maximus* L.) larvae? Aquacult Res 30:229–232

Ringø E, Myklebust R, Mayhew TM, Olsen RE (2007) Bacterial translocation and pathogenesis in the digestive tract of larvae and fry. Aquaculture 268:251–264

Ringø E, Løvmo L, Kristiansen M, Salinas I, Myklebust R, Olsen RE, Mayhew TM (2010) Lactic acid bacteria *vs*. pathogens in the gastrointestinal tract of fish: a review. Aquacult Res 41:451–467

Ringø E, Olsen RE, Gonzales Vecino JL, Wadsworth S, Song SK (2012) Use of immunostimulants and nucleotides in

aquaculture: a review. J Mar Sci Res Develop 2:104. doi:10.4172/2155-9910.1000104

Ringø E, Dimitroglou A, Hoseinifar SH, Davies SJ (2014) Prebiotics in finfish: an update. In: Merrifield D, Ringø E (eds) Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics. Wiley-Blackwell Publishing, Oxford (in press)

Rinkinen M, Westermarck E, Salminen S, Ouwehand AC (2003) Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus. Vet Microbiol 97:55–61

Robertson PAW, O'Dowd C, Burrells C, Williams P, Austin B (2000) Use of *Carnobacterium* sp as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture 185:235–243

Rodriguez-Estrada U, Satoh S, Haga Y, Fushimi H, Sweetman J (2009) Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharides and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout, *Oncorhynchus mykiss*. Aquacult Sci 57:609–617

Romero J, Feijoó CG, Navarrete P (2012) Antibiotics in aquaculture – use, abuse and alternatives. In: Carvalho E (ed) Health and environment in aquaculture, pp. 159–198. In-Tech, ISBN 978-953-51-0497-1

Saez MI, Martinez T, Alarcon J (2013) Effect of dietary inclusion of seaweeds on intestinal proteolytic acivity of juvenile sea bream, *Sparus aurata*. Int Aqua Feed, March/April 2013:4p

Sakai M (1999) Current research status of fish immunostimulants. Aquaculture 172:63–92

Salinas I, Cuesta A, Esteban MA, Meseguer J (2005) Dietary administration of *Lactobacillus delbrueckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. Fish Shellfish Immunol 19:67–77

Salinas I, Myklebust R, Esteban MA, Olsen RE, Meseguer J, Ringø E (2008a) *In vitro* studies of *Lactobacillus delbrueckii* subsp. *lactis* in Atlantic salmon (*Salmo salar* L.) foregut: tissue responses and evidence of protection against *Aeromonas salmonicida* subsp. *salmonicida* epithelial damage. Vet Microbiol 128:167–177

Salinas I, Abelli L, Bertoni F, Picchietti S, Roque A, Furones D, Cuesta A, Meseguer J, Esteban MA (2008b) Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead seabream (*Sparus aurato* L.). Fish Shellfish Immunol 25:114–123

Salma W, Zhou Z, Wang W, Askarian F, Kousha A, Ebrahimi MT, Myklebust R, Ringø E (2011) Histological and bacteriological changes in intestine of beluga (*Huso huso*) following ex vivo exposure to bacterial strains. Aquaculture 314:24–33

Salminen S, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau M-C, Roberfroid M, Rowland I (1998) Functional food science and gastrointestinal physiology and function. Br J Nutr 80(Suppl 1):S147–171

Salonius K, Simard N, Harland R, Ulmer JB (2007) The road to licensure of a DNA vaccine. Curr Opin Investig Drugs 8:635–641

Saulnier DMA, Spinler JK, Gibson GR, Versalovic J (2009) Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. Curr Opin Biotechnol 20:135–141

Sayre RT, Wagner RE, Siripornadulsil S, Farias C (2001) Transgenic algae for delivering antigens to an animal. WIPO Patentscope, publication no.: WO/2001/098335

Schumacher M, Kelkel M, Dicato M, Diederich M (2011) Gold from the sea: marine compounds as inhibitors of the hallmarks of cancer. Biotechnol Adv 29:531–547

Sealey WM, Barrows FT, Hang A, Johansen KA, Overturf K, LaPatra SE, Hardy RW (2008) Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*. Anim Feed Sci Technol 141:115–128

Seyfried EE, Newton RJ, Rubert KF, Pedersen JA, McMahon KD (2010) Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. Microb Ecol 59:799–807

Sheikhzadeh N, Reza A, Razi Allah JJ, Hossein T-N (2010) Effect of Ergosan on semen quality of male rainbow trout (*Oncorhynchus mykiss*) broodstock. Anim Reproduct Sci 122:183–188

Sheikhzadeh N, Pashaki AK, Nofouzi K, Heidarieh M, Tayefi-Nasrabadi H (2012) Effects of dietary Ergosan on cutaneous mucosal immune response in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 32:407–410

Skjåk-Bræk G, Flo T, Halaas Ø, Espevik T (2000) Immune stimulating properties of de-equatorially β (1–4) linked poly-uronides. Bioactive Carbohydrate Polymers. Kluwer, pp 85–93

Skjermo J, Vadstein O (1999) Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 177:333–343

Smith P (2008) Antimicrobial resistance in aquaculture. Rev Sci Tech Off Int Epiz 27:243–264

Soltanian S, Stuyven E, Cox E, Sorgeloos P, Bossier P (2009) Beta-glucans as immunostimulants in vertebrates and invertebrates. Crit Rev Microbiol 35:109–138

Sommerset I, Krossoy B, Biering E, Frost P (2005) Vaccines for fish in aquaculture. Expert Rev Vaccines 4:89–101

Son VM, Chang CC, Wu MC, Guu YK, Chiu CH, Cheng WT (2009) Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. Fish Shellfish Immunol 26:691–698

Sperstad S (2009) Characterisation of antimicrobial peptides from the spider crab, *Hyas araneus* (Decapoda, Crustacea). Ph.D. thesis. Norwegian College of Fishery Science, University of Tromsø, Norway

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

Suomalainen L-R, Bandilla M, Valtonen ET (2009) Immunostimulants in prevention of columnaris disease of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 32:723–726

Tacchi L, Bickerdike R, Douglas A, Secombes CJ, Martin SAM (2011) Transcriptomic responses to functional feeds in Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol 31:704–715

Tacchi L, Secombes CJ, Bickerdike R, Adler MA, Venegas C, Takle H, Martin SAM (2012) Transcriptomic and

physiological responses to fishmeal substitution with plant proteins in formulated feed in farmed Atlantic salmon (*Salmo salar*). BMC Genomics 13: 363, http://www.biomedcentral.com/1471-2164/13/363

Talpur AD, Ikhwanuddin M, Bolong A-MA (2013) Nutritional effects of ginger (*Zingiber officinale* Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. Aquaculture 400–401:46–52

Tamminen M, Karkman A, Lõhmus A, Muziasari WI, Takasu H, Wada S, Suzuki S, Virta M (2011) Tetracycline resistance gens persist in aquaculture farms in the absence of selection pressure. Environ Sci Technol 45:386–391

Tapia-Paniagua ST, Reyes-Becerril M, Ascencio-Valle F, Esteban MA, Clavijo E, Balebona MC, Morinigo MA (2011) Modulation of the intestinal microbiota and immune system of farmed *Sparus aurata* by the administration of the yeast *Debaryomyces hansenii* L2 in conjunction with inulin. J Aquacult Res Develop S 1:012. doi:10.4172/2155-9546.S1-012

Tonheim TC, Bøgwald J, Dalmo RA (2008) What happens to the DNA vaccine in fish? A review of current knowledge. Fish Shellfish Immunol 25:1–18

Torrecillas S, Makol A, Betancor MB, Montero D, Caballero MJ, Sweetman J, Izquierdo M (2013) Enhanced intestinal epithelial barrier health status on European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish Shellfish Immunol 34:1485–1495

Torrecillas S, Montero D, Izquierdo M (2014) Improved health and growth of fish fed mannan oligosaccharides: potential mode of action. Fish Shellfish Immunol 36:525–544

Tulli F, Chini Zittelli G, Giorgi G, Poli BM, Tibaldi E, Tredici MR (2012) Effect of the inclusion of dried *Tetraselmis suecica* on growth, feed utilization, and fillet composition of European sea bass juveniles fed organic diets. J Aquatic Food Prod Technol 21:188–197

Vadstein O (1997) The use of immunostimulation of marine larviculture: possibilities and challenges. Aquaculture 155:401–417

Van Muiswinkel WB (2008) A history of fish immunology and vaccination. I. The early days. Fish Shellfish Immunol 25:397–408

Viera MP, Gómez Pinchetti JL, Courtois de Vicose G, Bilbao A, Suárez S, Haroun RJ, Izquierdo MS (2005) Suitability of three macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve. Aquaculture 248:75–82

Villamil L, Tafalla C, Figueras A, Novoa B (2002) Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin Diag Lab Immunol 9:1318–1323

Villamil L, Figueras A, Planas M, Novoa B (2010) *Pediococcus acidilactici* in the culture of turbot (*Psetta maxima*) larvae: Administration pathways. Aquaculture 307:83–88

Vollstad D, Bøgwald J, Gaserød O, Dalmo RA (2006) Influence of high-M alginate on the growth and survival of Atlantic cod (*Gadus morhua* L.) and spotted wolffish (*Anarhichas minor* Olafsen) fry. Fish Shellfish Immunol 20:548–561

Volman JJ, Ramakers JD, Plat J (2008) Dietary modulation of immune function by β-glucans. Physiol. Behavior 94:276–284

von Gersdorff Jorgensen L, Sigh J, Kania PW, Holten-Andersen L, Buchmann K, Clark T, Rasmussen JS, Einer-Jensen K, Lorenzen N (2012) Approaches towards DNA vaccination against a skin ciliate parasite in fish. PLoS ONE 7:e48129

von Ossowski I, Reunanen J, Satokari R, Vesterlund S, Kankainen M, Huhtinen H, Tynkkynen S, Salminen S, de Vos WM, Palva A (2010) Mucosal adhesion properties of the probiotic *Lactobacillus rhamnosus* GG SpaCBA and SpaFED pilin subunits. Appl Environ Microbiol 76:2049–2057

Walker TL, Purton S, Becker DK, Collet C (2005) Microalgae as bioreactors. Plant Cell Rep 24:629–641

Wang YB, Tian ZQ, Yao JT, Li WF (2008) Effect of probiotics, *Enteroccus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. Aquaculture 277:203–207

Wijffels RH (2008) Potential of sponges and microalgae for marine biotechnology. Trend Biotechnol 26:26–31

Wu Y, Liu W-B, Li H-Y, Xu W-N, He J-X, Li X-F, Jiang G-Z (2013a) Effects of dietary supplementation of fructooligosaccharide on growth performance, body composition, intestinal activities and histology of blunt snout bream (*Megalobrama amblycephala*) fingerlings. Aquacult Nutr 19:886–894

Wu Z-x, Pang S-f, Chen X-x, Yu Y-m, Zhou J-m, Chen Y, Pang L-j (2013b) Effect of *Coriolus versicolor* polysaccharides on haematological and biochemical parameters and protection against *A. hydrophila* in allogynogenetic crucian carp (*Carassius auratus gibelio*). Fish Physiol Biochem 39:181–190

Wu Y-r, Gong Q-f, Fang H, Liang W-w, Chen M, He R-j (2013c) Effect of *Sophora flavescens* on non-specific immune response of tilapia (*Oreochromis niloticus*) and disease resistance against *Streptococcus agalactiae*. Fish Shellfish Immunol 34:220–227

Xu C, Mutoloki S, Evensen O (2012) Superior protection conferred by inactivated whole virus vaccine over subunit and DNA vaccines against salmonid alphavirus infection in Atlantic salmon (*Salmo salar* L.). Vaccine 30:3918–3928

Ye J-D, Wang K, Li F-D, Sun Y-Z (2011) Single or combined effects of fructo- and mannan oligosaccharides supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of Japanese flounder *Paralichthys olivaceus*. Aquacult Nutr 17:e902–e911

Zadeh HE, Hoseinifar S, Zaheh HV, Ringø E (2014) The effects of dietary inulin on growth performances, survival and digestive enzyme activities of common carp (*Cyprinus carpio*) fry. Aquacult Nutr accepted

Zhang J, Liu Y, Tian L, Yang H, Liang G, Xu D (2012a) Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol 33:1027–1032

Zhang M, Hu YH, Xiao ZZ, Sun Y, Sun L (2012b) Construction and analysis of experimental DNA vaccines against megalocytivirus. Fish Shellfish Immunol 33:1192–1198

Zhou Z, Wang W, Liu W, Gatlin DM, Zhang Y, Yao B, Ringø E (2012) Identification of highly-adhesive gut *Lactobacillus* strains in zebrafish (*Danio rerio*) by partial *rpo*B gene sequence analysis. Aquaculture 370–371:150–157