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Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments.

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Running title: Importance of lactic acid bacteria in fish farming

Abstract

Many recent papers have deepened the state of knowledge about lactic acid bacteria (LAB) in fish gut. In spite of high variability in fish microbiota, LAB are sometimes abundant in the intestine, notably in freshwater fish. Several strains of *Streptococcus* are pathogenic to fish. *S. iniae* and *Lactococcus garvieae* are major fish pathogens, against which commercial vaccines are available. Fortunately, most LAB are harmless, and some strains have been reported for beneficent effects on fish health. A major step forward of the recent years was those converging evidences that LAB can stimulate the immune system in fish. An open question is whether viability can affect immunostimulation. The issue is crucial to commercialise live probiotics rather than inactivated preparations or extracts. There was a regain of interest in allochthonous strains used as probiotics for terrestrial animals or human, due to economical and regulatory constraints, but the short survival in seawater may limit application to marine fish. If viability is required, alternative treatments may be the incorporation of prebiotics in feed, and other dietary manipulations that could promote intestinal LAB. Antagonism to pathogens is the other main feature of candidate probiotics, and there are many reports concerning mainly carnobacteria and *Enterococcus*. Some bacteriocins were characterized, which may be of interest not only for aquaculture, but also for food preservation.

Keywords: Fish · Gastrointestinal microbiota · Lactic acid bacteria · Pathogen · Probiotic

Introduction

Aquaculture is a fast growing industry, which represented one third of the world fisheries production in 2003 [Lowther, 2005]. The intensification of fish culture has caused the emergence of new pathogens, and the need for sustainable treatments and prophylactic measures. The interest of lactic acid bacteria (LAB) was highlighted in these regards, as the group includes fish pathogens, and also candidate probiotics that could improve fish health [Ringø and Gatesoupe, 1998]. In the meantime, the state of knowledge on fish gastrointestinal microbiota has amplified, and it may be opportune to update the importance of LAB in fish culture, in the light of recent advances. The reader is referred to the previous review [Ringø and Gatesoupe, 1998], while the present paper focused on fresh literature. In the recent years, the progress in molecular microbiology led to reassign the place of the bacterial group in microbiota. While new pathogens emerged, some vaccines have been commercialised, and many LAB have been tested for their probiotic potential.

Variability in natural occurrence

One of the most important features of gastrointestinal microbiota in fish is variability. Spanggaard et al. [2000] compared the intestinal microflora of rainbow trout in three different farms. These authors found *Carnobacterium* spp. dominant among the culturable isolates in one farm at one sampling date, but this genus was detected neither at another date in the same farm, nor in the other farms. Parallel samples were taken in the same three farms at the same dates for FISH and DGGE analyses, which led to different images of the bacterial community, while not culturable bacteria were found dominant in one fish [Huber et al., 2004]. This example illustrated the difficulty to delineate the actual incidence of LAB in fish intestine, but they are sometimes abundant. For instance, Cai et al. [1999] estimated LAB counts to 10^7 - 10^8 CFU/g in the intestine of common carp, mainly identified as *Lactococcus garvieae*, and also *Pediococcus acidilactici* and *Enterococcus faecium*. Diversity was particularly studied in freshwater fish [Cai et al., 1999; González et al., 2000; Hagi et al., 2004; Bucio et al., 2006], but LAB are also present in marine fish. Seppola et al. [2006] characterized as carnobacteria all the culturable isolates from the hindgut chamber of Atlantic cod, to which they ascribed a role of fermentation chamber.

Environmental fluctuations seemed the main cause of the variability observed in fish microbiota. Al-Harbi and Uddin [2003] showed the similarity of bacterial communities in sediment, freshwater and hybrid tilapia, though *Streptococcus* sp. was more abundant in brackish water and in Nile tilapia than in the pond sediment [Al-Harbi and Uddin, 2005]. Fish are poikilotherms, and temperature seemed the main cause of seasonal variation [Bucio et al., 2006]. Hagi et al. [2004] observed a shift from *Lactococcus raffinolactis* to *Lactococcus lactis* in fish intestine in summer, when temperature was above 20°C. The diet is also known to affect intestinal LAB. Ringø and Olsen [1999] isolated *Carnobacterium divergens* from the intestine of Arctic charr fed a low-carbohydrate diet, whereas other species of *Carnobacterium* were identified in fish fed wheat meal. Ringø et al. [2002a] noted that Arctic charr fed vegetable oil hosted *Carnobacterium* spp., unlike those fed with marine oil. Furthermore, some of these strains were found antagonistic to pathogens, possibly improving the resistance of fish to disease. These studies are particularly interesting in front of the need to replace fishmeal and fish oil in aqua feeds, due to resource shortage. The novel diets may thus affect microbiota in fish, and the interaction with fish health should be further investigated.

Pathogens

Two vaccines have been commercialised against the two major LAB pathogenic to fish, *Lactococcus garvieae* and *Streptococcus iniae* [Sommerset et al., 2005]. *L. garvieae* has many biovars, either pathogenic [Eldar et al., 1999] or not [Cai et al., 1999]. The efficiency of the vaccine may be affected by environmental conditions, e.g. the effect of salinity on barramundi vaccination against *S. iniae* [Delamare-Deboutteville et al., 2006]. Many fish species are

affected by these two major pathogens [Buller, 2004], and streptococcosis can spread with wild fish [Colorni et al., 2002]. Several other species of *Streptococcus* comprise fish pathogens [Buller, 2004]. *S. agalactiae* seemed of particular concern among the emerging epizootics [Evans et al., 2002], and a new vaccine has been proposed [Evans et al., 2004; Pasnik et al., 2005a, b, 2006]. *Carnobacterium piscicola* has been also frequently isolated from fish, but few strains seemed highly virulent [Buller, 2004]. Other genera of LAB have strains pathogenic to fish, but the incidence of the disease seemed generally limited to one fish species, or to one region, like *Vagococcus salmoninarum*, an emerging pathogen to rainbow trout in Europe [Ruiz-Zarzuela et al., 2005].

Though pathogenic strains are generally specific, some risk for human health might be caused by LAB harboured by fish. Virulence factors seemed widely distributed among the genus *Enterococcus*, and Semedo et al. [2003] recommended to screen for virulence even familiar strains before use by high risk population groups. *Streptococcus phocae*, a pathogen for aquatic mammals was isolated from clinical specimens of Atlantic salmon [Gibello et al., 2005]. The pathogeny has not been clearly established yet for salmon, but they could contaminate seals and other predators. Besides pathogenic strains, an overhanging threat is the spread antibiotic resistance due to livestock. Integrated broiler-fish farming seemed to increase the number of resistant isolates of *Enterococcus* spp., in comparison with farm culturing fish only [Petersen and Dalsgaard, 2003].

Autochthonous probiotics

L. garviae and *C. piscicola* were used as test pathogens to select candidate probiotics for fish among the intestinal bacteria with antagonistic features [Sugita et al., 2002; Vine et al., 2004]. Brunt and Austin [2005] protected rainbow trout against pathogenic *L. garviae* and *S. iniae* by incorporating into the feed an antagonistic strain of *Aeromonas sobria*, which stimulated the immune system of the trout. Limited protection was observed with the inactivated probiotic, but the live cells were the most effective.

Most LAB are harmless, and some strains are beneficent for fish health. The genus *Carnobacterium* was found particularly rich in strains antagonistic to pathogens, for instance many *C. piscicola*-like isolates from the gut of Atlantic salmon and Arctic charr inhibited the growth of *Aeromonas salmonicida* [Ringø et al., 2000, 2001]. Other isolates from gills of Atlantic salmon inhibited also *A. salmonicida* and *Vibrio anguillarum* [Ringø and Holzapfel, 2000]. Ringø et al. [2001] characterized *Carnobacterium divergens* isolates from the intestine of Atlantic salmon, Atlantic cod and wolf fish with inhibitory activity against *A. salmonicida*, like another strain from Arctic charr, which was also antagonistic to *V. anguillarum* and *Vibrio viscosus* [Ringø et al., 2002b]. It must be kept in mind that an in-vitro inhibition does not necessarily mean that the candidate probiotic will work in-vivo. Antagonistic *Carnobacterium* spp. did not protect against *V. anguillarum* the fish from where they were isolated, Atlantic cod or rainbow trout [Gildberg and Mikkelsen, 1998; Spanggaard et al., 2001]. Mortality was delayed during the challenge test when cod were fed a diet supplemented with another strain of *C. divergens*, isolated from Atlantic salmon [Gildberg and Mikkelsen, 1998]. The same strain was tested on turbot larvae challenged with *Vibrio pelagius*, but there was no improvement of survival [Ringø, 1999]. *Carnobacterium inhibens* isolated from Atlantic salmon [Jöborn et al., 1999] reduced mortality of the salmon and rainbow trout challenged with *A. salmonicida*, *Vibrio ordalii* and *Yersinia ruckeri*, but not *V. anguillarum* [Robertson et al., 2000]. *Carnobacterium* sp. BA211, isolated from rainbow trout, was found superior to *C. inhibens* for protecting the trout against *A. salmonicida* [Irianto and Austin, 2002]. Strain BA211 stimulated the immune system, and this was likely the main mode of action, since inactivated cells protected the trout against furunculosis [Irianto and Austin, 2003]. The inactivation seemed to decrease the effects on cellular immunity, except for the number of leucocytes, which was much greater with the inactivated form than with the live probiotic. The protection worked in 15-30 g juveniles, but not in 1-2 g fry. However, These challenge experiments did not reflect exactly what could happen in normal rearing conditions. In particular, the pathogen has been generally injected intraperitoneally to improve the repeatability of the test. The method is relevant to investigate the immune response after infection, but there is no information about the primary defenses against the infection in case of intestinal route. The importance of antagonism may be thus

underestimated, and the efficiency of candidate probiotics will need long-term application in fish farms to be validated.

Besides carnobacteria, few other LAB were tested as probiotics. For instance, *Weissella hellenica* isolated from the Japanese flounder was antagonistic to *Edwardsiella tarda*, *Pasteurella piscicida*, *Aeromonas hydrophila*, and *V. anguillarum*, but not *Streptococcus faecalis* [Cai et al., 1998]. When the candidate probiotic was added to the diet, the flounder had a higher growth than the control group, while less bacteria were counted in the intestine of the experimental group [Byun et al., 1997]. The survival of Atlantic halibut larvae was improved after incubation with *Lactobacillus plantarum* isolated from Atlantic cod, and the proliferation of mucous cells suggested a stimulation of cellular defenses [Ottesen and Olafsen, 2000].

Three strains of *C. piscicola* isolated from salmonid intestine (V1) and cold-smoked salmon (SF668, A9b) were investigated for their production of bacteriocin for biopreservation of fish products [Nilsson et al., 2002; Brillet et al., 2004]. The three strains were bactericidal to *Listeria monocytogenes* [Duffes et al., 1999; Duffes et al., 2000; Nilsson et al., 2002]. *C. divergens* V41 from salmonid intestine produced another bacteriocin, which seemed particularly promising for food protection [Métivier et al., 2000; Connil et al. 2002a, b; Brillet et al., 2004, 2005]. Three other bacteriocin producing LAB from turbot flesh inhibited *L. monocytogenes* and *Staphylococcus aureus* (*Lactococcus lactis*, *Enterococcus faecium*, and *Enterococcus mundtii* [Campos et al., 2006]). These heat-resistant bacteriocins worked in acidic conditions, and they may be of particular interest for processed food. Three other strains of the genus *Enterococcus* were isolated from the intestine of *Prochilodus argenteus*, with antagonistic properties against a wide range of bacteria [Silva et al., 2005]. *Pediococcus* sp. isolates from rohu fish intestine were also antagonistic to *Bacillus cereus*, *Escherichia coli*, and *S. aureus*, but the inhibitory cell-free supernatants should be treated with protease to confirm whether the effect was due to the bacteriocin production hypothesized by Halami et al. [1999]. Bacteriocin production can play a role in the antagonistic modes of action of probiotics, but the production of organic acids like lactic and acetic acids may be crucial too, according Vázquez et al. [2005] who tested turbot pathogens against allochthonous LAB in vitro. Tomé et al. [2006] confirmed that organic acids could account for the inhibitory activity of LAB in smoked salmon.

Allochthonous probiotics

Autochthonous bacteria may be a source of peculiarly fitted probiotics, but there has been regain of interest for the allochthonous strains that were already approved as probiotics for land animals or human, due to regulatory constraints [Gatesoupe, 2005]. Nikoskelainen et al. [2001a] evaluated seven such LAB strains by considering their adherence and penetration into fish mucus, their inhibition of pathogen growth and adhesion, and their resistance to fish bile. *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* were selected as the best candidate probiotics for rainbow trout. Mortality was significantly reduced when the trout were fed a diet supplemented with a high dose of *L. rhamnosus* (10^{12} CFU/g) [Nikoskelainen et al., 2001b]. The gastrointestinal tract was progressively colonized by the probiotic during one month of experimental feeding with a wide range of dosages (10^4 - 10^{11} CFU/g feed [Nikoskelainen et al., 2003]), up to becoming dominant in the culturable flora [Panigrahi et al., 2004]. Meanwhile, the immune system was stimulated [Nikoskelainen et al., 2003; Panigrahi et al., 2004]. As for *Carnobacterium* sp., the viability of the probiotic affected the immune response, and the heat-killed cells were less efficient than the live preparation [Panigrahi et al., 2005]. One or two weeks after probiotic treatment, the immune system came back to initial status [Nikoskelainen et al., 2003; Panigrahi et al., 2005]. A similar trend of immunostimulation was observed in gilthead seabream fed live *Lactobacillus delbrückei* [Salinas et al., 2005]. In this experiment, the viability of the probiotic was not checked in the intestine, but strong stimulation of seabream head-kidney leucocytes was observed in-vitro with heat-inactivated *L. delbrückei* [Salinas et al., 2006]. Villamil et al. [2002] showed the adhesion of *Lactococcus lactis* to turbot intestinal mucus in-vitro, and the antagonism to fish pathogens. The intestinal colonization seemed low in turbot fed the probiotic, but heat-killed cells of *L. lactis* stimulated the immune response [Villamil et al., 2002]. Allochthonous probiotics act therefore on the immune system of marine juvenile fish, but the viability did not seem essential, and their colonization potential needs confirmation in vivo. Vázquez et al. [2003] estimated between 3 and 21 h the half lives of allochthonous LAB in

seawater (35 g/l) at 20-30°C. This short survival time may not be favourable for colonization in marine fish, though probiotics kept alive in fish larvae fed live food organisms. *Pediococcus acidilactici* was relatively resistant to salinity [Vázquez et al., 2003], and it was retrieved in high numbers in larval pollack fed *Artemia* treated with the probiotic (10^5 CFU/larva [Gatesoupe, 2002]). *Lactobacillus plantarum* was similarly counted in gilthead seabream larvae before weaning (10^4 CFU/g larvae [Carnevali et al., 2004]). In this later experiment, *Artemia* were concomitantly treated with the allochthonous probiotic, and an autochthonous strain of *Lactobacillus fructivorans*, which colonised naturally the gut of seabream since weaning. By the end of the experiment, the counts of *L. fructivorans* in the fry fed the probiotics were one log higher than those of the control group (10^3 - 10^4 CFU/ g larvae). *L. plantarum* was not retrieved after weaning, though both probiotics were still supplied via the compound diet. This combined treatment increased fry survival, but it was not possible to know whether the effect was due to one or both probiotics. However, the number of immunocompetent cells associated with the gut was increased by this combined treatment [Picchiatti et al., 2006]. A commercial preparation with four probiotics, including *Lactobacillus acidophilus*, was also tested on the Japanese flounder [Taoka et al., 2006]. The consortium improved water quality, lysozyme activity, and resistance against heat shock and *V. anguillarum* infection. There was no evidence that the LAB contributed to these effects. Synergy may be expected from such association of probiotics, but that should be further investigated by comparing the effects of the single and multiple treatments.

Several allochthonous probiotics affected intestinal microbiota in freshwater fish, with various consequences on health and rearing performances. *P. acidilactici* limited the incidence of the vertebral column compression syndrome in rainbow trout [Aubin et al., 2005]. *Enterococcus faecium* protected European eel challenged with *Edwardsiella tarda* [Chang and Liu, 2002]. Some probiotic LAB were qualified as growth promoters, like *Streptococcus faecium* for common carp [Bogut et al., 1998], *E. faecium* for *Silurus glanis* [Bogut et al., 2000], or a commercial mix with *S. faecium* and *Lactobacillus acidophilus* for Nile tilapia [Lara-Flores et al., 2003]. In this later case, it was not possible to discriminate the efficiency of the two strains. The same remark applies to another commercial consortium with *Lactobacillus coagulans* and *Saccharomyces cerevisiae*, which stimulated the growth of Indian carps, *Catla catla* and *Cirrhinus mrigala* [Mohanty et al., 1996; Swain et al., 1996].

Conclusion

A major step for the rational use of LAB in aquaculture was brought by the converging evidences of their effects on the immune system of fish. However, many questions are open before practical application. Bricknell and Dalmo [2005] proposed the pulse administration of immunostimulants to avoid possible adverse effects of continuous supply. The long term effects of LAB as dietary supplement are still unknown, and the optimum duration of treatment should be estimated. Whether viability is essential for health benefits should be further investigated by comparing the effects of live probiotics to those of inactivated cells, cellular fractions, culture supernatants, and purified compounds like bacteriocins or organic acids. Where viability would be required, the commercialisation of new probiotics may be hindered by economical and regulatory impediments, especially with unfamiliar autochthonous strains, which should be certified exempt from virulence factors. Thus, the prebiotic approach may be worth developing too, but the applicability to fish awaits confirmation [Burr et al., 2005; Gatesoupe, 2005]. It may be worth exploring new aqua feed ingredients for their richness in carbohydrates that could be fuelled selectively by LAB.

References

- Al-Harbi AH, Uddin N: Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquac Res* 2003;34:43-48.
- Al-Harbi AH, Uddin N: Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture* 2005;250:566-572.
- Aubin J, Gatosoupe FJ, Labbé L, Lebrun L: Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquac Res* 2005;36:758-767.
- Bogut I, Milakovic Z, Bukvic Z, Brkic S, Zimmer R: Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in carp (*Cyprinus carpio*). *Czech J Anim Sci* 1998;43:231-235.
- Bogut I, Milakovic Z, Brkic S, Novoselic D, Bukvic Z: Effects of *Enterococcus faecium* on the growth rate and content of intestinal microflora in sheat fish (*Silurus glanis*). *Vet Med-Czech* 2000;45:107-109.
- Bricknell I, Dalmo RA: The use of immunostimulants in fish larval aquaculture. *Fish Shellfish Immunology* 2005;19:457-472.
- Brillet A, Pilet MF, Prevost H, Bouttefroy A, Leroi F: Biodiversity of *Listeria monocytogenes* sensitivity to bacteriocin-producing *Carnobacterium* strains and application in sterile cold-smoked salmon. *J Appl Microbiol* 2004;97:1029-1037.
- Brillet A, Pilet MF, Prevost H, Cardinal M, Leroi F: Effect of inoculation of *Carnobacterium divergens* V41, a biopreservative strain against *Listeria monocytogenes* risk, on the microbiological, chemical and sensory quality of cold-smoked salmon. *Int J Food Microbiol* 2005;104:309-324.
- Brunt J, Austin B: Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis* 2005;28:693-701.
- Bucio A, Hartemink R, Schrama JW, Verreth J, Rombouts FM: Presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system. *Food Microbiol* 2006;23:476-482.
- Buller NB: *Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual*. Wallingford, UK, CABI Publishing, 2004.
- Burr G, Gatlin D, Ricke S: Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. *J World Aquacult Soc* 2005;36:425-436.
- Byun JW, Park SC, Benno Y, Oh TK: Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). *J Gen Appl Microbiol* 1997;43:305-308.
- Cai Y, Benno Y, Nakase T, Oh TK: Specific probiotic characterization of *Weissella hellenica* DS-12 isolated from flounder intestine. *J Gen Appl Microbiol* 1998;44:311-316.
- Cai Y, Suyanandana P, Saman P, Benno Y: Classification and characterization of lactic acid bacteria isolated from the intestines of common carp and freshwater prawns. *J Gen Appl Microbiol* 1999;45:177-184.
- Campos CA, Rodríguez O, Calo-Mata P, Prado M, Barros Velázquez J: Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (*Psetta maxima*). *Food Res Int* 2006;39:356-364.
- Carnevali O, Zamponi MC, Sulpizio R, Rollo A, Nardi M, Orpianesi C, Silvi S, Caggiano M, Polzonetti AM, Cresci A: Administration of probiotic strain to improve sea bream wellness during development. *Aquac Int* 2004;12:377-386.
- Chang CI, Liu WY: An evaluation of two probiotic bacterial strains, *Enterococcus faecium* SF68 and *Bacillus toyoi*, for reducing edwardsiellosis in cultured European eel, *Anguilla anguilla* L. *J Fish Dis* 2002;25:311-315.
- Colorni A, Diamant A, Eldar A, Kvitt H, Zlotkin A: *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. *Dis Aquat Organ* 2002;49:165-170.
- Connil N, Plissoneau L, Onno B, Pilet MF, Prevost H, Dousset X: Growth of *Carnobacterium divergens* V41 and production of biogenic amines and divercin V41 in sterile cold-smoked salmon extract at varying temperatures, NaCl levels, and glucose concentrations. *J Food Protect* 2002a;65:333-338.

- Connil N, Prevost H, Dousset X: Production of biogenic amines and divercin V41 in cold smoked salmon inoculated with *Carnobacterium divergens* V41, and specific detection of this strain by multiplex-PCR. *J Appl Microbiol* 2002b;92:611-617.
- Delamare-Deboutteville J, Wood D, Barnes AC: Response and function of cutaneous mucosal and serum antibodies in barramundi (*Lates calcarifer*) acclimated in seawater and freshwater. *Fish Shellfish Immunol* 2006;21:92-101.
- Duffes F, Corre C, Leroi F, Dousset X, Boyaval P: Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins of *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. *J Food Protect* 1999;62:1394-1403.
- Duffes F, Leroi F, Dousset X, Boyaval P: Use of a bacteriocin producing *Carnobacterium piscicola* strain, isolated from fish, to control *Listeria monocytogenes* development in vacuum-packed cold-smoked salmon stored at 4 degrees C. *Sci Aliment* 2000;20:153-158.
- Eldar A, Goria M, Ghittino C, Zlotkin A, Bercovier H: Biodiversity of *Lactococcus garvieae* strains isolated from fish in Europe, Asia, and Australia. *Appl Environ Microbiol* 1999;65:1005-1008.
- Evans JJ, Klesius PH, Gilbert PM, Shoemaker CA, Al Sarawi MA, Landsberg J, Duremdez R, Al Marzouk A, Al Zenki S: Characterization of beta-haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *J Fish Dis* 2002;25:505-513.
- Evans JJ, Klesius PH, Gilbert PM, Shoemaker CA: Efficacy of *Streptococcus agalactiae* (group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. *Vaccine* 2004;22:3769-3773.
- Gatesoupe FJ: Probiotic and formaldehyde treatments of *Artemia* nauplii as food for larval pollack, *Pollachius pollachius*. *Aquaculture* 2002;212:347-360.
- Gatesoupe FJ: Probiotics and prebiotics for fish culture, at the parting of the ways. *Aqua Feeds: Formulation & Beyond* 2005;2(3):3-5.
- Gibello A, Mata AI, Blanco MM, Casamayor A, Domínguez L, Fernández-Garayzabal JF: First Identification of *Streptococcus phocae* Isolated from Atlantic Salmon (*Salmo salar*). *J Clin Microbiol* 2005;43:526-527.
- Gildberg A, Mikkelsen, H: 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* 1998;167:103-113.
- González CJ, Encinas JP, García-López ML, Otero A: Characterization and identification of lactic acid bacteria from freshwater fishes. *Food Microbiol* 2000;17:383-391.
- Hagi T, Tanaka D, Iwamura Y, Hoshino T: Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. *Aquaculture* 2004;234: 335-346.
- Halami PM, Chandrashekar A, Joseph R: Characterization of bacteriocinogenic strains of lactic acid bacteria in fowl and fish intestines and mushroom. *Food Biotechnol* 1999;13:121-136.
- Huber I, Spanggaard B, Appel KF, Rossen L, Nielsen T, Gram L: Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *J Appl Microbiol* 2004;96:117-132.
- Irianto A, Austin B: Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis* 2002;25:333-342.
- Irianto A, Austin B: Use of dead probiotic cells to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis* 2003;26:59-62.
- Jöborn A, Dorsch M, Olsson JC, Westerdahl A, Kjelleberg S: *Carnobacterium inhibens* sp nov., isolated from the intestine of Atlantic salmon (*Salmo salar*). *Int J Syst Bacteriol* 1999;49:1891-1898.
- Lara-Flores M, Olvera-Novoa MA, Guzmán-Méndez BE, López-Madrid W: Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 2003;216:193-201.
- Lowther A: Highlights from the FAO database on aquaculture production statistics. *FAO Aquaculture Newsletter* 2005;33:22-24.
- Métivier A, Boyaval P, Duffes F, Dousset X, Compoin JP, Marion D: Triton X-114 phase partitioning for the isolation of a pediocin-like bacteriocin from *Carnobacterium divergens*. *Lett Appl Microbiol* 2000;30:42-46.

- Mohanty SN, Swain SK, Tripathi SD: Rearing of catla (*Catla catla* Ham.) spawn on formulated diets. *J Aqua Trop* 1996;11:253-258.
- Nikoskelainen S, Salminen S, Bylund G, Ouwehand AC: Characterization of the properties of human- and dairy-derived probiotics for prevention of infectious diseases in fish. *Appl Environ Microbiol* 2001a;67:2430-2435.
- Nikoskelainen S, Ouwehand AC, Salminen S, Bylund G: Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. *Aquaculture* 2001b;198:229-236.
- Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius EM: Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). *Fish Shellfish Immunol* 2003;15:443-452.
- Nilsson L, Nielsen MK, Ng Y, Gram L: Role of acetate in production of an autoinducible class IIa bacteriocin in *Carnobacterium piscicola* A9b. *Appl Environ Microbiol* 2002;68:2251-2260.
- Ottesen OH, Olafsen JA: Effects on survival and mucous cell proliferation of Atlantic halibut, *Hippoglossus hippoglossus* L., larvae following microflora manipulation. *Aquaculture* 2000;187:225-238.
- Panigrahi A, Kiron V, Kobayashi T, Puangkaew J, Satoh S, Sugita H: Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. *Vet Immunol Immunopathol* 2004;102:379-388.
- Panigrahi A, Kiron V, Puangkaew J, Kobayashi T, Satoh S, Sugita H: The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 2005;243:241-254.
- Pasnik DJ, Evans JJ, Panangala VS, Klesius PH, Shelby RA, Shoemaker CA: Antigenicity of *Streptococcus agalactiae* extracellular products and vaccine efficacy. *J Fish Dis* 2005a;28:205-212.
- Pasnik DJ, Evans JJ, Klesius PH: Abstract Duration of protective antibodies and correlation with survival in Nile tilapia *Oreochromis niloticus* following *Streptococcus agalactiae* vaccination. *Dis Aquat Organ* 2005b;66:129-34.
- Pasnik DJ, Evans JJ, Klesius PH: Passive immunization of Nile tilapia (*Oreochromis niloticus*) provides significant protection against *Streptococcus agalactiae*. *Fish Shellfish Immunol* DOI: 10.1016/j.fsi.2006.01.001.
- Petersen A, Dalsgaard A: Antimicrobial resistance of intestinal *Aeromonas* spp. and *Enterococcus* spp. in fish cultured in integrated broiler-fish farms in Thailand. *Aquaculture* 2003;219:71-82.
- Picchiatti S, Mazzini M, Taddei AR, Renna R, Fausto AM, Mulero V, Carnevali O, Cresci A, Abelli L: Effects of administration of probiotic strains on GALT of larval gilthead seabream: immunohistochemical and ultrastructural studies. *Fish Shellfish Immunol* DOI: 10.1016/j.fsi.2006.03.009.
- Ringø E: Does *Carnobacterium divergens* isolated from Atlantic salmon, *Salmo salar* L., colonize the gut of early developing turbot, *Scophthalmus maximus* L., larvae? *Aquac Res* 1999;30:229-232.
- Ringø E, Gatesoupe FJ: Lactic acid bacteria in fish: a review. *Aquaculture* 1998;160:177-203.
- Ringø E, Holzapfel W: Identification and characterization of carnobacteria associated with the gills of Atlantic salmon (*Salmo salar* L.). *Syst Appl Microbiol* 2000;23:523-527.
- Ringø E, Olsen RE: The effect of diet on aerobic bacterial flora associated with intestine of Arctic charr (*Salvelinus alpinus* L.). *J Appl Microbiol* 1999;86:22-28.
- Ringø E, Bendiksen HR, Wesmajervi MS, Olsen RE, Jansen PA, Mikkelsen H: Lactic acid bacteria associated with the digestive tract of Atlantic salmon (*Salmo salar* L.). *J Appl Microbiol* 2000;89:317-322.
- Ringø E, Wesmajervi MS, Bendiksen HR, Berg A, Olsen RE, Johnsen T, Mikkelsen H, Seppola M, Strøm E, Holzapfel W: Identification and characterization of carnobacteria isolated from fish intestine. *Syst Appl Microbiol* 2001;24:183-191.
- Ringø E, Lødemel JB, Myklebust R, Jensen L, Lund V, Mayhew TM, Olsen RE: The effects of soybean, linseed and marine oils on aerobic gut microbiota of Arctic charr *Salvelinus alpinus* L. before and after challenge with *Aeromonas salmonicida* ssp. *salmonicida*. *Aquac Res* 2002a;33:591-606.

- Ringø E, Seppola M, Berg A, Olsen RE, Schillinger U, Holzapfel W: Characterization of *Carnobacterium divergens* strain 6251 isolated from intestine of Arctic Charr (*Salvelinus alpinus* L.). Syst Appl Microbiol 2002b;25:120-129.
- Robertson PAW, O'Dowd C, Burrells C, Williams P, Austin B: Use of *Carnobacterium* sp as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture 2000;185:235-243.
- Ruiz Zarzuela I, de Blas I, Gironés O, Ghittino C, Múzquiz, JL: Isolation of *Vagococcus salmoninarum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), broodstocks: Characterization of the pathogen. Vet Res Commun 2006;29:553-562.
- Salinas I, Cuesta A, Esteban MA, Meseguer J: Dietary administration of *Lactobacillus delbrueckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. Fish Shellfish Immunol 2005;19:67-77.
- Salinas I, Díaz-Rosales P, Cuesta A, Meseguer J, Chabrillón M, Moriñigo MA, Esteban MA: Effect of heat-inactivated fish and non-fish derived probiotics on the innate immune parameters of a teleost fish (*Sparus aurata* L.). Vet Immunol Immunopathol DOI: 10.1016/j.vetimm.2006.01.020.
- Semedo T, Almeida Santos M, Silva Lopes-MF, Figueiredo Marques-JJ, Barreto Crespo-MT, Tenreiro-R: Virulence factors in food, clinical and reference enterococci: A common trait in the genus? Syst Appl Microbiol 2003;26:13-22.
- Seppola M, Olsen RE, Sandaker E, Kanapathippillai P, Holzapfel W, Ringø E: Random amplification of polymorphic DNA (RAPD) typing of carnobacteria isolated from hindgut chamber and large intestine of Atlantic cod (*Gadus morhua* L.). Syst Appl Microbiol 2006;29:131-137.
- Silva FCP, Brito MFG, Farias LM, Nicoli JR: Composition and antagonistic activity of the indigenous intestinal microbiota of *Prochilodus argenteus* Agassiz. J Fish Biol 2005;67:1686-1698.
- Sommerset I, Krossoy B, Biering, E, Frost, P: Vaccines for fish in aquaculture. Expert Rev Vaccines 2005;4:89-101.
- Spanggaard B, Huber I, Nielsen J, Nielsen T, Appel KF, Gram L: The microflora of rainbow trout intestine: a comparison of traditional and molecular identification. Aquaculture 2000;182:1-15.
- Spanggaard B, Huber I, Nielsen J, Sick EB, Pipper CB, Martinussen T, Slierendrecht WJ, Gram L: The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. Environ Microbiol 2001;3:755-765.
- Sugita H, Okano R, Suzuki Y, Iwai S, Mizukami M, Akiyama N, Matsuura S: Antibacterial abilities of intestinal bacteria from larval and juvenile Japanese flounder against fish pathogens. Fisheries Sci 2002;68:1004-1011.
- Swain SK, Rangacharyulu PV, Sarkar S, Das KM: Effect of a probiotic supplement on growth, nutrient utilization and carcass composition in mrigal fry. J Aqua 1996;4:29-35.
- Taoka Y, Maeda H, Jo JY, Jeon MJ, Bai SC, Lee WJ, Yuge K, Koshio S: Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fisheries Sci 2006;72:310-321.
- Tomé E, Teixeira P, Gibbs PA: Anti-listerial inhibitory lactic acid bacteria isolated from commercial cold smoked salmon. Food Microbiol 2006;23:399-405.
- Vázquez JA, Cabo ML, González MP, Murado MA: Survival of lactic acid bacteria in seawater: A factorial study. Curr Microbiol 2003;47:508-513.
- Vázquez JA, González MP, Murado MA: Effects of lactic acid bacteria cultures on pathogenic microbiota from fish. Aquaculture 2005;245:149-161.
- Villamil L, Tafalla C, Figueras A, Novoa B: Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin Diagn Lab Immunol 2002;9:1318-1323.
- Vine NG, Leukes WD, Kaiser H: In vitro growth characteristics of five candidate aquaculture probiotics and two fish pathogens grown in fish intestinal mucus. FEMS Microbiol Lett 2004;231:145-152.