Probiotics in Aquaculture

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ABSTRACT

The skin, lateral line, gills and gastrointestinal tract or a combination of these organs are suggested to be infection routes in fish. This presentation will present some information on pathogenesis, protection against bacterial adhesion, autochthonous microbiota in the gastrointestinal tract and prebiotics. This information is important when discussing the use of probiotics in aquaculture.

Intensive fish production has increased the risk of infectious diseases and there is a growing need to find alternatives to antibiotic treatment for disease control as indiscriminate use of antibiotics in many parts of the aquaculture industry has led to the development of antibiotic resistance in bacteria. Today, a range of microorganisms have been suggested as or evaluated as fish probiotics. These include lactic acid bacteria, *Bacillus* species, *Pseudomonas* species, *Vibrio* species and other Gram-negatives. However, research in probiotics for aquaculture is at an early stage and much work is still needed.

Another aspect on fish health is the use of prebiotics to increase the population level of already beneficial bacteria colonizing the gastrointestinal tract and the effect of diet in disease resistance.

INTRODUCTION

Before presenting the hard facts on probiotics in aquaculture a short background about pathogenesis, protection against bacterial adhesion and the autochthonous microbiota in the gastrointestinal tract is first provided here. This background information is important when discussing the use of probiotics in aquaculture. Pathogenic microorganisms have evolved mechanisms to target skin, gills or gastrointestinal tract as a points of entry, and today it is generally accepted that the three major routes of infection in fish are through: (a) skin (Kawai *et al*., 1981; Muroga and de la Cruz, 1987; Kanno *et al*., 1990; Magarinos *et al*., 1995; Svendsen and Bøgvald, 1997; Spanggard *et al*., 2001); (b) gills (Hjeltnes *et al*., 1987; Baudin Laurencin and Germon, 1987; Svendsen *et al*., 1999); and (c) gastrointestinal tract (Sakai, 1979; Rose *et al*., 1989; Chair *et al*., 1994; Olsson, 1995; Grisez *et al*., 1996; Olsson *et al*., 1996*;* Romalde *et al*., 1996; Jöborn *et al*., 1997; Robertson *et al*., 2000; Lødemel *et al*., 2001).

Pathogenicity can be divided into four different phases (Birkbeck and Ringø, 2002): (1) the initial phase where the pathogen enters the host's environment, including the gastrointestinal tract; (2) the exponential phase where the pathogen adheres to and colonizes mucosal surfaces, replicates to sufficient numbers and translocate into host enterocytes; (3) the stationary phase where the pathogen replicates within the host and circumvents the host defence system. In this phase the host is moribund and this can quickly be followed by (4) the death phase.

In aquatic ecosystems, the intimate relationships between microorganisms and other biota and the constant flow of water through the gastrointestinal tract of fish and invertebrates will also affect their indigenous microbiota. Against this background, we may assume that the natural microbiota on eggs, larvae, fry, juveniles and adult fish may help to protect against colonization by a harmful microbiota. Another aspect seen from a microbial point of view is the fact that the microbiota of intensive rearing systems differs dramatically from that in the sea, and it is influenced by many factors such as; rearing techniques, nutrient, disinfection techniques and the use of antibiotics.

In order to adhere successfully, colonize and produce disease the pathogen must overcome the host defence system. It is well known that stress from environmental factors, such as oxygen tension, water temperature and water salinity, are important in increasing the susceptibility of fish to microbial pathogens. The water milieu can also facilitate transmission of these pathogens.

The pathogenesis of *Vibrio* infections in mammals is primarily a gut infection, and it is therefore logical to ask whether the same is true in fish. Fish pathogenic bacteria, such as *Vibrio salmonicida* and *V. anguillarum*, have been shown *in vivo* to adhere to the intestinal epithelium of fish larvae and to promote severe destruction of microvilli (Olafsen and Hansen, unpublished results, cited in Knudsen *et al.*, 1999). Severe damage with loss of cellular integrity was also noted in midgut of spotted wolffish (*Anarhichas minor* Olafsen) fry infected by V. *anguillarum* (Ringø, Mikkelsen and Myklebust, unpublished data, cited in Ringø *et al.*, 2002a) (Fig. 1), compared to normal enterocytes (Fig. 2) (Olsen, Myklebust and Ringø, unpublished data). Scanning electron microscopy investigations of human intestinal mucosa infected with enteropathogenic *Escherichia coli* (EPEC), showed that EPEC adhere intimately in microcolonies and cause gross alterations at the apical surface of infected enterocytes (Knutton *et al.*, 1987; Knutton, 1995).
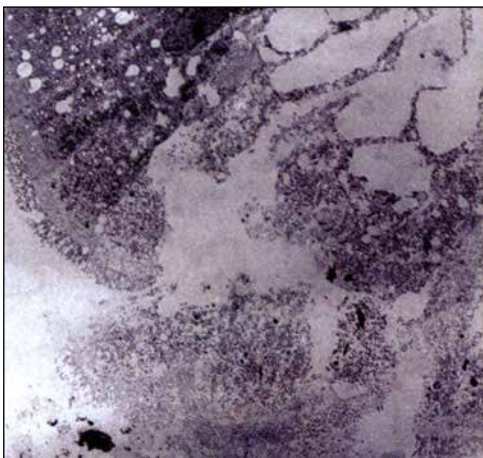

Figure 1.   Spotted   wolffish (*Anarhichas minor* Olafsen)   fry   infected   by *Vibrio anguillarum.* Notice the severe cellular damage. (after Ringø, Mikkelsen and Myklebust, unpublished data)

Figure 2. Normal enterocytes. Cells display the normal columnar arrangement with intact intracellular junctions and a regular, well-defined microvillous brush border (mv) at the cell apex. (after Olsen, Myklebust and Ringø, unpublished data)
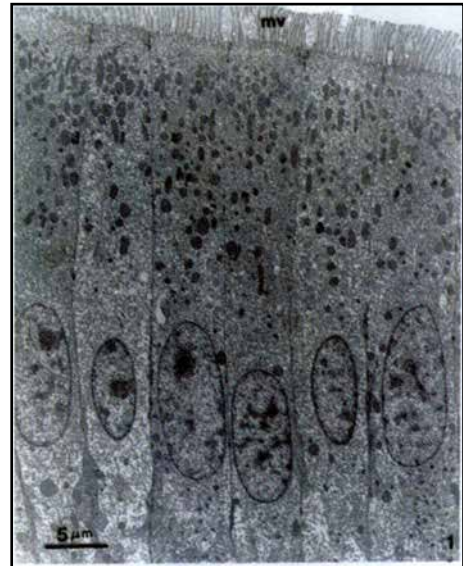
The susceptibility of early life stages of turbot and Atlantic halibut (*Hippoglossus hippoglossus* L.) to *Aeromonas salmonicida* ssp. *salmonicida* was studied in challenge experiments (Bergh *et al*., 1997). Larvae of both species experienced high mortality during the yolk sac stage, and the authors suggested that this was as a result of the challenge test. However, the bacterium could not be recovered from the larvae by culture, but the pathogen was shown to be present in the intestinal lumen of some turbot larvae examined using immunohistochemical techniques (Fig. 3). Based on this result, the authors (Bergh *et al*., 1997) proposed that A. *salmonicida* ssp. *salmonicida* may persist in the larvae.
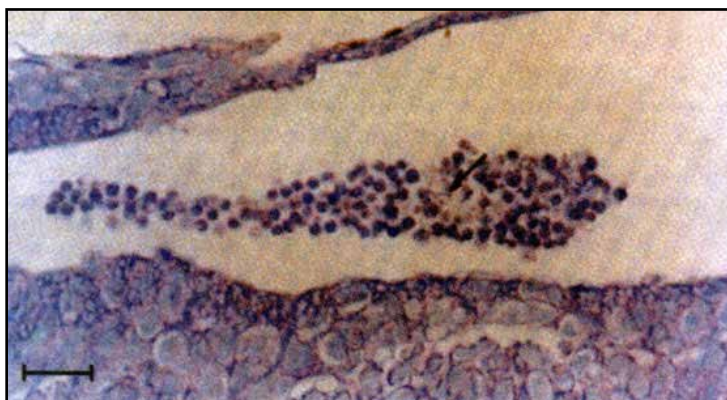

Figure 3. Semithin sections from turbot (*Scophthalmus maximus L.*) larva of group As-A, challenged with *Aeromonas salmonicida* ssp. *salmonicida* stained with Giemsa. Cell-like structures can be seen associated with positively red-stained bacteria-like structures (arrow). Scale bar is 10 μm. (after Bergh *et al*., 1997)

Endocytosis of bacteria by enterocytes has been observed in the hindgut of several fish species (Ringø *et al*., 2002a). In their study on turbot (*Scophthalmus maximus* L.) larvae, Grisez *et al.* (1996) reported free *V. anguillarum* from an endosome in the lamina propria by immunohistochemical staining (Fig. 4).
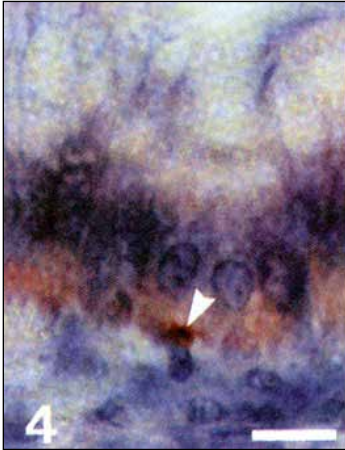

Figure 4.   *Vibrio anguillarum* infection in turbot (*Scophthalmus maximus L.*) after oral challenge. *Vibrio anguillarum* attached to the microvilli (brush border) of the intestinal epithelium. (after Grisez *et al*., 1996)

Readers with special interest in pathogenesis and the gastrointestinal tract of growing fish are referred to the review of Birkbeck and Ringø (2002).

PROTECTION AGAINST BACTERIAL ADHESION MUCUS

The internal surface of the host is the first defence barrier to infection. Intestinal mucins secreted by specialized epithelial goblet cells located in the intestinal enterocytes form a viscous, hydrated blanket on the surface of the intestinal mucosa that protects the delicate columnar epithelium. This is thought to be a vital component of the intestinal mucosal barrier in prevention of colonization by pathogens in both fish and endothermic animals (Florey, 1962; Forstner, 1978; Westerdahl *et al*., 1991; Maxson *et al*., 1994; Mims *et al*., 1995; Henderson *et al*., 1999). Gastrointestinal mucus is thought to have three major functions: (1) protection of the underlying mucosa from chemical and physical damage; (2) lubrication of the mucosal surface; and (3) to provide a barrier against enteroadherence of pathogenic organisms to the underlying mucosal epithelium.

It is well known that intestinal mucus is composed almost entirely of water (90-95%) and the electrolyte composition is similar to plasma, accounting for about 1% of the mucus weight. The remaining 4-10% is composed of high-molecular-weight glycoproteins (mucins), consisting of a protein core with numerous carbohydrate (fucose and galactose) sidechains. Hydrolysis of intestinal mucus material of rainbow trout liberated increased amounts of N-acetylgalactosamine and N-acetylglucosamine (O'Toole *et al*., 1999), indicated that these carbohydrates may be present as mucin-bound moieties in fish intestinal mucus as the case for mucus from other animal species (Roussel *et al*., 1988). The majority of intestinal mucus-associated lipids in rainbow trout partitioned to the organic phase during extraction with chloroform/methanol and was found to contain

saturated and unsaturated free fatty acids, phospholipids, bile acid, cholesterol, and monoglycerides and diglycerides (O'Toole *et al.*, 1999).

Olsson *et al.* (1992) put forward the hypothesis that the gastrointestinal tract is a site of colonization of *V. anguillarum* as the pathogen could utilize diluted turbot intestinal mucus as sole nutrient source. In a later study, Garcia *et al.* (1997) concluded that Atlantic salmon (*Salmo salar* L.) intestinal mucus is an excellent growth medium of *V. anguillarum.* This result is an important aspect of the pathogenesis of this pathogen.

The mucous blanket is constantly renewed by the secretion of high molecular weight glycoproteins from individual goblet cells throughout the epithelium. Goblet cells differentiate in the lower portion of the crypts of both small and large intestine and gradually migrate onto the villi or mucosal surface.

In an early study on histopathology changes caused by *V. anguillarum*, Ransom *et al.* (1984) found large amounts of goblet (mucus producing) cells in the anterior part of gastrointestinal tract of infected chum salmon (*Oncorhynchus keta* Walbaum). The first reaction of Arctic charr (*Salvelinus alpinus* L.), a salmonid fish, infected by pathogenic bacteria (A. *salmonicida* ssp. *salmonicida*) the causative agent of furunculosis is to peel off the infected mucus by increased goblet (mucus producing) cell production compared to uninfected fish (Lødemel *et al.*, 2001) (Fig. 5). A similar reaction to that found in infected Arctic charr, is also observed in rabbit and rats infected by pathogenic bacteria (Mantle *et al.*, 1989, 1991; Enss *et al.*, 1966), and this reaction may be considered a normal host response to particular intestinal infections (Mims *et al.*, 1995).
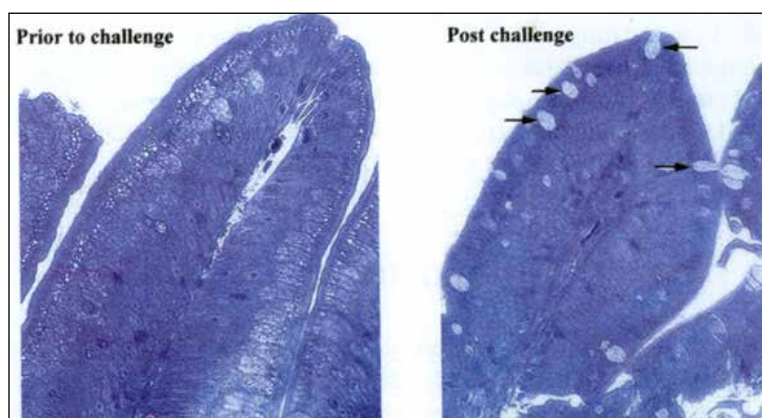

Figure 5.   Light microscopic view of villi in the midgut from Arctic charr (*Salvelinus alpinus* L.) fed soybean oil prior to challenge and post challenge with the fish pathogen *Aeromonas salmonicida* ssp. *salmonicida*. Note the substantially more conspicuous goblet (mucus producing) cells (arrows) along the villi of infected fish. (after Lødemel *et al.*, 2001)

Furthermore, the peel off of mucus might lead to loss of the autochthonous (indigenous) microbiota closely associated with the intestinal epithelium forming one of the first defence to limit colonization of pathogenic bacteria.

AUTOCHTHONOUS MICROBIOTA

Savage (1983) defined bacteria isolated from the digestive tract as either autochthonous (indigenous) or allochthonous (transient). Recently, Ringø and Birkbeck (1999) presented a list of criteria (found in healthy animals, colonize early stages and persist throughout life, found in both free-living and hatchery-cultured fish, grow anaerobically, and found associated with epithelial mucosal in the digestive tract) for testing autochthony of bacteria from the gastrointestinal tract of fish. To define the presence of autochthonous microbiota in fish, electron microscope investigations are a useful tool (Ringø *et al.* 2002a). One might put forward the hypothesis that autochthonous microbiota associated closely with the intestinal epithelium form a barrier serving as the first defence to limit direct attachment or interaction of pathogenic bacteria to the mucosa as reported for endothermic animals (van der Waaij *et al.*, 1972; Forstner, 1978; Slomiany *et al.*, 1994; Henderson *et al.*, 1996). A remarkable feature of the indigenous intestinal microbiota of fish is that situations like stress, antibiotic administration, and even small dietary changes, affect the microbial community of the digestive tract. The stability of the intestinal flora is an extremely important factor in the natural resistance of fish to infections produced by bacterial pathogens in the digestive tract. Interest in the phenomenon of resistance provided by flora components against colonization by pathogens has existed for many years in the endothermic literature (for review see Hentges, 1983; Hentges, 1992; Tancrède, 1992; Salminen *et al.*, 1996). A fundamental question when discussing translocation from the gastrointestinal tract in fish is if the autochthonous microbiota has a protective role against pathogenic bacteria by producing antibacterial substances. Information about the existence of antibacterial substances produced by bacteria isolated from the digestive tract of fish has been demonstrated in several comprehensive reviews (Ringø and Gatesoupe, 1998; Gatesoupe, 1999; Hansen and Olafsen, 1999; Ringø and Birkbeck, 1999; Gomez-Gill *et al.*, 2000; Gram and Ringø, 2002; Ringø *et al.*, 2002b). However, it is not yet known to what extent the natural microbiota of fish may be protective towards pathogen colonization. This is an important subject to clarify as the aquaculture industry is plagued by many disease problems, and an important goal for the microbiologist should therefore be to increase colonization of the gut by bacteria with an antibacterial potential against fish pathogens.

PROBIOTICS

The use of food containing live microorganisms with beneficial properties has been known for centuries. O'Sullivan *et al.* (1992) referee to Plino who advocated the use of fermented milk products in the treatment of various gastrointestinal infections as early as 76 BC. In modem time the term "probiotic" was first used in 1954 by Vergin, but since then, many different variations of the definition have been proposed (Table 1).

Antibiotic treatment to prevent infection is not recommended because of selection for strains resistant to chemotherapy but also because of concern about environmental risk and that resistant strains in the environment may transfer R plasmid to human intestinal microbiota. Nevertheless, its use is still a practical measure even methods do not prevent pathogenic proliferation in the system. Furthermore, the use of antibiotics may dramatically change the intestinal microflora of the fish and, thus impair its first-line defences (Austin and Al-Zahrani, 1988; Strøm and Ringø, 1993). An alternative method to antibiotic treatment would be the use of

Table 1. Definitions of probiotics by various authors (after Gram and Ringø, 2002)

Definition	Reference	Comments
microbial compounds which promotes body functions and beneficial microorganisms	Vergin, 1954	products - not live culture?
microbially produced "factors" which promote growth of other organisms	Lilly and Stillwell, 1965	growth promotion
animal feed supplements – organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance	Parker, 1974	only feed what is "microbial balance"
live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance	Fuller, 1989	only feed what is "microbial balance"
a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract	Naidu *et al.*, 1999	only feed what is "microbial balance"
live microorganisms supplemented in food or feed which give beneficial effects on the intestinal microbial balance	Gildberg *et al.*, 1997	only feed what is "microbial balance"
a live microbial supplement which beneficially affects the host animal by improving its microbial balance is based on elimination of harmful microflora from the animal's digestive tract	Gram *et al.*, 1999; Bogut *et al.*, 1998; Havenaar *et al.*, 1992	what is "microbial balance" elimination? only dietary tract
mono- or mixed cultures of live microorganisms which when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora	Conway, 1996; Holzapfel *et al.*, 1998; Salminen *et al.*, 1998	what are "properties of indigenous microflora"
viable microorganisms (bacteria or yeasts) that exhibit a beneficial effect on the health of the host when they are ingested		
beneficial bacteria which may override pathogens by producing inhibitory substances, or by preventing pathogenic colonization of the host	Riquelme *et al.*, 2000	what is "override"
beneficial bacteria that displace pathogens by competitive processes or by release of growth inhibitors	Moriarty, 1997	
live intestinal bacteria that are added to promote the viability of the host, but the term is also proper for bacteria able to regulate colonization of the outer surfaces	Skjermo and Vadstein, 1999	
live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment	Verschuere *et al.*, 2000	how is "colonization" regulated?
oral probiotics are living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition	Guarner and Schaafsma, 1998	
live microbial cultures added to feed or environment (water) to increase viability (survival) of the host	Gram and Ringø, 2002	

probiotics, or beneficial bacteria, which override pathogens by producing inhibitory substances, or by preventing pathogenic colonization in the host.

Table 2 shows the effect of addition of probiotic microorganisms (*Vibrio pelagius*, *V. mediterranei* Q40, *Aeromonas media*, *Pseudomonas* ssp. *Vibrio* ssp. and *Thalassobacter utilis*) on fish and crustacean larval survival.

Table 2.   Effect of addition of probiotic microorganisms on fish and crustacean larval survival (after Gram and Ringø, 2002)

Presumed probiont	Pathogen	Host organisms	Effect on survival	Reference
Vibrio pelagius	not known	Turbot larvae	Increase accumulated survival from 6-9% on day 12 and from day 0-3% on 16 after hatching	Ringø and Vadstein, 1998
Vibrio mediterranei Q40	not known	Turbot larvae	Increase accumulated survival (5 days post hatching) in 5 separate experiments (*e.g.* 14 to 55% in trial lor 75 to 81% in trial 4).	Huys *et al*., 2001
Aeromonas media	*Vibrio tubiashii*	Oyster larvae	Increase survival after 6 days from 4-100%	Gibson *et al*., 1998
Pseudomonas and unknown strain	*Vibrio anguillarum* like	Scallop larvae	Increase survival from 5-60% after 14 days	Riquelme *et al*., 1997
Pseudomonas and *Vibrio*	field trial? not known	Scallop larvae	Same survival after 48 hours as antibiotic treated tanks	Riquelme *et al*., 2001
Thalassobacter utilis	field trial not known (*Vibrio* spp.)	Crab larvae	Increase survival from 16 to 26%	Nogami and Maeda, 1992 Nogami *et al*., 1997

Gatesoupe (1997) tested in his study a siderophore-producing *Vibrio* as probiotic by feeding infected turbot larvae with rotifers enriched by the *Vibrio*. He reported improved larval survival 48 hours after infection. However, 10 days post-infection, no difference was seen in survival. In contrast to these results, Ringø and Vadstein (1998) reported that addition of *V. pelagius*, originally isolated from turbot larvae, to early developing turbot larvae had no short term effect, but caused a slight increase in survival after 12-16 days compared to larvae exposed to *Aeromonas caviae*. In an earlier study, Ringø *et al.* (1996) suggested by using an enzyme-linked immunosorbent assay that *V. pelagius* seems to colonize the larval gut when the bacteria was added to the tank water at the day of hatching but it was, however, not shown whether the

bacterium could persist in the gut if the larvae were removed from the *V. pelagius*-containing tank water.

In a recent study, Huys *et al.* (2001) searched for beneficial bacterial strains for turbot larviculture and reported increased survival of larvae by addition of a *V. mediterranei* Q40 originally isolated from sea bream larvae and an unknown organism isolated from turbot larvae at a concentration of 10^5 bacteria per ml water.

Some information is available on bacterial probiotics in culturing of Pacific oyster (*Crassostrea gigas* Thunberg) larvae. Gibson *et al.* (1998) used an *Aeromonas* media-like strain, originally isolated from Koi carp (*Cyprinus carpio*) from the Hawksbury River (Gibson, pers. com., 2001 to Lone Gram, Danish Institute for Fisheries Research, Denmark), by adding the probiont to waters of oyster larvae which had been challenged with the pathogen *V. tubiashii*. The challenge test caused an increase in numbers of the pathogen and a complete kill of the oyster population within five days, but by adding the *Aeromonas* media strain together with the pathogen, reduced numbers of *V. tubiashii* and resulted in complete survival of the population. These results are in accordance with earlier results demonstrating that additions of both algae (McCausland *et al.*, 1999) and bacteria (strain CA2) (Douillet and Langdon, 1994) improved growth of the Pacific oyster larvae.

Riquelme *et al.* (1997) investigated 506 bacterial isolates, obtained from laboratory and hatchery sources for their potential probiotic effect in Chilean scallop (*Argopecten purpuratus* Lamarck 1819) larval culture. Initially, both a *Pseudomonas* isolate showing *in vitro* activity against a larval pathogen a *V. anguillarum*-related strain and an unidentified isolate with no *in vitro* inhibitory activity were found to improve survival from 5% in the non-probiotic treated to 60% in the probiotic treated over a 14 day period. Screening the 506 bacterial strains, the authors found that only 11 isolates were able to inhibit growth of a *V. anguillarum* related bacterium associated with mortality of scallop larvae. However, several strains showing *in vitro* activity increased mortality of scallop larvae. Thus, this study clearly demonstrates the importance of *in vivo* testing, as strains with *in vitro* effect may be dangerous to the animals, and strains with no *in vitro* effect may have probiotic effects *in vivo*.

Recently, Riquelme *et al.* (2001) reported that growth and survival in field trials with Chilean scallop larvae treated with pathogen-antagonizing bacteria (*Vibrio* sp. C33 and *Pseudomonas* sp. 11) at 10^3 colony forming units per ml (CFU/ml) were the same as when the larvae were treated with antibiotics. The antagonizing bacteria were added to the water at the initiation of the experiment and again after 48 hours. Controls with no treatment were not included as the commercial producer experienced rapid mortality when no treatment was used. In an earlier investigation, Riquelme *et al.* (2000) studied the uptake of pathogen-inhibiting bacterial cultures in Chilean scallop larvae, and found that an *Arthrobacter* was ingested in significant numbers. This can be a way of continuously adding the probiotic culture to the scallop larvae. The *Arthrobacter* strain was not tested in *in vivo* infection trials.

In two studies, Nogami and Maeda (1992) and Nogami *et al.* (1997) added 10^5-10^6 CFU/ml of bacterial culture isolated from shrimp pond to seawater used for crab (*Portunus trituberculatus*) culture. The strain was a Gram-negative, non-fermentative, motile rod identified as *Thalassobacter utilis* (Maeda and Liao, 1992; Nogami *et al.*, 1997). The culture, which was added once every

seven days, was selected based on its ability to improve survival in *in vivo* infection trials. The organism also inhibited growth of *V. anguillarum*, *in vitro.* By adding the culture, a decline in concentration of *Vibrio* spp. in the seawater occurred and crab survival was significantly improved. It should be emphasized that the addition of five other microbial cultures (e.g. a *Bacillus subtilis*) accelerated mortality of the larvae (Nogami and Maeda, 1992).

Prevention of bacterial disease in growing fish is somewhat easier than in larvae, fry and juveniles. However, vaccination of fish smaller than 35 g (Intervet International, The Netherlands; Aqua Health Ltd, Canada) is not recommended, making the smaller stages of fish still susceptible to infection. For a range of bacterial pathogen-host combinations, good vaccines have been developed, and their optimization and use will be facilitated as further understanding of the pathogen virulence factors and of the host immune system emerges. The studies described in Table 3 should be regarded as trials of the probiotic concept rather than as suggestions for actual use of probiotics. Both the bacteriophage, *Tetraselmis*, Gram-positive bacteria (bacilli and carnobacteria, a lactic acid bacteria) and Gram-negative bacteria such as *Vibrio* and *Pseudomonas* have been evaluated as potential probionts and the experiments cover both additions to the rearing water or incorporation in the feed (Table 3).

The pathogens which are most frequently used include; *Pseudomonas plecoglossicida*, *anguillarum*, *V. ordalli*, *Yersinia ruckeri*, and *A. salmonicida*. In most of the cited studies in Table 3 increased survival of the host organisms were observed.

Readers who want more information about probiotics in aquaculture are referred to the reviews of Gatesoupe (1999), Gomez-Gil *et al.* (2000), Verschuere *et al.* (2000) and Gram and Ringø (2002).

LACTIC ACID BACTERIA (LAB)

It is suggested that lactic acid bacteria (LAB) along with other bacteria that belong to the autochthonous (indigenous) microbiota of aquatic animals might be an important part of the defence mechanism against colonization of fish pathogens in the gastro-intestinal tract. In addition to the antagonistic microorganisms colonizing the mucus surface in the natural microbial defence mechanisms, it has been shown that the surface mucus also plays a role in the prevention of colonization by parasites, bacteria and fungi.

Effects of LAB administration on intestinal microbiota

It is well known that LAB under normal circumstances are not numerically dominant in the digestive tract of fish (Ringø and Gatesoupe, 1998). In order to increase the proportion of LAB, some investigations have attempted to increase their population level by dietary factors such as: (1) chromic oxide (Ringø, 1993); (2) different oils (Ringø *et al.*, 1998; Ringø *et al*., 2002b); (3) high and low dietary lipids (Ringø and Olsen, 1999); and (4) inulin (Ringø, Myklebust and Olsen, unpublished results). Another important criterion for the use of LAB in commercial aquaculture, is the colonization potential of LAB in the fish gut, as Vibrionaceae may also persist

Table 3. Effect of addition of probiotic microorganisms on fish survival. (after Gram and Ringø, 2002)

Presumed probiont	Pathogen	Host organisms	Effect on survival	Reference
bacteriophage[1]	*Pseudomonas plecoglossicida*	ayu	increase survival from 35-75%	Park *et al.*, 2000
Tetraselmis suecica	Several Gram-negatives	salmon	increase survival from 0-15% to 20-100%	Austin *et al.*, 1992
Bacillus spp.	field trial not known	channel catfish	increase survival from 56-80%	Queiroz and Boyd, 1998
Carnobacterium sp.	*Vibrio anguillarum*	salmon	no effect on survival	Robertson *et al.*, 2000
	Vibrio ordalii		increase survival from 23-74%	
	Yersinia ruckeri		increase survival from 42-71%	
	Aeromonas salmonicida		increase survival from 0-20%	
	Aeromonas salmonicida	trout	increase survival from 32-74%	
Vibrio alginolyticus	*Aeromonas salmonicida*	salmon	increase survival from 0-82%	Austin *et al.*, 1995
	Vibrio anguillarum		increase survival from 10-26%	
	Vibrio ordalii		increase survival from 0-26%	
	Yersinia ruckerii		no effect on survival	
Pseudomonas fluorescens	*Aeromonas salmonicida*	salmon	increase healthy fish from 25-70 to 90-100%	Smith and Davey, 1993
Pseudomonas fluorescens	*Vibrio anguillarum*	trout	increase survival from 50-70%	Gram *et al.*, 1999
Pseudomonas spp.	*Vibrio anguillarum*	trout	increase survival (some strains)	Spanggaard *et al.*, 2001
			no effect (some strains)	
Pseudomonas fluorescens	*Aeromonas salmonicida*	salmon	no effect on survival	Gram *et al.*, 2001

[1] It is debatable whether or not a bacteriophage qualifies as a probiont "a live microbial...." due to the inert nature of viruses

for days or weeks in fish (Austin *et al.*, 1995; Munro *et al.*, 1995; Ringø and Vadstein, 1998). Some recent studies have demonstrated that carnobacteria strains are able to survive for several days in the intestine of larval and juvenile fish (Strøm and Ringø, 1993; Jöborn *et al.*, 1997; Gildberg and Mikkelsen, 1998; Ringø, 1999). Three of these studies (Jöborn *et al.*, 1997; Gildberg and Mikkelsen, 1998; Ringø, 1999) have suggested that there is apparently no host specificity with regard to colonization of the fish gut with carnobacteria, contrary to endothermic animals where adhesion of LAB appears to be complicated by host specificity (Lin and Savage, 1984; Fuller, 1986, 1989; Conway, 1989). However, the colonization site in the fish gut is also an important criterion. In a recent study, Gildberg and Mikkelsen (1998) administered two *Carnobacterium divergens* strains originally isolated from the intestine of mature Atlantic cod (*Gadus morhua* L.) and Atlantic salmon, to Atlantic cod juveniles via the food. When the Atlantic cod isolate was used, the authors only detected *C. divergens* in pyloric caeca, while the concentration of the bacteria was approximately ten fold higher in the pyloric caeca than in the intestine when the salmon isolate was used.

Transient bacteria may also be efficient if the cells are introduced at high dose. Moreover, as LAB may exert antibacterial effects against undesirable microbes, some investigators have attempted to increase the proportion of LAB associated with the fish digestive tract. In a study with four days old Atlantic cod larvae, Strøm and Ringø (1993) used an antagonistic LAB strain which, when added to the rearing water, favourably influenced the intestinal microbiota of the larvae by increasing the proportion of LAB from approximately 5% up to 70% and by a subsequent decrease in the proportion of the bacteria genera *Pseudomonas*, Cytophaga/*Flexibacter* and *Aeromonas* (Table 4). These results indicate that the LAB are able to colonize and may comprise a major part of the autochthonous microbiota in the gut of the larvae. A similar increase in intestinal LAB was also found in Atlantic cod fry fed a diet containing C. *divergens* (Gildberg *et al.*, 1997) (Table 4). In a study with Atlantic salmon fry, Gildberg *et al.* (1995) demonstrated that administration of LAB reported as *Lb. plantarum*, but later reclassified as *C. divergens* (Ringø *et al.*, 2001a) increased the proportion of adherent LAB to intestinal wall from nil to 100% (Table 4).

Recently, Byun *et al.* (1997) evaluated the effect of LAB (*Lactobacillus* sp. DS-12) administration via the feed on the intestinal microbiota of flounder (*Paralichthys olivaceus)* after one month of feeding (Table 4). *Lactobacillus* sp. DS-12 was not detected in the intestine of the control group, but 10^7/g LAB were found in the GIT when the fish were fed a LAB supplemented feed.

In a recent study, Bogut *et al.* (2000) evaluated the effect of *Enterococcus faecium* on the intestinal microbiota of Sheat fish (*Silurus glanis*). In this study, the fish were exposed to *E. faecium* by including the bacteria in the diet. After approximately two months of feeding, some interesting differences in the intestinal microbiota were observed between the two rearing groups. *Enterococcus faecium*-administration decreased the population level of *Staphylococcus aureus*, *Escherichia coli* and other bacteria of the family Enterobacteriaceae, and resulted in complete elimination of *Clostridium* spp. (Table 4).

Only one investigation has evaluated the influence of a commercial LAB preparation on the allochtonous intestinal microbiota. Supplementation of one gram of *E. faecium* M74 per 100 kg feed influenced the intestinal microbiota of 0+ Israeli carp (*Cyprinus carpio*) to some extent (Bogut *et al.*, 1998). While *E. coli* disappeared from the intestinal microbiota of the fish after 14

Table 4. Effect of lactic acid bacteria (LAB) administration on intestinal microbiota. (after Ringø et al., 2002)

Fish species	LAB used	Bacterial genera isolated and proportion of microflora population before administration (control)	after administration	after challenge	References
Atlantic cod - larvae	C. divergens	Pseudomonas 42.5; Cytophaga/Flexibacter 42.5 Aeromonas 10; C. divergens 5	C. divergens 70; Pseudomonas 20	*	Strøm and Ringø, 1993
Atlantic cod - fry	C. divergens	No information was given	No information was given	C. divergens 75 Pseudomonas-like 25	Gilberg et al., 1997
Atlantic salmon-fry	C. divergens	Pseudomonas, Enterobacteriaceae Gram positive cocci	C. divergens 100	A. salmonicida 90	Gildberg et al., 1995
Turbot - larvae	C. divergens	C. divergens n.d.	C. divergens (8×10^3)	C. divergens 10 *	Ringø, 1999
Flounder[1]	Lactobacillus sp.	Enterobacteriaceae 4.3 (5/5); G(+) 4.6 (5/5)	Enterobacteriaceae 4.8 (5/5); G(+) 4.3 (5/5)	*	Byun et al., 1997
	DS-12	Yeast 4.6 (5/5); Hemolytic bacteria 5.8 (2/5) Mucoid colony form 4.8 (1/5); Aerobes 8.5 (5/5) Anaerobes 7.6 (5/5) Aerobes 7.3 (5/5); Anaerobes 6.6 (5/5)	Lactobacillus sp. DS-12 7.0 (3/5) Clostridium 4.3 (1/5); Yeast 4.3 (1/5) Hemolytic bacteria 5.1 (1/5)		
Carp[b]	Ent. faecium	Enterobacteriaceae 6.2: E. coli 4.2 Ent. faecalis 3.3; Staph. aureus 3.7 Bacillus spp. 7.0; Clostridium spp. 2.9	Enterobacteriaceae 6.2: E. coli n.d. Ent. faecalis 3.5; Staph. aureus 4.0 Bacillus spp. 7.0; Clostridium spp. 2.7	*	Bogut et al., 1998
Sheat fish[c]	Ent. faecium	Escherichia coli 3.1; Enterobacteriaceae 3.0 Staph. aureus 4.7 Bacillus 6.0; Clostridium 2.1	Escherichia coli 1.1; Enterobacteriaceae 1.9 Staph. aureus 1.4 Bacillus 5.6; Clostridium n.d	*	Bogut et al., 2000

[a] - Data are presented as log 10 and frequency are shown in parentheses; [b] - Data are presented as log 10 after 4 weeks of feeding
[c] - Data are presented as log 10 after 58 days of feeding; n.d - not detected; * - challenge test not done

days and onwards by feeding the probiotic preparation (Table 4), the population level of Enterobacteriaceae, *E. faecalis*, *S. aureus*, *Bacillus* spp. and *Clostridium* spp. were not reduced as a result of including *E. faecium* into the diet (Bogut *et al*., 1998). The authors suggested a high adhesive ability in the epithelium of carp digestive tract for *E. faecium*. However, as they isolated the allochthonous (transient) intestinal microbiota, convincing experimental evidence was not provided.

When dealing with the potential of probiotics (for example LAB) in aquaculture the fundamental question arises whether it is possible to colonize and maintain the probiotic bacteria within the digestive tract. This is particularly important when long-term exposure may be required for the probiotic effect. In this respect, electron microscope investigations are a useful tool (Ringø *et al*., 2001b, 2002a).

During the last decade some reports have been published on the nutritional contribution of LAB to the production rate of rotifer *Brachionus plicatilis* (Gatesoupe, 1990; Gatesoupe, 1991), while the control of the microbiota of rotifer cultures has received less attention.

Challenges in vivo

The major factors involved in the biocontrol of bacterial pathogens in the gastrointestinal tract are primarily those regulating the composition, functions and interactions of indigenous microbial populations with the animal tissues. This concept is supported by repeated observations that strains of transient enteropathogens can colonize intestinal habitats of endothermic animals. The fact that fish contain intestinal microbiota with antagonistic effects against fish pathogens has prompted investigators to conduct challenge experiments with LAB during the last decade (Gatesoupe, 1994; Gildberg *et al*., 1995, 1997; Gildberg and Mikkelsen, 1998; Harzevili *et al*., 1998). However, in these studies some conflicting results on the mortality were reported when the control group was compared with probiotic treatment (Table 5).

Gatesoupe (1994) suggested that *in vivo* experiments with turbot larvae using rotifers grown on LAB strains (resembling those of *Lactobacillus plantarum* or *Carnobacterium* sp.) improved the disease resistance in challenge tests with pathogenic *Vibrio* (*V. splendidus* strain VS 11). However, the results reported in this study were registered after 48 and 72 hours, beyond which the mortality pattern was not discussed. In three papers, Gildberg and Mikkelsen (1998) and Gildberg *et al.* (1995; 1997) have used two LAB strains originally isolated from Atlantic salmon and Atlantic cod by Strøm (1988). These two isolates were recently identified by 16S rDNA and AFLP™ fingerprinting as *C. divergens* (Ringø *et al*., 2001a). In challenge trials with cohabitants with *A. salmonicida*, Gildberg *et al.* (1995) in contrast to the expectations, registered highest mortality of Atlantic salmon fry with fish given the diet containing *C. divergens*, originally isolated from Atlantic salmon intestine. In their study with Atlantic cod fry, Gildberg and Mikkelsen (1998) observed the same cumulative mortality independent whether the *C. divergens* isolates supplemented to the commercial feed were originally isolated from the digestive tract of Atlantic cod or Atlantic salmon, when the fish were bath exposed to *V. anguillarum*. On the other hand, an improved disease resistance of Atlantic cod fry was observed by supplementing a commercially dry feed with a strain of *C. divergens* originally isolated from the cod (Gildberg *et al*., 1997). The

Table 5. Challenge tests of fish with lactic acid bacteria (LAB) (after Ringø et al., 2002)

LAB isolate used	Host	Pathogen	Way of administration	Effect in challenge test	Suggested mode of action	Reference
Carnobacterium spp.[a]	Turbot - larvae	V. splendidus	Enrichment of rotifers	+	Antagonism and/or improved nutritional value of the rotifers	Gatesoupe, 1994
C. divergens[b]	Atlantic salmon - fry	A. salmonicida	Addition to the diet	-		Gildberg et al., 1995
C. divergens[c]	Atlantic cod - juveniles	V. anguillarum	Addition to the diet	+	Not specified	Gildberg et al., 1997
C. divergens[b]	Atlantic cod - fry	V. anguillarum	Addition to the diet	+[e]	Antagonism	Gildberg and Mikkelsen, 1998
C. divergens[c]	Atlantic cod - fry	V. anguillarum	Addition to the diet	-		Gildberg and Mikkelsen, 1998
Lb. rhamnosus[d]	Rainbow trout	A. salmonicida	Addition to the diet	+		Nikoskelainen et al., 2001

+ improved disease resistance; - no significant effect

* - isolated from rotifer; [b]- isolated from intestine of Atlantic salmon (Strøm, 1988); [c] - isolated from intestine of Atlantic cod (Strøm, 1988); [d] - a probiotic for human use

[e] - Twelve days after infection significant reduced cumulative mortality was recorded in fish given feed supplemented with C. divergens isolated from Atlantic salmon, but no effect was detected four weeks after infection

explanation for these conflicting results has not been elucidated. Gildberg and Mikkelsen (1998) put forward a hypothesis that bacteriocin production can be inducible and may not occur if the bacteria are not frequently challenged with inhibitors as previously demonstrated by Schrøder *et al.* (1980). Furthermore, a recent study by Nikoskelainen *et al.* (2001) used the human probiotic *Lactobacillus rhamnosus* in a challenge test with *A. salmonicida* with promising results (Table 5). These results should stimulate fish microbiologist to use human probiotic LAB in future studies.

If the intestine is involved in infection as suggested by several authors, the fundamental question arises whether there are differences between the posterior part of the intestine and the hindgut region of the intestine? It is well established that the intestine in an immature or inflammatory state has an enhanced capacity to absorb intact macromolecules (for review see Olsen and Ringø, 1997). Furthermore, some studies report endocytosis of bacteria by enterocytes in the epithelial border of hindgut of herring (*Clupea harengus*) larvae (Hansen *et al.*, 1992; Hansen and Olafsen, 1999), herring and Atlantic cod larvae (Olafsen and Hansen, 1992) and 36 days old juvenile turbot (Grisez *et al.*, 1996). It is generally accepted that mature and non-inflammatory intestines of adult salmonids are not permeable to microparticulates in contrast to the mammalian gastrointestinal tract where M-cells are active in phagocytosis. However, a recent study demonstrated endocytosis of bacteria by enterocytes in the epithelial border of hindgut of adult salmonid fish (Fig. 6a), as well as in the posterior part of the intestine (pyloric caeca) (Fig. 6b) (Ringø *et al.*, 2002b). These results are in accordance with observations made by Vigneulle and Laurencin (1991) and Tamura *et al.* (1993) who measured phagocytosis of fixed *V. anguillarum* in the posterior intestine of rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*) and eel (*Anguilla anguilla*).
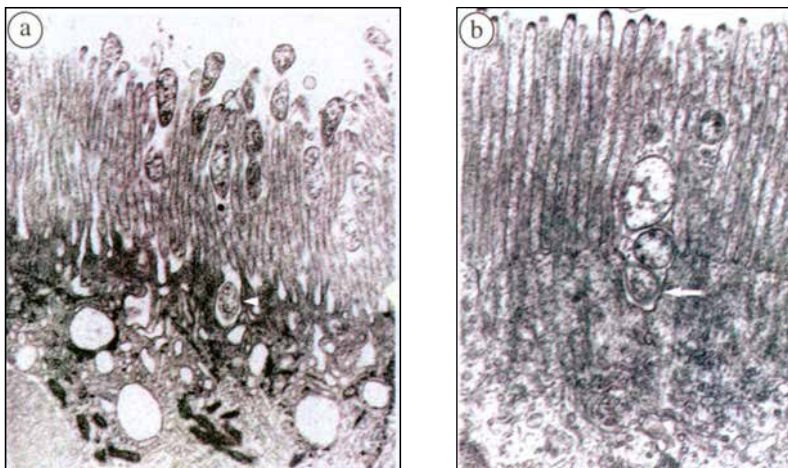

Figure 6.   Endocytosis of bacteria demonstrated in the hindgut region (a) and pyloric caeca (b) of Arctic charr (*Salvelinus alpinus* L.). (after Ringø *et al.*, 2002b)

The observations of Vigneulle and Laurencin (1991), Tamura *et al.* (1993) and Ringø *et al.* (2001b, 2002a, 2002b) indicate that the intestine is involved in bacterial translocation. Yet no clear evidence is available on possible differences between different parts of the intestine with regard to bacterial infection.

It is well known that rotifers are often suspected of being a vector for bacterial infections to the predating organisms (Perez-Benavente and Gatesoupe, 1988; Tanasomwang and Muroga, 1988; Nicolas *et al.*, 1989). It is therefore surprising that studies dealing with the proliferation of larval pathogens in rotifer cultures are so scarce (Gatesoupe, 1991; Hazevili *et al.*, 1998). Gatesoupe (1991) reported that the proliferation of *A. salmonicida* that accidentally appeared in the experimental rotifer culture was inhibited by treatment with *Lb. plantarum.* Hazevili *et al.* (1998) reported that administration of the probiotic strain *Lac. lactis* AR21 under sub-optimal feeding regime, counteracted the growth inhibition of the rotifers due to *V. anguillarum.*

Readers with special interest in lactic acid bacteria in fish are referred to the comprehensive reviews of Ringø and Gatesoupe (1998) and Ringø *et al.*, 2002c.

PREBIOTICS

Specific bacterial pathogens can be an important cause of mortality as intensive husbandry practices often result in breakdown of natural barriers between the host and pathogens. Nowadays, the prevention and control of these diseases has concentrated on good husbandry practices and the use of vaccines and antibiotics. However, treatment by feeding antibiotics may cause the development of resistant bacteria through plasmids or bacteriophages (Towner, 1995). Therefore, there is an increased interest within the aquaculture industry in the control or elimination of antimicrobial use. Alternative methods need to be developed to maintain a healthy microbial environment. Two such methods that are gaining acceptance within the industry are the use of probiotic bacteria or prebiotics to control potential pathogens.

During the last decade, several reviews have dealt with the potential of probionts in aquaculture (Ringø and Gatesoupe, 1998; Gatesoupe, 1999; Hansen and Olafsen, 1999; Ringø and Birkbeck, 1999; Gomez-Gill *et al.*, 2000; Verschuere *et al.*, 2000; Gram and Ringø, 2002; Ringø *et al.*, 2002c). It is therefore a pertinent question whether it is possible to colonize and maintain probiotic bacteria within the digestive tract. This is particularly important when long-term exposure is required for the probiotic effect. However, to date, there is no real evidence demonstrating the preventive effect of probionts against colonization and adherence of fish pathogenic bacteria in aquaculture. The reason for this may be that the probionts used are unable to colonize the mucus layer of the digestive tract or external surfaces. Examination of adhesion has become a standard procedure for selecting new probiotic strains for human application (Salminen *et al.*, 1996), but it is less common in aquaculture.

The addition of high doses of probiotic strains (for example, lactic acid bacteria) to established microbial communities of fish provoked a temporary change in the composition of the intestinal microbial community. However, within a few days after administration had stopped, the added strains showed a sharp decrease and were lost from the gastrointestinal tract (Jöborn *et al.*,

1997; Ringø and Gatesoupe, 1998). Another way to colonize and increase the population level of beneficial bacteria with antagonistic ability is the use of prebiotics. The modern concept of prebiotics implies the use of selective agents to favour growth of the protective indigenous gut microbiota. Dietary fiber is a prebiotic that belongs to the broad category of carbohydrates. Burkitt *et al.* (1972) defined dietary fiber as "*the sum of polysaccharides and lignin which are not digested by the endogenous secretions of the human gastrointestinal tract.*" They can be classified into soluble (*e.g.*, inulin and oligofructose), insoluble (*e.g.*, cellulose) or mixed (*e.g.*, bran). It is well known from endothermic investigations that dietary fibers are fermented by the anaerobic intestinal microbiota, primarily those colonizing the large intestine (Roberfroid, 1993; Gibson *et al.*, 1995; Roberfroid, 1995; Gibson, 1998; Rumessen and Gudmand-Høyer, 1998), leading to the production of lactic acid, short chain fatty acids (SCFA-acetate, propionate, and butyrate) and gases (H_2, CO_2 and CH_4) (Roberfroid, 1993) that are utilized by the host (Schneeman. 1999). Inulin, is a polydisperse carbohydrate consisting mainly of β (2→1) fructosyl-fructose links, generally referred to as fructan and is found in various edible fruits and vegetables such as wheat, onions, leeks, garlic, asparagus, artichokes and bananas (Roberfroid, 1993; Van Loo *et al.*, 1995). Although inulin is not a natural fiber in fish diet, the prebiotic potential of inulin and other dietary fibers may also have interesting applications in aquaculture. Some information is available about fermentation of inulin by fish gut microbiota, notably, *Carnobacterium piscicola* (Ringø *et al.*, 1998), *C. mobile* (Ringø and Olsen, 1999), and *Carnobacterium* spp. (Ringø and Olsen, 1999; Ringø *et al.*, 2001a). It is also known that dietary inulin resulted in damage to intestinal enterocytes of the salmonid fish Arctic charr (Fig. 7) (Olsen *et al.*, 2001) compared to normal enterocytes (Fig. 8), and that dietary inulin alters the adherent gut microbiota of Arctic charr (Ringø, unpublished results). However, the effect of dietary inulin on fish welfare is not yet known.
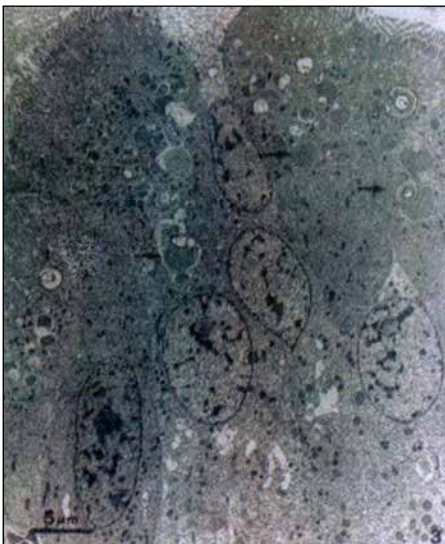

Figure 7.   The epithelium in the hindgut of Arctic charr (*Salvelinus alpinus* L.) fed dietary inulin. The cells are highly vacuolated and many of the vacuoles have lamellar content (small arrows) which may be inulin. The apical surface of these cells shows sign of damage including loss of membrane and microvilli (large arrows). (after Olsen *et al.*, 2001)

Figure 8. Epithelial cells in the hindgut of Arctic charr (*Salvelinus alpinus* L.) fed control diet (dietary dextrin). The columnar cells typically show numerous vacuoles varying in size and electron density (arrows). Cytoplasm, microvilli and intracellular organelles appear normal. In the upper middle part of the field, an effete enterocyting is probably being shed as part of normal epithelial turnover. (after Olsen *et al.*, 2001)

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REFERENCES

Austin, B. and Al-Zahrani, A.M.J. 1988. The effect of antimicrobial compounds on the gastrointestinal microflora of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis., 33:1-14.

Austin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, I. and Griffith, D.R.W. 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*. J. Fish Dis., 18:93-96.

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

Baudin Laurencin, F. and Germon, E. 1987. Experimental infection of rainbow trout, *Salmo gairdneri* R., by dipping in suspensions of *Vibrio anguillarum*: ways of bacterial penetration: influence of temperature and salinity. Aquaculture, 67:203-205.

Bergh, Ø., Hjeltnes, B., Skiftesvik, A.B. 1997. Experimental infections of turbot *Scophthalmus maximus* and halibut *Hippoglossus hippoglossus* yolk sac larvae with *Aeromonas salmonicida* subsp. *salmonicida*. Dis. Aquat. Org., 29:13-20.

Birkbeck, T.H. and Ringø, E. 2002. Pathogenesis and the GI tract of growing fish. *In*: Microbial Ecology of the Growing Animal. W. Holzapfel and P. Naughton (eds.). Elsevier, Amsterdam, Netherlands. (in press).

Bogut, I., Milakovic, Z., Brkic, S., Novoselic, D. and Bukvic, Z. 2000. Effects of *Enterococcus faecium* on the growth rate and content of intestinal microflora in sheat fish (*Silurus glanis*). Veterinari Medicina, 4:107-109.

Bogut, I., Milakovic, Z., Bukvic, Z., Brkic, S. and Zimmer, R. 1998. Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in carp (*Cyprinus carpio*). Czech J. Anim. Sci., 43:231-235.

Burkitt, D.P., Walker, A.R.P. and Painter, N.S. 1972. Effects of dietary fibre on stools and transit-times, and its role in the causation of disease. Lancet ii, 1408-1412.

Byun, J.W., Park, S.C., Benno, Y. and Oh, T.K. 1997. Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). J. Gen. Appl. Microbiol., 43:305-308.

Chair, M., Dehasque, M., van Poucke, S., Nelis, H., Sorgeloos, P. and de Leenher, A.P. 1994. An oral challenge for turbot larvae with *Vibrio anguillarum*. Aquacult. Int., 2:270-272.

Conway, P.L. 1989. Lactobacilli: fact and fiction. *In*: The Regulatory and Protective Role of the Normal Microflora. R. Grubb, T. Midtvedt, E. Norrin (eds.), pp. 263-282. Macmillan Press Ltd., London.

Conway, P.L. 1996. Selection criteria for probiotic microorganisms. Asia Pacific J. Clin. Nutr., 5:10-14.

Douillet, P.A. and Langdon, C.J. 1994. Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture, 119:25-40.

Enss, M.L., Müller, H., Schmidt-Witting, U., Kownatzki, R., Coenen, M. and Hedrich, H.J. 1966. Effects of perorally applied endotoxin on colonic mucins of germfree rats. Scan. J. Gastro., 31:868-874.

Florey, H.W. 1962. The secretion and function of intestinal mucus. Gastroenterol., 43:326-336.

Forstner, J.F. 1978. Intestinal mucins in health and disease. Digestion, 17:234-264.

Fuller, R. 1986. Probiotics. J. Appl. Bacteriol. suppl., 1S-7S.

Fuller, R. 1989. Probiotics in man and animal. J. Appl. Bacteriol., 66:365-378.

Garcia, T., Otto, K., Kjelleberg, S. and Nelson, D.R. 1997. Growth of *Vibrio anguillarum* in salmon intestinal mucus. Appl. Environ. Microbiol., 63:1034-1039.

Gatesoupe, F.J. 1990. The continuous feeding of turbot larvae, *Scophthalmus maximus*, and control of bacterial environment of rotifers. Aquaculture, 89:139-148.

Gatesoupe, F.J. 1991. The effect of three strains of lactic acid bacteria on the production rate of rotifers, *Brachionus plicatilis*, and their dietary value for larval turbot, *Scophthalmus maximus.* Aquaculture, 96:335-342.

Gatesoupe, F.J. 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic *Vibrio.* Aquatic Living Res., 7:277-282.

Gatesoupe, F.J. 1997. Siderophore production and probiotic effect of *Vibrio* sp. associated with turbot larvae, *Scophthalmus maximus.* Aquatic Living Res., 10:239-246.

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

Gibson, G.R., 1998. Dietary modulation of the human gut microflora using prebiotics. Br. J. Nutr., 80:S209-S212.

Gibson, G.R., Beaty, E.R., Wang, X. and Cummings, J.H. 1995. Selective stimulation of bifidobacterium in the colon by oligofructose and inulin. Gastroenterology, 108:975-982.

Gibson, L.F., Woodworth, J. and George, A.M. 1998. Probiotic activity of *Aeromonas media* on the pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii.* Aquaculture, 169:111-120.

Gildberg, A., Johansen, A. and Bøgvald, J. 1995. Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida.* Aquaculture, 138:23-34.

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

Gildberg, A., Mikkelsen, H., Sandaker, E. and Ringø, E. 1997. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). Hydrobiologia, 352:279-285.

Gomez-Gill, B., Roque, A. and Turnbull, J.F. 2000. The use and selection of prebiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture, 191:259-270.

Gram, L., Melichiorsen, J., Spanggard, B., Huber, I. and Nielsen, T.F. 1999. Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* strain AH2, a possible probiotic treatment of fish. Appl. Environ. Microbiol., 65:969-973.

Gram, L., Løvold, T., Melchiorsen, J., Nielsen, J. and Spanggard, B. 2001. *In vitro* antagonism of the probiont *Pseudomonas fluorescens* strain AH2 against *Aeromonas salmonicida* does not confer protection of salmon against furunculosis. Aquaculture, 199:1-11.

Gram, L. and Ringø, E. 2002. Prospects of fish probiotics. *In*: Microbial Ecology of the Growing Animal. W. Holzapfel and P. Naughton (eds.). Elsevier, Amsterdam, Netherlands. (in press)

Grisez, L., Chair, M., Sorgeloos, P. and Ollevier, F. 1996. Mode of infection and spread of *Vibrio anguillarum* in turbot *Scophthalmus maximus* larvae after oral challenge through live feed. Dis. Aquatic Org., 26:181-187.

Guarner, F. and Schaafsma, G.J. 1998. Probiotics. Int. J. Food Microbiol., 39:237-238.

Hansen, G.H. and Olafsen, J.A. 1999. Bacterial interactions in early life stages of marine cold water fish. Microb. Ecol., 38:1-26.

Hansen, G.H., Strøm, E. and Olafsen, J.A. 1992. Effect of different holding regimes on the intestinal microflora of herring (*Clupea harengus*) larvae. Appl. Environ. Microbiol., 58:461-470.

Harzevili, A.R.S., Van Duffel, H., Dhert, P., Swings, J. and Sorgeloss, P. 1998. Use of a potential probiotic *Lactococcus lactis* AR21 strain for the enhancement of growth in the rotifer *Brachionus plicatilis* (Müller). Aquacult. Res., 29:411-417.

Havenaar, R., Teten Brink, B. and Huis In't Veld, J.H.J. 1992. Selection of strains for probiotic use. *In*: Probiotics. The Scientific Basis. R. Fuller (ed.), pp. 209-224. Chapman and Hall, London.

Henderson, B., Wilson, M. and Mcnab Lax, A.J. 1999. Cellular Microbiology. John Wiley and Sons, Chichester.

Henderson, B., Poole, S. and Wilson, M. 1996. Microbial host interactions in health and disease: Who controls the cytokine network? Immunopharmacol., 35:1-21.

Hentges, D.J. 1983. Role of the intestinal microflora in host defence against infection. *In*: Human Intestinal Microflora in Health and Disease. D.J. Hentges (ed.), pp. 311-331. Academic Press, New York, U.S.A.

Hentges, D.J. 1992. Gut flora and disease resistance. *In*: Probiotics. The Scientific Basis. R. Fuller (ed.), pp. 87-100. Chapman & Hall, London.

Hjeltnes, B., Andersen, K., Ellingsen, H.M. and Egedius, E. 1987. Experimental studies on pathogenicity of a *Vibrio* sp. isolated from Atlantic salmon, *Salmo salar* L., suffering from Hitra disease. J. Fish Dis., 10:21-27.

Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U. and Huis In't Veld, J.H.J. 1998. Overview of gut flora and probiotics. Int. J. Food Microbiol., 41:85-101.

Huys, L., Dhert, P., Robles, R., Ollevier, F., Sorgloos, P. and Swings, J. 2001. Search for beneficial bacterial strains for turbot (*Scophthalmus maximus* L.) larviculture. Aquaculture, 193:25-37.

Jöborn, A., Olsson, J.C., Westerdahl, A., Conway, P.L. and Kjelleberg, S. 1997. Colonization in the fish intestinal tract and production of inhibitory substances in intestinal mucus and faecal extracts by *Carnobacterium* sp. strain K1. J. Fish Dis., 20:383-392.

Kanno, T., Nakai, T. and Muroga, K. 1990. Mode of transmission of vibriosis among ayu *Plecoglossus alvelitis.* J. Aquatic Animal Health, 1:2-6.

Kawai, K., Kusuda, R. and Itami, T. 1981. Mechanisms of protection in ayu orally vaccinated for vibrios. Fish Pathol., 15:257-262.

Knudsen, G., Sørum, H., McL Press, C. and Olafsen, J.A. 1999. *In situ* adherence of *Vibrio* spp. to cryosections of Atlantic salmon, *Salmo salar* L., tissue. J. Fish Dis., 22:409-418.

Knutton, S. 1995. Electron microscopical methods in adhesion. Methods in Enzymology, 253:145-158.

Knutton, S., Lloyd, D.R. and McNeish, A.S. 1987. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect. Immun., 55:69-77.

Lilly, D.M. and Stillwell, R.H. 1965. Probiotics: growth-promoting factors produced by microorganisms. Science, 147:747-748.

Lin, J.H.C. and Savage, D.C. 1984. Host specificity of the colonization of murine gastric epithelium by lactobacilli. FEMS Microbiol. Lett., 24:67-71.

Lødemel, J.B., Mayhew, T.M., Myklebust, R., Olsen, R.E., Espelid, S. and Ringø, E. 2001. Effect of three dietary oils on disease susceptibility in Arctic charr (*Salvelinus alpinus* L.) during cohabitant challenge with *Aeromonas salmonicida* ssp. *salmonicida.* Aquacult. Res., 32:935-945.

Maeda, M. and Liao, I.C. 1992. Effect of bacterial population on the growth of prawn larva, *Penaeus monodon.* Bull. Natl. Res. Inst. Aquacult., 21:25-29.

Magarinos, B., Pazos, F., Santos, Y., Romalde, J.L. and Toranzo, A.E. 1995. Response of *Pasteurella piscicida* and *Flexibacter maritimus* to skin mucus of marine fish. Dis. Aquat. Org., 21:103-108.

Mantle, M., Thakore, E., Hardin, J. and Gall, D.G. 1989. Effect of *Yersinia enterocolitica* on intestinal mucin secretion. Am. J. Physiol., 256:G319-G327.

Maxson, R.T., Dunlap, J.P., Tryka, F., Jackson, R.J. and Smith, S.D. 1994. The role of the mucus gel layer in intestinal bacterial translocation. J. Surgical Res., 57:682-686.

McCausland, M.A., Malcolm, R.B., Barrett, S.M., Diemar, J.A. and Heasman, M.P. 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture, 174:323-342.

Mims, C.A., Dimmock, N.J., Nash, A. and Stephen, J. 1995. Mims' Pathogenesis of Infectious Disease. 4th ed. Academic Press, London. 414 p.

Moriarty, D.J.W. 1997. The role of microorganisms in aquaculture ponds. Aquaculture, 151:333-349.

Munro, P.D., Barbour, A. and Birkbeck, T.H. 1995. Comparison of the growth and survival of larval turbot in the absence of culturable bacteria with those in the presence of *Vibrio anguillarum*, *Vibrio alginolyticus*, or a marine *Aeromonas* sp. Appl. Environ. Microbiol., 61:4425-4428.

Muroga, K. and de la Cruz, M.C. 1987. Fate and location of *Vibrio anguillarum* in tissues of artificially infected ayu (*Plecoglossus altivelis*). Fish Pathol., 22:99-103.

Naidu, A.S., Bidlack, W.R. and Clemens, R.A. 1999. Probiotic spectra of lactic acid bacteria (LAB). Crit. Rev. Food Sci. Nutr., 38:13-126.

Nicolas, J.L., Robic, E. and Ansquer, D. 1989. Bacterial flora associated with a trophic chain consisting of micro-algae, rotifers and turbot larvae: influence of bacteria on larval survival. Aquaculture, 83:237-248.

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

Nogami, K. and Maeda, M. 1992. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus triberculatus.* Can. J. Fish. Aquat. Sci., 49:2373-2376.

Nogami, K., Hamasaki, K., Maeda, M. and Hirayama, K. 1997. Biocontrol method in aquaculture for rearing the swimming crabs larvae *Portunus trituberculatus.* Hydrobiologia, 358:291-295.

Olafsen, J.A. and Hansen, G.H. 1992. Intact antigen uptake in intestinal epithelial cells of marine fish larvae. J. Fish Biol., 40:141-156.

Olsen, R.E., Myklebust, R., Kryvi, H., Mayhew, T.M. and Ringø, E. 2001. Damaging effect of dietary inulin on intestinal enterocytes in Arctic char (*Salvelinus alpinus* L.). Aquacult. Res., 32:931-934.

Olsen, R.E. and Ringø, E. 1997. Lipid digestibility in fish: A review. Recent Res. Devl. Lipid Res., 1:199-264.

Olsson, C. 1995. Bacteria with inhibitory activity and *Vibrio anguillarum* in fish intestinal tract. Fil. Dr. thesis. Gothenburg University, Sweden, ISBN 91-628-1850-3. 141 p.

Olsson, J.C., Jöborn, A., Westerdahl, A., Blomberg, L., Kjelleberg, S. and Conway, P.L. 1996. Is the turbot, *Scophthalmus maximus* L., intestine a port of entry for the fish pathogen *Vibrio anguillarum*? J. Fish Dis., 19:225-234.

Olsson, J.C., Westerdahl, A., Conway, P.L. and Kjelleberg, S. 1992. Intestinal characterization potential of turbot (*Scophthalmus maximus*) and dab (*Limanda limanda*)- associated bacteria with inhibitory effects against *Vibrio anguillarum*. Appl. Environ. Microbiol., 58:551-556.

O'Sullivan, M.G., Thornton, G., O'Sullivan, G.C. and Collins, J.K. 1992. Probiotic bacteria: Myth or reality? Trends Food Sci. Technol., 3:309-314.

O'Toole, R., Lundberg, S., Fredrikson, S.A., Jansson, A., Nilsson, B. and Wolf-Watz, H. 1999. The chemotactic response of *Vibrio anguillarum* to fish intestinal mucus is mediated by a combination of multiple mucus components. J. Bacteriol., 181:4308-4317.

Park, S.C., Shimamura, I., Fukunaga, M., Mori, K-I. and Nakai, T. 2000. Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plectoglossicida*, as a candidate for disease control. Appl. Environ. Microbiol., 66:1416-1422.

Parker, R.B. 1974. Probiotics, the other half of antibiotic story. Anim. Nutr. Health, 29:4-8.

Perez-Benavente, G. and Gatesoupe, F.J. 1988. Bacteria associated with cultures rotifers and *Artemia* are detrimental to larval turbot, *Scophthalmus maximus* L. Aquacult Eng., 118:289-293.

Queiroz, J.F. and Boyd, C.E. 1998. Effects of a bacterial inoculum in channel catfish ponds. J. World Aquacult. Soc., 29:67-73.

Ransom, D.P., Lannan, C.N., Rohovecm, J.S. and Fryer, J.L. 1984. Comparison of histopathology caused by *Vibrio anguillarum* and *Vibrio ordalii* in three species of Pacific salmon. J. Fish Dis., 7:107-115.

Ringø, E. 1993. The effect of chromic oxide (Cr_2O_3) on aerobic bacterial populations associated with the epithelial mucosa of Arctic charr, *Salvelinus alpinus* (L.). Can. J. Microbiol., 39:1169-1173.

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., Bendiksen, H.R., Gausen, S.J., Sundsfjord, A. and Olsen, R.E. 1998. The effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa and from faecalia of Arctic charr, *Salvelinus alpinus* (L.). J. Appl. Microbiol., 85:855-864.

Ringø, E. and Birkbeck, T.H. 1999. Intestinal microflora of fish larvae and fry. Aquacult. Res., 30:73-93.

Ringø, E., Birkbeck, T.H., Munro, P.D., Vadstein, O. and Hjelmeland, K. 1996. The effect of early exposure to *Vibrio pelagius* on the aerobic flora of turbot, *Scophthalmus maximus* (L.). J. Appl. Bacteriol., 81:207-211.

Ringø, E. and Gatesoupe, F.J. 1998. Lactic acid bacteria in fish: a review. Aquaculture, 160:177-203.

Ringø, E., Lødemel, J.B., Myklebust, R., Jensen, L., Lund, V., Mayhew, T.M. and Olsen, R.E. 2002b. The effect of soybean, linseed and marine oils on aerobic gut microbiota of Arctic charr (*Salvelinus alpinus* L.) prior to and post challenge with *Aeromonas salmonicida* ssp. *salmonicida.* Aquacult. Res., 3. (in press)

Ringø, E., Lødemel, J.B., Myklebust, R., Kaino, T., Mayhew, T.M. and Olsen, R.E. 2001b. Epithelium-associated bacteria in the gastrointestinal tract of Arctic charr (*Salvelinus alpinus* L.). An electron microscopical study. J. Appl. Microbiol., 90:294-300.

Ringø, E. and Olsen, R.E. 1999. The effect of diet on aerobic bacterial flora associated with intestine of Arctic charr (*Salvelinus alpinus* L.). J. Appl. Microbiol., 86:22-28.

Ringø, E., Olsen, R.E., Mayhew, T.M. and Myklebust, R. 2002a. Electron microscopy of the intestinal microflora of fish. Aquaculture. (submitted paper).

Ringø, E., Schillinger, U. and Holzapfel, W. 2002c. Antibacterial abilities of lactic acid bacteria isolated from aquatic animals and the use of lactic acid bacteria in aquaculture. *In*: Microbial Ecology of the Growing Animal. W. Holzapfel and P. Naughton (eds.). Elsevier, Amsterdam, Netherlands. (in press.)

Ringø, E. and Vadstein, O. 1998. Colonization of *Vibrio pelagius* and *Aeromonas caviae* in early developing turbot (*Scophthalmus maximus* L.) larvae. J. Appl. Microbiol., 84:227-233.

Ringø, E., Wesmajervi, M.S., Bendiksen, H.R., Berg, A., Olsen, R.E., Johnsen, T., Mikkelsen, H., Seppola, M., Strøm, E. and Holzapfel, W. 2001a. Identification and characterization of carnobacteria isolated from fish intestine. Sys. Appl. Microbiol., 24:183-191.

Riquelme, C., Araya, R. and Escribano, R. 2000. Selective incorporation of bacteria by *Argopecten purpuratus* larvae: implications for the use of probiotics in culture systems of the Chilean scallop. Aquaculture, 181:25-36.

Riquelme, C., Araya, R., Vergara, N., Rojas, A., Guaita, M. and Candia, M. 1997. Potential probiotic strains in the culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). Aquaculture, 154:17-26.

Riquelme, C., Jorquera, M.A., Rojas, A.I., Avendano, R.E. and Reyes, N. 2001. Addition of inhibitor-producing bacteria to mass cultures of *Argopecten purpuratus* larvae (Lamarck, 1819). Aquaculture, 192:111-119.

Roberfroid, M.B. 1993. Dietary fiber, inulin, and oligofructose - A review comparing their physiological effects. Crit. Rev. Food Sci. Nutr., 33:103-148.

Roberfroid, M.B. 1995. A functional food. Chicory fructooligosaccharides: A colonic food with prebiotic activity. World Ingr. Mar., pp. 42-44.

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

Romalde, J.L., Magarinos, B., Nunez, S., Barja, J.L. and Toranzo, A.E. 1996. Host range susceptibility of *Enterococcus* sp. strains isolated from diseased turbot: Possible routes of infection. Appl. Environ. Microbiol., 62:607-611.

Rose, A.S., Ellis, A.E. and Munro, A.L.S. 1989. The infectivity by different routes of exposure and shedding rates of *Aeromonas salmonicida* subsp. *salmonicida* in Atlantic salmon, *Salmo salar* L., held in seawater. J. Fish Dis., 12:573-578.

Roussel, P., Lamblin, G., Lhermitte, M., Houdret, N., Lafitte, J-J., Perini, J-M., Klein, A. and Scharfman, A. 1988. The complexity of mucins. Biochimie, 70:1471-1482.

Rumessen, J.J. and Gudmand-Hoyer, E. 1998. Fructans of chocory: Intestinal transport and fermentation of different chain lengths and relation to fructose and sorbitol malabsorption. Amer. J. Clin. Nutr., 68:357-364.

Sakai, D.K. 1979. Invasive routes of *Aeromonas salmonicida* subsp. *salmonicida*. Sci. Rep. Hokkaido Fish Hatch., 34:61-89.

Salminen, S., von Wright, A., Morelli, L., Marteau, P., Brassart, D., de Voss, W.M., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.E. and Mattila Sandholm, T. 1998. Demonstration of safety of probiotics-a review. Int. J. Food Microbiol., 44:93-106.

Salminen, S., Isolauri, E. and Salmonen, E. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains for future challenges. Antonie van Leeuwenhoek, 70:347-358.

Savage, D.C. 1983. Mechanisms by which indigenous microorganisms colonize gastrointestinal epithelial surface. Prog. Food Nutr. Sci., 7:65-74.

Schneeman, B.O. 1999. Fiber, inulin and oligifructose: Similarities and differences. J. Nutr., 129:S1424-S1427.

Schrøder, K., Clausen, E., Sandberg, A.M. and Raa, J. 1980. Psychrotrophic *Lactobacillus plantarum* from fish and its ability to produce antibiotic substances. *In*: Advances in Fish Science and Technology. J.J. Connell (ed.), pp. 480-483. Fishing News Books, Farnham, Surrey, England.

Skjermo, J. and Vadstein, O. 1999. Techniques for microbial control in intensive fish larval rearing. Aquaculture, 177:333-343.

Slomiany, B.L., Murty, V.L.N., Piotrowski, J. and Slomiany, A. 1994. Gastroprotective agents in mucosal defense against *Helicobacter pylori.* Gen. Pharmacol., 5:833-841.

Smith, P. and Davey, S. 1993. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-induced furunculosis by a fluorescent pseudomonad. J. Fish Dis., 16:521-524.

Spanggard, B., Huber, I., Nielsen, J., Sick, E.B., Slierendrecht, W.J. and Gram, L. 2001. Antagonism of the indigenous fish microflora against *Vibrio anguillarum* and subsequent use of non-pathogenic cultures in protection against vibriosis. Environ. Microbiol., 3:755-765.

Strøm, E. 1988. Melkesyrebakterier i fisketarm. Isolasjon, karakterisering og egenskaper. Master's Thesis. The Norwegian College of Fishery Science, University of Tromsø, Norway. (In Norwegian).

Strøm, E. and Ringø, E. 1993. Changes in the bacterial flora in early developing cod, *Gadus morhua* (L.), larvae after an inoculation of *Lactobacillus plantarum* in the water. *In*: Physiological and Biochemical Aspects of Fish Development. B. Walter and H.J. Fyhn (eds.), pp 226-228. University of Bergen, ISBN 82-992402-0-4.

Svendsen, Y. S. and Bøgwald, J. 1997. Influence of artificial wound and non-intact mucus layer on mortality of Atlantic salmon (*Salmo salar* L.) following a bath challenge with *Vibrio anguillarum* and *Aeromonas salmonicida.* Fish Shellfish Immunol., 7:317-325.

Svendsen, Y.S., Dalmo, R.A. and Bøgwald, J. 1999. Tissue localization of *Aeromonas salmonicida* in Atlantic salmon, *Salmo salar* L., following experimental challenge. J. Fish Dis., 22:125-131.

Tamura, S., Shimizu, T. and Ikegami, S. 1993. Endocytosis in adult eel intestine: immunological detection of phagocytic cells in the surface epithelium. Biol. Bull., 184:330-337.

Tanasomwang, V. and Muroga, K. 1988. Intestinal microflora of larval and juvenile stages in Japanese flounder (*Paralichthys olivaceus*). Fish Pathol., 23:77-83.

Tancrède, C. 1992. Role of human microflora in health and disease. Eur. J. Clin. Microbiol. Infect. Dis., 11:1012-1015.

Towner, K.J. 1995. The genetics of resistance. *In*: Antimicrobial Chemotherapy. D. Greenwood (ed.), pp. 159-167. Oxford Univ. Press, Oxford.

van der Waaij, D., Berghuid-de Vries, J.M. and Lekkerkerk-van der Wees, J.E.C. 1972. Colonization resistance of the digestive tracts of mice during systemic antibiotic treatment. J. Hyg., 70:405411.

Van Loo, J., Coussement, P., Delenheer, L., Hoebregs, H. and Smiths, G. 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit. Rev. Food Sci. Nutr., 35:525-552.

Vergin, F. von. 1954. Anti-und probiotika. Hippokrates heft, 4:116-119.

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture - a review. Microbiol. Mol. Biol. Rev., 64:655-671.

Vigneulle, M. and Baudin Laurencin, F. 1991. Uptake of *Vibrio anguillarum* bacterin in the posterior intestine of rainbow trout *Oncorhynchus mykiss*, sea bass *Dicentrarchus labrax* and turbot *Scophthalmus maximus* after oral administration or anal intubation. Dis. Aquat. Org., 11:85-92.

Westerdahl A., Olsson, C.J., Kjelleberg, S. and Conway, P.L. 1991. Isolation and characterization of turbot (*Scophthalmus maximus*) - associated bacteria with inhibitory effects against *Vibrio anguillarum.* Appl. Environ. Microbiol., 57:2223-2228.