Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 274 (2008) 1 – 14
Aquaculture
www.elsevier.com/locate/aqua-online

Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes

Aditya Kesarcodi-Watson [a,*], Heinrich Kaspar [a], M. Josie Lategan [b], Lewis Gibson [b]

[a] *Cawthron Institute, Private Bag 2, Nelson, 7001, New Zealand*
[b] *Department of Medical and Molecular Biosciences, Faculty of Science, University of Technology Sydney (UTS), PO Box 123, Broadway, NSW, 2007, Australia*

Received 27 July 2007; received in revised form 11 November 2007; accepted 12 November 2007

Abstract

Aquaculture production of molluscs is worth US$11 billion per year and represents 65% of World mollusc product. A significant limitation to the industry is loss of stock through bacterial disease. Traditional methods to combat disease with antibiotics have been questioned and alternatives have been sought. The field of probiotics as well as the screening methods used to acquire probiotic strains for the alternative management of disease in aquaculture is discussed. This review provides a comprehensive summary of probiotics in aquaculture with special reference to mollusc culture.

Keywords: Probiotics; Aquaculture; Review; Mollusc; Screening process

Contents

1. Introduction

Forty percent of World aquatic product (including capture fisheries) derives from aquaculture, being valued at US$78 billion. Aquaculture produced molluscs account for 21% of total aquaculture product, and make up 65% of total mollusc product when capture fisheries are considered (FAO, 2007). Importance of aquaculture product is set to increase dramatically as a result of overfishing of the world's waters and an increasing demand for seafood. A significant issue affecting production is the loss of stock through disease. Diseases caused by *Vibrio* spp. and *Aeromonas* spp. are commonly implicated in episodes of mortality.

When faced with disease problems, the common response has been to turn to antimicrobial drugs (hereafter referred to as ADs). The livestock and aquaculture industries have

* Corresponding author. Tel.: +64 3 548 2319; fax: +64 3 546 9464.
E-mail address: aditya.kesarcodi-watson@cawthron.org.nz (A. Kesarcodi-Watson).

0044-8486/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2007.11.019

experienced widespread use of ADs in their practices. While the use of such products has an obvious benefit to treat animals infected by bacterial disease, the use of ADs has been either prophylactic (preventative), or for growth enhancement (Van den Bogaard and Stobberingh, 2000). Certain ADs have been shown to positively influence growth of livestock and used widely (Acar et al., 2000; Witte, 2000; Wierup, 2001; Phillips et al., 2004). Given this, and the desire to prevent establishment of pathogenic bacteria, it is argued that ADs have been widely overused (Aarestrup, 1999; Schwarz et al., 2001). Schwarz et al. (2001) provided a good overview of AD use in animals and the potential hazards associated with this.

The use of ADs in agriculture and aquaculture has led to the emergence of antibiotic resistant bacteria (hereafter referred to as ARB) (Schwarz et al., 2001; Akinbowale et al., 2006). In aquaculture this was felt most dramatically in the shrimp industry where massive increases in production, overcrowding of animals and unchecked antibiotic usages led to the emergence of numerous ARB and production crashes in many Asian countries (Karunasagar et al., 1994; Moriarty, 1999). For example, production figures for shrimp in the Philippines dropped by 55% in 2 years; from 90,000 t to 41,000 t between 1995 and 1997. In fact, it has never recovered and, in 2002, a mere 37,000 t was produced. An industry previously worth US $760 million is now worth only $240 million (FAO, 2007). Similarly, Thai shrimp production dropped by 40% between 1994 and 1997 due to disease problems (Moriarty, 1999); bacterial pathogens and shrimp viruses. Within aquaculture, there are numerous reports of ARB of farm origin (Karunasagar et al., 1994; Son et al., 1997; Molina-Aja et al., 2002; Chelossi et al., 2003; Sahul Hameed et al., 2003; Alcaide et al., 2005).

However, the risk is not just the potential loss to the farmer. The emergence of ARB on aquaculture farms could pose a risk to human health. There are many reports illustrating the transferral of resistant genes between bacteria (Son et al., 1997; Aarestrup, 1999; Van den Bogaard and Stobberingh, 2000; Witte, 2000; Schwarz et al., 2001). This process means ARB originating from a shrimp farm could potentially transfer plasmids to bacteria involved in human health problems. This is an area of current debate. Studies point to a farm animal origin in certain ARB genes that have made their way into human bacteria (Van den Bogaard and Stobberingh, 2000; Witte, 2000; Schwarz et al., 2001). However, recent reports argue this phenomenon (Acar et al., 2000; Phillips et al., 2004). The argument is based on the view that, although ARB have arisen in animal husbandry through use of antimicrobials, there is insufficient data to show a linkage to resistant gene transferral to humans. They argue in favour of the beneficial role antibiotics play in farming, and caution against premature, unscientific decisions in the restriction of antibiotic usage.

Regardless of which argument represents the true situation, governments and organizations have introduced much tighter restrictions for antibiotic usage in animal production. The European Union (EU) initially put a ban on the use of avoparcin in 1997, and in 1999, included virginiamycin, spiramcin, tylosin and bacitracin as banned growth promoters in animal feed (Turnidge, 2004; Delsol et al., 2005). In 2005, the EU implemented a ban on the use of all non-therapeutic antimicrobials in animal production (Delsol et al., 2005).

The US has been less stringent. There was a proposal in 2000 to introduce a ban on the use of fluoroquinolone and there was concern also about the use of virginiamycin (Nawaz et al., 2001). More recently a bill called "Preservation of antibiotics for medical treatment act of 2005" was presented in the US congress. If passed this act would see a ban on the non-therapeutic use of a drug intended for human use, in the production of feed animals. This act would be enforced two years from the date of being passed (Martin, 2005).

Other countries which currently have less antibiotic control, such as many of the Asian countries, are likely to be pressured through foreign restrictions, via the export markets being tightly controlled for antibiotic-contaminated products. Despite chloramphenicol being banned in Thailand since 1999 as a result of worldwide concern over its use in animal production, trace levels are still detected in shrimp from Thailand, causing a temporary ban by the EU for Thai shrimp (Heckman, 2004). Chloramphenicol has also been detected in shrimp from Myanmar, India, Pakistan and Vietnam, highlighting the continuing misuse of ADs in Asian shrimp farming.

A leading example in the eradication of antibiotic use can be seen in the Norwegian salmon industry. After concern about the use of antibiotics in the late 1980s, there has been a 95% drop in usage from 50 tonnes to 1 tonne annually. During the same period, salmon production has increased 10-fold from about 5500 tonnes to 55,000 tonnes. Reasons for the turnaround have been attributed to the use of vaccines, better husbandry and selective breeding programs (Maroni, 2000).

There is a developing social attitude against unnecessary use of ADs and where possible, it is the move away from non-essential AD use that the responsible farmer now seeks. Given the threat that both ADs and bacterial pathogens pose to farmers, as well as in human health, alternatives are being sought. Probiotics is one field commanding considerable attention.

2. Probiotics: definition and principles

The term, probiotic, simply means "for life", originating from the Greek words "pro" and "bios" (Gismondo et al., 1999). The most widely quoted definition was made by Fuller (1989). He defined a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". This definition is still widely referred to, despite continual contention with regard to the correct definition of the term. Current probiotic applications and scientific data on mechanisms of action indicate that non-viable microbial components act in a beneficial manner and this benefit is not limited just to the intestinal region (Salminen et al., 1999). Fuller's definition was a revision of the original probiotic concept which referred to protozoans producing substances that stimulated other protozoans (Lilly and Stillwell, 1965). Yet, although probiotics have been an area of much interest and research in the past 30 years, the original idea was possibly formed by Metchnikoff in the early 1900s. Metchnikoff (1907) theorized that human health could be aided through the

ingestion of fermented milk products. The concept of probiotic activity has its origins in the knowledge that active modulation of the gastrointestinal tract (GIT) could confer antagonism against pathogens, help development of the immune system, provide nutritional benefits and assist the intestinal mucosal barrier (Vaughan et al., 2002).

Today probiotics are quite commonplace in health promoting "functional foods" for humans, as well as therapeutic, prophylactic and growth supplements in animal production and human health (Mombelli and Gismondo, 2000; Ouwehand et al., 2002; Sullivan and Nord, 2002; Senok et al., 2005). Typically, the lactic acid bacteria (LAB) have been widely used and researched for human and terrestrial animal purposes, and LAB are also known to be present in the intestine of healthy fish (Ringø and Gatesoupe, 1998; Hagi et al., 2004). Interest in LAB stems from the fact that they are natural residents of the human GIT with the ability to tolerate the acidic and bile environment of the intestinal tract. LAB also function to convert lactose into lactic acid, thereby reducing the pH in the GIT and naturally preventing the colonization by many bacteria (Mombelli and Gismondo, 2000; Klewicki and Klewicka, 2004). The most widely researched and used LAB are the lactobacilli and bifidobacteria (Corcoran et al., 2004; Ross et al., 2005; Senok et al., 2005).

Other commonly studied probiotics include the spore forming *Bacillus* spp. and yeasts. *Bacillus* spp. have been shown to possess adhesion abilities, produce bacteriocins (antimicrobial peptides) and provide immunostimulation (Cherif et al., 2001; Cladera-Olivera et al., 2004; Duc et al., 2004; Barbosa et al., 2005). The strains appear to be effective probiotics and commercial products containing such strains have been demonstrated to improve shrimp production to a level similar to that when antimicrobials are used (Decamp and Moriarty, 2006). *Bacillus* spp. hold added interest in probiotics as they can be kept in the spore form and therefore stored indefinitely on the shelf (Hong et al., 2005). The yeast, *Saccharomyces cerevisiae*, also has been commonly studied whereby immunostimulatory activity was demonstrated and production of inhibitory substances shown (Castagliuolo et al., 1999; Dahan et al., 2003; Van der Aa Kühle et al., 2005).

Multiple ways exist in which probiotics could be beneficial and these could act either singly or in combination for a single probiotic. These include: inhibition of a pathogen via production of antagonistic compounds, competition for attachment sites, competition for nutrients, alteration of enzymatic activity of pathogens, immunostimulatory functions, and nutritional benefits such as improving feed digestibility and feed utilization (Fuller, 1989; Fooks et al., 1999; Bomba et al., 2002). It is often reported that a probiotic must be adherent and colonize within the GIT, it must replicate to high numbers, it must produce antimicrobial substances, and it must withstand the acidic environment of the GIT (Ziemer and Gibson, 1998; Dunne et al., 1999; Gismondo et al., 1999; Mombelli and Gismondo, 2000). However, these descriptions are misleading. These beliefs are based on the understanding that a probiotic must become a permanent member of the intestinal flora. While bacteria with this capacity are common and much probiotic research focuses on attachment capacity of bacteria, it has actually been demonstrated that transient bacteria can also exert beneficial effects (Isolauri et al., 2004). Additionally, contrary to the requisite of being able to attach to mucus and produce antimicrobial substances, a probiotic need only possess one mode of action. Multistrain and multi-species probiotics have proven that it is possible to provide synergistic bacteria with complementary modes of action to enhance protection (Timmerman et al., 2004).

3. Probiotics in aquaculture

3.1. Extended definition

When looking at probiotics intended for an aquatic usage it is important to consider certain influencing factors that are fundamentally different from terrestrial based probiotics. Aquatic animals have a much closer relationship with their external environment. Potential pathogens are able to maintain themselves in the external environment of the animal (water) and proliferate independently of the host animal (Hansen and Olafsen, 1999; Verschuere et al., 2000a). These potential pathogens are taken up constantly by the animal through the processes of osmoregulation and feeding. A study with Atlantic halibut, *Hippoglossus hippoglossus*, showed the transition from a prevailing *Flavobacterium* spp. intestinal flora to an *Aeromonas* spp./*Vibrio* spp. dominant flora occurred when first feeding commenced (Bergh et al., 1994). This study highlighted the impact that the external environment and feeding had on the microbial status of the fish. However, the same study also found that the larvae did maintain a specific intestinal flora different to that of the external tank flora. This showed that, although there were ever-present external environmental factors influencing the microbial flora inside an aquatic animal, they could still maintain a host specific flora at any given time. It was suggested that this ability did not apply to bivalve larvae (Jorquera et al., 2001). Their work demonstrated that the transit time of bacteria in bivalve larvae was too short to allow the establishment of a bacterial population different from that of the surrounding water.

Based on the intricate relationship an aquatic organism has with the external environment when compared with that of terrestrial animals, the definition of a probiotic for aquatic environments needs to be modified. Verschuere et al. (2000a) suggested the definition "a 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". Apart from the requirement of the probiotic to be a live culture, this definition is a lengthy way of describing a probiotic as defined by Irianto and Austin (2002a) thus "a probiotic is an entire or components(s) of a micro-organism that is beneficial to the health of the host". The latter definition is in accordance with that given by Salminen et al. (1999). The non-requirement of being a live culture would allow for certain suggested immunostimulants (Itami et al., 1998; Smith et al., 2003), which are bacterial derivatives, such

as peptidoglycan and lipopolysaccharides, to be included as probiotics. Although there is some dispute about what an aquatic probiotic actually is, all definitions differ to that of Fuller (1989) in that there is no longer the requisite for the probiotic to be acting in the GIT. Therefore, modes of action such as competition for nutrients and production of inhibitory substances could occur in the culture water. Additional effects of probiotic action should also be considered, given the modified definition, including change of the water quality and interaction with phytoplankton (Verschuere et al., 2000a).

Phytoplankton are capable of producing substances toxic to other bacteria and could potentially act in a beneficial manner. For example, *Skeletonema costatum*, a common microalga used in mollusc and crustacean larviculture, has been shown to produce an organic extract capable of inhibiting the growth of *Listonella anguillarum* and three other vibrios (Naviner et al., 1999). Another study has shown a microalga, *Caulobacter* sp., produced the antibiotic thiotropocin (Kawano et al., 1997). This compound was shown not only to be inhibitory towards the fish pathogen *Lactococcus garvieae*, but also had antimicroalgal activity against *Skeletonema costatum* and *Heterosigma akashiwo*.

Perhaps of more importance is the consideration of what effect adding a probiotic bacterium will have upon phytoplankton. Microalgae are required for most larviculture in aquaculture and, in fact, certain bacteria can stimulate microalgal growth (Haines and Guillard, 1974; Ukeles and Bishop, 1975; Barker and Herson, 1978; Fukami et al., 1992; Suminto and Hirayama, 1996; Fukami et al., 1997; Suminto and Hirayama, 1997). Thus, probiotics could be specifically targeted for microalgae production; however, the subsequent effects of such bacteria towards the larvae must be established. The more realistic approach is using probiotics aimed at improving the health of the larvae and then determining whether this bacterium has an effect upon the microalgae. It would be very desirable to discover a probiotic that benefited the larvae and was either beneficial to or did not impair the microalgae. Consequently, the bacteria could be co-cultured with the microalgae as the entry-point into the larviculture system. This was done by Gomez-Gil et al. (2002) who found the shrimp probiotic, C7b, could be co-cultured with shrimp larvae food, *Chaetoceros muelleri*, without affecting the microalga. Similarly, Avendaño and Riquelme (1999) investigated the growth of seven bacterial strains with *Isochrysis galbana*. Four of these strains did not affect growth of the microalgae, while co-culture significantly improved ingestion of bacterium C33 by larval scallop, *Argopecten purpuratus*.

3.2. Modes of action

Several studies have demonstrated certain modes of probiotic action in effect in the aquatic environment. Bairagi et al. (2002) assessed aerobic bacteria associated with the GIT of nine freshwater fish. They determined that selected strains produced digestive enzymes, thus facilitating feed utilization and digestion. Ramirez and Dixon (2003) reported on the enzymatic properties of anaerobic intestinal bacteria isolated from three fish species, showing the potential role a probiotic could play. In a recent paper by Bairagi et al. (2004) the benefit of adding *B. subtilis* and *B. circulans* to the diet of rohu, *Labeo rohita*, was shown. In the search to replace fish meal with leaf meal in fish feed, they found that addition of the two fish intestinal *Bacillus* spp. increased performance as judged by a number of factors including growth, feed conversion ratio, and protein efficiency ratio. They attributed this to the extracellular cellulolytic and amylolytic enzyme production by the bacteria.

Although competition for adhesion sites has been widely suggested as a mode of action, there is little evidence in the literature to demonstrate this. There are studies reporting an adhesion of certain bacteria to intestinal mucus *in vitro*, but transferral of these to *in vivo* models has not produced supporting results (Hansen and Olafsen, 1999). Attachment ability of potential probiotics seen *in vitro* cannot be assumed to demonstrate the real effect *in vivo*. Additionally, while studies to date have demonstrated the ability of certain bacteria to adhere to intestinal mucus *in vitro* (Krovacek et al., 1987; Olsson et al., 1992; Garcia et al., 1997, Jöborn et al., 1997), they failed to assess a competitive exclusion effect. More recently, Vine et al. (2004a) demonstrated a competitive exclusion effect with five probiotics versus two pathogens on fish intestinal mucus. They found that the presence of one of the probiotics on the mucus inhibited the attachment of one of the pathogens tested. Interestingly, pre-colonization with the other probiotics encouraged attachment of the two pathogens. However, the general trend from their study showed that post treatment with the probiotics displaced the pathogen.

Although not directly concerning attachment competition, Yan et al. (2002) demonstrated that the production of antibiotic substances by two seaweed-associated *Bacillus* spp. was dependent on biofilm formation by the bacteria. This study highlighted a factor which might be important for some bacteria to be effective probiotics, i.e. surface attachment. This observation concurred with Fuller's (1989) definition of a probiotic, i.e. the requirement for GIT colonization. It has been proposed that the mechanism of competitive exclusion for attachment sites could be given a distinct advantage via addition of probiotic bacteria during the initial egg fertilization steps of larviculture, thereby "getting in there first" (Irianto and Austin, 2002a). This concept was not supported by Makridis et al. (2000) who found no difference between the concentrations of two bacteria in the gut of turbot larvae when these bacteria were administered at hatching and two days post hatching.

Several studies have attributed a probiotic effect to competition for energy sources (Rico-Mora et al., 1998; Verschuere et al., 1999; Verschuere et al., 2000b). Beneficial growth and survival was found in *Artemia* sp. pre-exposed to nine strains of bacteria before challenge with *V. proteolyticus* (Verschuere et al., 1999). It was concluded that the effect was not caused by extracellular products, but required the live bacterial cell. Although it was not specifically tested, they hypothesized that the protective effect probably resulted from competition for energy sources and for adhesion sites. Competition for iron has been reported as an important factor in marine bacteria (Verschuere et al., 2000a). Iron is needed by most bacteria for growth, but is generally limited in the tissues

and body fluids of animals and in the insoluble ferric Fe^{3+} form (Verschuere et al., 2000a). Iron-binding agents, siderophores, allow acquisition of iron suitable for microbial growth. Siderophore production is a noted mechanism of virulence in some pathogens (Gram et al., 1999). Equally, a siderophore-producing probiotic could deprive potential pathogens of iron under iron limiting conditions. This was shown by Gram et al. (1999), who found that a culture supernatant of *Pseudomonas fluorescens*, grown in iron-limited conditions, inhibited growth of *V. anguillarum*, whereas the supernatant from iron-available cultures did not.

Itami et al. (1998) found that addition of *Bifidobacterium thermophilum* derived peptidoglycan to kuruma shrimp increased significantly their survival when they were challenged with *V. penaeicida*. They attributed this to an immunostimulatory effect, as the phagocytic activity of shrimp granulocytes was significantly higher in the treated shrimp compared with those of the control animals. A study by Gullian et al. (2004) differed slightly in its approach of an immunostimulating probiotic. Rather than testing bacterial derivatives such as glycans or lipopolysaccharides, they tested immunostimulation by a live *Vibrio* sp. (P62) and *Bacillus* sp. (P64), using *V. alginolyticus* as a positive control. They concluded that P64 and *V. alginolyticus* were immunostimulants. However, this conclusion was based on only two of the nine immunological parameters they presented showing significant differences between treatments, and stemmed from standardizing all parameters into one index for one statistical conclusion, i.e. immunostimulatory or not. A recent review by Smith et al. (2003) provided important information on the potential problems associated with immunostimulants in crustacean aquaculture. They argued that the prolonged use of immunostimulants was in fact detrimental to the host and that much more research was needed before their use during critical periods could be considered safe.

Possibly the most studied mode of probiotic action in aquatic animals is the production of inhibitory substances; this will be discussed in the next section.

3.3. Previous research and methodology

A summary of past research into aquaculture probiotics is given in Table 1. It should be reiterated that, by definition, a probiotic need only benefit the host, and this could be either nutritional or a change in its immediate environment. Yet, screening to date has concentrated on the search for probiotics active against a pathogen; perhaps because of the problems bacterial pathogens can cause in the aquaculture environment. In screening for potential probiotics, most of this research employed identification of inhibitory activity *in vitro* (Dopazo et al., 1988; Westerdahl et al., 1991; Sugita et al., 1996a,b; Bly et al., 1997; Sugita et al., 1997a,b, 1998, 2002; Burgess et al., 1999; Jorquera et al., 1999; Spanggaard et al., 2001; Chythanya et al., 2002; Hjelm et al., 2004a,b). Currently, there are four methods commonly employed to screen for inhibitory substances *in vitro*; the double layer method, the well diffusion method, the cross-streak method, and the disc diffusion method. All methods are based on the principle that a bacterium (the producer) produces an extracellular substance which is inhibitory to itself or another bacterial strain (the indicator). The inhibitory activity is displayed by growth inhibition of the indicator in agar medium.

In some cases, initial *in vitro* screening was followed by small scale testing of short-listed candidates *in vivo* for either pathogenicity to the host (Makridis et al., 2000; Chythanya et al., 2002; Hjelm et al., 2004a) or host protection when challenged with a pathogen (Rengpipat et al., 1998; Robertson et al., 2000; Gram et al., 2001; Irianto and Austin, 2002b; Lategan and Gibson, 2003; Vaseeharan et al., 2004; Lategan et al., 2004a,b). Apart from the study by Gram et al. (2001), a positive protective effect was seen in all *in vivo* studies following positive antagonism assays *in vitro*. In other studies, *in vitro* short-listed probiotics have been tested further for properties such as bile resistance (Chabrillón et al., 2006), attachment capacity (Olsson et al., 1992; Hjelm et al., 2004a), immunostimulation (Gullian et al., 2004; Rengpipat et al., 2000; Irianto and Austin, 2003), competition for adhesion sites (Vine et al., 2004a; Chabrillón et al., 2006) and competition for nutrients (siderophore production) (Gram et al., 1999). In practice, these latter studies test whether or not a probiotic that produces diffusible inhibitory substances also possesses other modes of probiotic action.

Screening for production of an inhibitory substance *in vitro* and then taking likely candidates into the further testing stage limits the short list to those isolates which exhibit only one of the various modes of probiotic action, namely production of diffusible inhibitory substances. Although production of an inhibitory substance has been shown to work very well in probiotics and this screening method has identified very good probiotics in aquaculture (Irianto and Austin, 2002b; Lategan and Gibson, 2003; Vaseeharan et al., 2004; Lategan et al., 2004a,b), there are two major limitations to this approach. The first is that other modes of probiotic activity (e.g. immunostimulation, digestive enzymes production, competition for attachment site, or nutrients) will not be expressed in the laboratory on an agar plate and, hence, a major source of potential beneficial action will be overlooked. The second drawback is that positive results *in vitro* fail to determine the real *in vivo* effect. This means that a bacterium which is antagonistic in the laboratory might not be inhibitory when associated with the animal in question. For example, using *P. fluorescens* strain AH2, a probiotic proven to be successful in protecting rainbow trout from *V. anguillarum*, Gram et al. (2001) found that this bacterium was also inhibitory to the salmon pathogen *A. salmonicida in vitro*. However, no protective effect was found when transferring the probiotic to an *in vivo* challenge experiment with salmon and this pathogen. The same effect was seen by Ruiz-Ponte et al. (1999), when *in vitro* antagonism was not able to protect scallop larvae challenged by a pathogen in an *in vivo* situation. Similarly, a bacterium which is not inhibitory in the laboratory might actually be antagonistic *in vivo*. This occurrence was shown in a study with rainbow trout (Raida et al., 2003) where they assessed a commercial probiotic product, BioPlus2B, in

Table 1
Summary of research towards probiotics for aquaculture

Animals tested	Potential probiotic	Pathogen tested or type of study conducted	Test method	Reference
	A. media	*Ed. tarda*, *V. anguillarum*, *Y. ruckeri*, *A. salmonicida*, *Lactococcus garvieae*, *Saprolegnia parasitica*	*In vitro*	Lategan et al. (2006)
	Alt. haloplanktis	*V. anguillarum*, *V. alginolyticus*, *V. ordalii*, *A. hydrophila*	*In vitro*	Riquelme et al. (1996)
	Aerobic bacteria from GIT of freshwater fish	Enzyme production study	*In vitro*	Bairagi et al. (2002)
	Antibiotic producing *Alt.* sp.	Non-antibiotic producing *Alt.* sp.	*In vitro*	Lemos et al. (1991)
	B. spp.	*V. anguillarum*, *V. vulnificus*, *Pa. piscicida*, *Ent. seriolicida*	*In vitro*	Sugita et al. (1996a)
	Carp intestinal bacteria	*A. hydrophila*, *A. salmonicida*, *E. coli*, *S. aureus*	*In vitro*	Sugita et al. (1997b)
	Carnobacterium piscicola	*Carnobacteria* spp., *Lactobacillus* spp., *Pediococci* sp., *L.* spp.	*In vitro*	Stoffels et al. (1992)
	Freshwater bacteria	*A.* spp.	*In vitro*	Sugita et al. (1996b)
	6 terrestrial LAB (*L. rhamnosus* (ATCC 53103), *L. rhamnosus* (LC705), *L. casei*, *L. bulgaricus*, *L. johnsonii*, *Bif. lactis*, *Ent. faecium*	*A. salmonicida*, *V. anguillarum*, *Fl. psychrophilum*	*In vitro*	Nikoskelainen et al. (2001b)
	Marine bacteria	*V. anguillarum*	*In vitro*	Westerdahl et al. (1991)
	Marine bacteria	*V. anguillarum*	*In vitro*	Olsson et al. (1992)
	Marine bacteria	*L. garvieae*, *Pa. piscicida*, *V. anguillarum*, *V. vulnificus*	*In vitro*	Sugita et al. (2002)
	Marine bacteria	*A. hydrophila*, *V. alginolyticus*	*In vitro*	Vine et al. (2004a)
	Marine bacteria	*A. hydrophila*, *V. alginolyticus*	*In vitro*	Vine et al. (2004b)
	Psalt. undina	IHNV, *V. anguillarum*	*In vitro*	Maeda et al. (1997)
	Psalt. spp., B. spp.	*A. hydrophila*, *V. anguillarum*, *S. epidermidis*, *Pr.* spp., *Ca. albicans*, *Ent. faecalis*	*In vitro*	Ivanova et al. (1998)
	Ps. flourescens	*Saprolegnia* spp.	*In vitro*	Bly et al. (1997)
	Ps. spp., *Alt.* spp.	*V.* spp., *A.* spp., *Pa.* spp., *Ed.* spp., *Y. ruckeri*, *Ps. aeruginosa*	*In vitro*	Dopazo et al. (1988)
	Ps. spp., *A.* spp., *V.* spp.	IHNV	*In vitro*	Kamei et al. (1988)
	Ps. spp.	*A. hydrophila*	*In vitro*	Das et al. (2006)
	Roseobacter sp.	*Proteobacteria* spp., *Fl.* spp., *Actinobacteria* spp.	*In vitro*	Brinkhoff et al. (2004)
	Roseobacter spp., *V.* spp.	*V. anguillarum*, *V. splendidus*	*In vitro*	Hjelm et al. (2004b)
	V. anguillarum	Growth in salmon mucus study	*In vitro*	Garcia et al. (1997)
	V. sp. (strain NM10)	*Pa. piscicida*	*In vitro*	Sugita et al. (1997b)
	V. spp.	IHNV, OMV	*In vitro*	Direkbusarakom et al. (1998)
	V. spp., *B.* sp., coryneform	*V. vulnificus*	*In vitro*	Sugita et al. (1998)
	V. sp.	*V. anguillarum*	*In vitro*	Jorquera et al. (1999)
	123 *V.* spp.	*V. tapetis*	*In vitro*	Castro et al. (2002)
	V. mediterranei 1	*V. parahaemolyticus*	*In vitro*	Carraturo et al. (2006)
Finfish				
Atlantic cod	*Carnobacterium divergens*	*V. anguillarum*	*In vitro* and *in vivo*	Gildberg et al. (1997)
Atlantic cod	*Carnobacterium divergens*	*V. anguillarum*	*In vitro* and *in vivo*	Gildberg and Mikkelsen (1998)
Atlantic salmon	*Lactobacillus plantarum*	*A. salmonicida*	*In vitro* and *in vivo*	Gildberg et al. (1995)
Atlantic salmon	*Carnobacterium* sp. (K1)	*V. anguillarum*, *A. salmonicida*	*In vitro* and *in vivo*	Jöborn et al. (1997)
Atlantic salmon	*Ps. fluorescens*	*A. salmonicida*	*In vitro* and *in vivo*	Gram et al. (2001)
Atlantic salmon, rainbow trout	*Carnobacterium* sp.	*V. anguillarum*, *V. ordalii*, *Y. ruckeri*, *A. salmonicida*	*In vitro* and *in vivo*	Robertson et al. (2000)
Eel	Commercial product: Cernivet® LBC (Ent. Faecium SF68), Toyocerin® (B. toyoi)	*Ed. tarda*	*In vivo*	Chang and Liu (2002)
Eel	*A. media*	*Saprolegnia* sp.	*In vitro* and *in vivo*	Lategan and Gibson (2003)
Eel	*A. media*	*Saprolegnia parasitica*	*In vivo*	Lategan et al. (2004b)

Table 1 (*continued*)

Animals tested	Potential probiotic	Pathogen tested or type of study conducted	Test method	Reference
Finfish				
Gilthead sea bream	*Cytophaga* sp., *Roseobacter* sp., *Ruergeria* sp., *Paracoccus* sp., *A.* sp., *Shewanella* sp.	Natural larval survival study	*In vivo*	Makridis et al. (2005)
Gilthead sea bream	*V.* spp., *Micrococcus* sp.	*L. anguillarum*	*In vitro* and *in vivo*	Chabrillón et al. (2006)
Goldfish	Dead cells of *A. hydrophila*	*A. salmonicida*	*In vivo*	Irianto et al., 2003
Indian major carp	*B. subtilis*	*A. hydrophila*	*In vivo*	Kumar et al. (2006)
Nile tilapia	*Str. faecium*, *Lactobacillus acidophilus*, *Sacc. cerevisiae*	Growth study	*In vivo*	Lara-Flores et al. (2003)
Pollack	Commercial product: Bactocell (*Pediococcus acidilactici*), Levucell (*Sacc. cerevisiae*)	Pollack growth study using enriched *Artemia*	*In vivo*	Gatesoupe (2002)
Rainbow trout	*Ps. fluorescens*	*V. anguillarum*	*In vitro* and *in vivo*	Gram et al. (1999)
Rainbow trout	*Lactobacillus rhamnosus*	*A. salmonicida* ssp. *salmonicida* (furunculosis)		Nikoskelainen et al. (2001a)
Rainbow trout	*Ps.* spp.	*V. anguillarum*	*In vitro* and *in vivo*	Spanggaard et al. (2001)
Rainbow trout	*A. hydrophila*, *V. fluvialis*, *Carnobacterium* sp.	*A. salmonicida*	*In vitro* and *in vivo*	Irianto and Austin (2002a)
Rainbow trout	Dead cells of *A. hydrophila*, *V. fluvialis*, *Carnobacterium* sp.	*A. salmonicida*	*In vivo*	Irianto and Austin (2003)
Rainbow trout	*Lactobacillus rhamnosus*	Immune enhancement paper	*In vivo*	Nikoskelainen et al. (2003)
Rainbow trout	Commercial product: BioPlus2B (*B. subtilis*, *B. licheniformis*)	*Y. ruckeri*	*In vivo*	Raida et al. (2003)
Rainbow trout	*Lactobacillus rhamnosus*	Natural immunostimulation measured	*In vivo*	Panigrahi et al. (2004)
Rainbow trout	*Pediococcus acidilactici*, *Sacc. boulardii*	Prevention of vertebral column compression syndrome	*In vivo*	Aubin et al. (2005)
Rainbow trout	*A. sobria*	*L. garvieae*, *Str. iniae*	*In vivo*	Brunt and Austin, 2005
Rainbow trout	*Lactobacillus rhamnosus*	Natural immunostimulation measured	*In vivo*	Panigrahi et al. (2005)
Rohu	*B. circulans*, *B. subtilis*	Digestive enzyme study	*In vivo*	Bairagi et al. (2004)
Sea bass	*Debaryomyces hansenii*, *Sacc. cerevisiae*	Digestive enzyme study	*In vivo*	Tovar et al. (2002)
Senegalese sole	*V.* spp., *Ps.* spp., *Micrococcus* sp.	*V. harveyi*	*In vitro* and *in vivo*	Chabrillón et al. (2005)
Silver perch	*A. media*	*Saprolegnia* sp.	*In vivo*	Lategan et al. (2004a)
Tilapia	Commercial product: Alchem Poseidon, Korea	*Ed. tarda*	*In vivo*	Taoka et al. (2006)
Turbot	2 unidentified marine bacteria	GIT colonization study	*In vivo*	Makridis et al. (2000)
Turbot	Marine bacteria	Natural survival study	*In vivo*	Huys et al. (2001)
Turbot	*Roseobacter* spp., *V.* spp.	*V. anguillarum*, *V. splendidus*, *Psalt.* sp.	*In vitro* and *in vivo*	Hjelm et al. (2004a)
Crustaceans				
F.W. prawns	*Lactobacillus* spp.	Gram negative bacteria	*In vivo*	Venkat et al. (2004)
Shrimp embryos	*Alt.* sp.	Lagenidum callinectes (fungus)	*In vivo*	Gil-Turnes et al. (1989)
Shrimp	*Bif. thermophilum* derived peptidoglycan	*V. penaeicida*	*In vivo*	Itami et al. (1998)
Shrimp	Commercial product: DMS 1000, 1100, 2000	Pond culture survival study	*In vivo*	Moriarty (1998)
Shrimp	*B.* sp.	*V. harveyi*	*In vivo*	Rengpipat et al. (1998)
Shrimp	Commercial product: BioStart™ HB-1 (*B. subtilis*, *B. megaterium*, *B. polymyxa*) BioStart™ HB-2 (*B. licheniformis*)	Natural growth and survival study	*In vivo*	McIntosh et al. (2000)
Shrimp	*B.* sp. (S11)	*V. harveyi*	*In vivo*	Rengpipat et al. (2000)
Shrimp	*Ps. aeruginosa*	*V. harveyi*, *V. fluvialis*, *V. parahaemolyticus*, *V. damsela*, *V. vulnificus*	*In vitro* and *in vivo*	Chythanya et al. (2002)
Shrimp	*B.* spp.	*V. harveyi*	*In vivo*	Meunpol et al. (2003)
Shrimp	*B. subtilis*	*V. harveyi*	*In vitro* and *in vivo*	Vaseeharan and Ramasamy (2003)
Shrimp	*V.* spp., *B.* sp.	*V. harveyi*	*In vitro* and *in vivo*	Gullian et al. (2004)
Shrimp	*Ps.* sp. (PM 11), *V. fluvalis* (PM 17)	Natural immunostimulation study	*In vitro* and *in vivo*	Alavandi et al. (2004)
Shrimp	Commercial product: unidentified	Pond occurrence of *L. anguillarum* study	*In vivo*	Vaseeharan et al. (2004)
Shrimp	*Ps.* sp. PS-102	112 bacterial pathogens	*In vitro*	Vijayan et al. (2006)

(continued on next page)

Table 1 (*continued*)

Animals tested	Potential probiotic	Pathogen tested or type of study conducted	Test method	Reference
Crustaceans				
Shrimp larvae	Arthrobacter XE-7	*V. parahaemolyticus*, *V. anguillarum*, *V. nereis*	*In vitro* and *in vivo*	Li et al. (2006)
Swimming crab larvae	*Thalassobacter utilis*	*V. anguillarum*, *Haliphthoros* sp. (fungus)	*In vivo*	Nogami et al. (1997)
Mollusc				
Abalone	Unidentified: 1 yeast and 1 bacterium	Growth study and challenge with *V. anguillarum*	*In vivo*	Macey and Coyne (2005)
Pacific oyster larvae	*Alt.* sp. (CA2)	Growth and natural survival experiment	*In vivo*	Douillet and Langdon (1993)
Pacific oyster larvae	*Alt.* sp. (CA2)	Growth and natural survival experiment	*In vivo*	Douillet and Langdon (1994)
Pacific oyster larvae	*A. media*	*A.* spp., *V.* spp., *P. damsella*, *Y. ruckeri*, *V. tubiashii*	*In vitro* and *in vivo*	Gibson et al. (1998)
Scallop larvae	Marine bacteria	*V. anguillarum*	*In vitro* and *in vivo*	Riquelme et al. (1997)
Scallop larvae	Marine bacteria	*V. anguillarum*		Avendaño and Riquelme (1999)
Scallop larvae	*Roseobacter* sp.	Variety-including *V.* spp., *A.* spp.	*In vitro* and *in vivo*	Ruiz-Ponte et al. (1999)
Scallop larvae	*V.* sp. (C33), *Ps.* sp. (strain 11), *Arthrobacter* sp. (strain 77)	Natural survival and ingestion study	*In vivo*	Riquelme et al. (2000)
Scallop larvae	*V.* sp. (C33), *Ps.* sp. (strain 11), *B.* Sp. (B2)	Natural survival experiment in mass culture	*In vivo*	Riquelme et al. (2001)
Live food				
Artemia	9 marine bacteria	Natural survival and growth study	*In vivo*	Verschuere et al. (1999)
Artemia	Commericial product: 9 commercial products and 8 laboratory cultures (including mainly *B.* spp. and *Ps.* spp.)	Natural growth study	*In vivo*	Douillet (2000a)
Artemia	*A.* spp., *V.* spp.	*V. proteolyticus*	*In vivo*	Verschuere et al. (2000a)
Artemia	*Microbacterium* spp., *Exiguobacterium* sp.	Natural survival study	*In vivo*	Orozco-Medina et al. (2002)
Artemia	*Sacc. boulardii* (yeast)	*V. harveyi*	*In vivo*	Patra and Mohamed (2003)
Artemia	LAB	*V. alginolyticus*	*In vitro* and *in vivo*	Villamil et al. (2003)
Chaetoceros ceratosporum	Marine bacteria	Microalgae growth study	*In vivo*	Fukami et al. (1992)
Chaetoceros gracilis	*Fl.* sp.	Co-culture study	*In vivo*	Suminto and Hirayama (1996)
Chaetoceros gracilis, *Isochrysis galbana*, *Pavlova lutheri*	*Fl.* sp.	Co-culture study	*In vivo*	Suminto and Hirayama (1997)
Chaetoceros muelleri	*V. alginolyticus*	Co-culture study	*In vivo*	Gomez-Gil et al. (2002)
Isochrysis galbana	7 inhibitory substance-producing marine bacteria	Co-culture study	*In vivo*	Avendaño and Riquelme (1999)
Rotifers	LAB	Turbot growth study	*In vivo*	Gatesoupe (1991)
Rotifers	Mixed culture	Growth experiment	*In vivo*	Hirata et al. (1998)
Rotifers	*L. lactis*	*V. anguillarum*	*In vivo*	Shiri Harzevili et al. (1998)
Rotifers	*Alt.* sp., 3 unidenitfied spp.	Growth study	*In vivo*	Douillet (2000b)
Rotifers	7 terrestrial LABs	Growth study	*In vivo*	Planas et al. (2004)
Skeletonema costatum	Putative *A.* sp (SK-05)	*V. alginolyticus*	*In vivo*	Rico-Mora et al. (1998)

Ps. = *Pseudomonas*, *A.* = *Aeromonas*, *V.* = *Vibrio*, *Ps.* = *Pseudomonas*, *Alt.* = *Alteromonas*, *Pa.* = *Pasteurella*, *Ed.* = *Edwardsiella*, *Y.* = *Yersinia*, *Psalt.* = *Pseudoalteromonas*, *S.* = *Staphylococcus*, *Pr.* = *Proteus*, *Ca.* = *Candida*, *Ent.* = *Enterococcus*, *E.* = *Escherichia*, *L.* = *Lactococcus*, *P.* = *Photobacterium*, *Bif.* = *Bifidobacterium*, *Fl.* = *Flavobacterium*, *Str.* = *Streptococcus*, *Sacc.* = *Saccharomyces*, *B.* = *Bacillus*, IHNV = infectious hematopoietic necrosis virus, OMV = *Onchorhynchus masou* virus.

rainbow trout challenged with *Yersinia ruckeri*. Although the product was shown to enhance survival of the challenged fish, no inhibitory effect was found via *in vitro* antagonism assays.

The most likely reasons for the research approaches taken in the past are cost, ease of experimentation and lack of test animals and space. Setting up initial screening experiments involves very large numbers of tests in order to screen as many isolates as possible in the hope of obtaining good probiotics. In reality, setting up this phase of experimentation with a suitable number of animals per replicate, a sufficient number of replicates per treatment, and screening even the modest number of 100 isolates, presents a huge demand for number of animals needed and also the space and resources to carry out these experiments *in vivo*. In view of this, it is not surprising that a laboratory component is added to the screening before challenging animals

with a substantially reduced short-list. Contrary to the *in vitro* approach of identifying probiotics, Makridis et al. (2005) recently adopted a direct *in vivo* approach. They isolated six bacteria from healthy cultures of gilthead sea bream larvae food, *Artemia* sp. and rotifers. They then tested these food-sourced bacteria with the sea bream larvae. They found that addition of the bacteria significantly improved larval survival. Similarly, search for *Artemia* sp. probiotics by Verschuere et al. (1999) implemented *in vivo* experiments using bacteria sourced from healthy *Artemia* sp. cultures. Based on growth and survival figures of these monoxenic cultures, nine out of eighteen strains tested were chosen for *in vivo* challenge experiments against *V. proteolyticus* CW8T2 (Verschuere et al., 2000b). All nine strains demonstrated a significant protective effect. The encouraging results from these two studies highlighted the benefit of including test animals at the initial stages of the screening process.

Another popular approach used for identifying aquaculture probiotics included testing of proven human and agricultural probiotics such as LAB and yeasts. The research approach consisted of either selecting and testing of LAB from the GIT of aquatic animals (Stoffels et al., 1992; Gildberg et al., 1995, 1997; Gildberg and Mikkelsen, 1998; Shiri Harzevili et al., 1998; Tovar et al., 2002; Bairagi et al., 2004), or using probiotics developed for terrestrial animals (Nikoskelainen et al., 2001a,b, 2003; Lara-Flores et al., 2003;; Patra and Mohamed, 2003; Panigrahi et al., 2004, 2005; Planas et al., 2004; Venkat et al., 2004; Aubin et al., 2005). Such research, therefore, limits the identification of novel probiotic bacteria. However, research of this type is definitely warranted as the evidence to date has shown LAB to be just as useful in aquatic animals as in terrestrial animals.

3.4. Probiotic research in mollusc aquaculture

There has been moderate past research effort into probiotics for bivalve molluscs. This has included work on the Pacific oyster, *Crassostrea gigas*; the scallop, *Pecten maximus*; the Chilean scallop, *Argopecten purpuratus*; and the Manila clam, *Ruditapes philippinarum* (Table 1). It is noteworthy that, apart from Douillet and Langdon (1993, 1994), all work published to date on bivalve probiotics originated as a consequence of screening for diffusible inhibitory substances *in vitro*.

Work on larvae of the Chilean scallop has been the most sustained published information on probiotics in bivalve mollusc culture (Riquelme et al., 1996, 1997, 2001; Avendaño and Riquelme, 1999; Jorquera et al., 1999; Riquelme et al., 2000; Jorquera et al., 2001). The initial published work (Riquelme et al., 1996) identified a bacterium, *Alteromonas haloplanktis*, capable of reducing mortality when larvae were exposed to 10^3 colony forming units ml^{-1} (CFU ml^{-1}) of *V. anguillarum* (VAR). It was found in the same study that only stationary phase supernatants of the probiotic were inhibitory to VAR *in vitro* when compared with log phase supernatants. Despite this moderate success, *A. haloplanktis* was not pursued in further published research. Jorquera et al. (1999) then set out to isolate the antimicrobial fractions of C33 (*Vibrio* sp.) using thin layer chromatography. This bacterium had previously shown good antimicrobial activities *in vitro*. Avendãno and Riquelme (1999) tested the co-culture and administration of seven potential probiotics to larvae through the microalga *I. galbana*, including one bacterium (strain 11) capable of providing larval protection against VAR for 24 h (Riquelme et al., 1997). They found that the previously non-ingested bacterium, C33, was ingested by the larvae after co-culture thereby providing a vector for introduction. The next two published reports by this group provided useful information. Of the three bacteria tested (strains 11, 77 and C33), Riquelme et al. (2000) demonstrated that only two of these were ingested by the larvae (strains 11 and 77). They also determined that when the probiotics were given at 10^6 CFU ml^{-1}, a period of 6 h was needed for significant ingestion to occur. Additionally, with the one strain tested further (strain 77), 24 h was needed for it to become the dominant member of the larval microbiota when administered at 10^6 CFU ml^{-1} and 48 h was required if given at 10^4 CFU ml^{-1}. The next report (Riquelme et al., 2001) incorporated probiotics into a commercial scale hatchery production using the bacterial strains C33 (*Vibrio* sp.), strain 11 (*Pseudomonas* sp.), and *Bacillus* sp. (strain B2). This study determined that the probiotics allowed completion of the larval cycle without the need to use antibiotics.

The first work on probiotics in bivalves was conducted by Douillet and Langdon (1993). Unlike most research, their approach did not include *in vitro* agar-based tests. Instead, they applied twenty-one strains of bacteria directly to axenic Pacific oyster larvae, *Crassostreas gigas*, to determine the growth and survival characteristics of the bacterium added. Larvae were never challenged by a pathogen under the experimental protocol; the study looked specifically at the monoxenic effect of each bacterium. Of the tested strains, CA2, a putative *Alteromonas* sp., was identified as consistently enhancing both larval growth and survival. This work was followed up by determining the effect of adding different concentrations of CA2 to non-axenic cultures of the larvae and the effect of CA2 with different species of microalgae (Douillet and Langdon, 1994). They showed that although seasonal variation in seawater microbiota did not affect the growth advantages from CA2, seasonal variation in growth and survival caused by different broodstock cohorts was apparent. Based on the lower numbers of slow growing larvae in treatments receiving CA2, they also proposed that it might provide some nutritional benefit. Another study on *C. gigas* was conducted by Gibson et al. (1998). They found a bacterium, *Aeromonas media* (strain A199), capable of inhibiting 89 of the 90 strains tested *in vitro* using the cross-streak method. Tested strains mainly comprised vibrios and aeromonads, with also two strains of *Y. ruckeri*, two of *Photobacterium damsella*, three of *L. anguillarum* and one of *L. garvieae*, the non-affected bacterium. This widespread antagonism was then tested in bioassays with oyster larvae. It was found that A199 was able to prevent larval death when challenged with up to 10^5 CFU ml^{-1} of *V. tubiashii*, if A199 was inoculated 1 h earlier at 10^4 CFU ml^{-1}. More recently, Elston et al. (2004) determined two potential probiotics for *C. gigas* larval production, P02-45 and P02-1. They determined that both killed bacteria and cell-free

extracts were inhibitory *in vitro*. They further tested the co-culture of these strains with microalgae, and found that a protective effect against *V. tubiashii* could be established via co-culture with *I. galbana* (T-Iso) and *Rhodomonas* sp. Probiotic addition in larval challenge experiments was at a concentration of 10^5 CFU ml^{-1} and was not detrimental to larval survival until exceeding a concentration of 10^7 CFU ml^{-1}.

Other mollusc research has been conducted on the scallop, *P. maximus* (Ruiz-Ponte et al., 1999). Ruiz-Ponte et al. (1999) found a strain of *Roseobacter* sp. (BS107) that produced *in vitro* antagonism only when the probiotic was cultured in the presence of either another bacterium producing a proteinaceous molecule, or the molecule itself. This molecule was thought to act in effecting the antibacterial activity of BS107. In larval bioassays, BS107 did not enhance survival either in monoculture or when larvae were challenged with *V. pectenicida*. However, BS107 cell extracts did enhance survival of larvae in normal culture, but not when challenged with a pathogen. This suggested that substances produced by live BS107 could have been toxic to the larvae and that BS107 was not effective when high concentrations of the pathogen were used.

Work on the Manila clam, *R. philippinarum*, established *Vibrio* spp. microbiota associated with the mollusc over a one-year period (Castro et al., 2002). The most common species were found to be *V. tubiashii*, *V. splendidus* and *V. harveyi*. In screening isolates against a bacterium, *V. tapetis*, implicated in brown ring disease, they found that five strains of *V. tubiashii* or *V. tubiashii*-like bacteria were able to inhibit the growth of the pathogen. However, the significance of this finding remains to be seen as *V. tubiashii* alone has been shown to be pathogenic to certain bivalves. An interesting observation to come out of this study was that the *in vitro* antagonism by *V. tubiashii* was demonstrated only when the producer was grown on Mueller–Hinton agar highlighting another consideration with *in vitro* based screening.

3.5. Developing probiotics for aquaculture

It has been widely published that a probiotic must possess certain properties (Verschuere et al., 2000a). These properties were proposed in order to aid in correct establishment of new, effective and safe products. The properties include:

1. the probiotic should not be harmful to the host it is desired for,
2. it should be accepted by the host, e.g. through ingestion and potential colonization and replication within the host,
3. it should reach the location where the effect is required to take place,
4. it should actually work *in vivo* as opposed to *in vitro* findings,
5. it should preferably not contain virulence resistance genes or AB resistance genes.

The list of these requisites is given to allow step-wise examination of potential probiotics. However, the sum of many of these properties could be tested quickly via *in vivo* experimentation with the target animal. In essence, these properties are describing one simple question, "does the potential probiotic provide an overall health benefit when given to the animal?"

It was stated previously that there are inherent limitations with the past and current *in vitro* screening procedures and problems with changing the initial screening phase to *in vivo* experiments. Despite this, the possibility of being able to answer the question "does the potential probiotic provide a health benefit when given to the animal?" in the screening phase offers great simplicity, directness and an all-encompassing allowance for probiotics acting by any mode of probiotic action to be identified. For these reasons, the prospect of including test animals in initial screening by means of challenge tests is very appealing. Such a screening model was recently described while using nematodes to screen for antimicrobials (Bhavsar and Brown, 2006). Twenty-five compounds were found to be effective in promoting nematode survival. In addition, they were shown to act by different mechanisms that may have been overlooked in a more classical screening procedure. Future research into novel probiotics for aquaculture would benefit from adoption of these principles as opposed to a total focus on screening for the production of inhibitory substances.

The future application for probiotics in aquaculture looks bright. There is an ever-increasing demand for aquaculture products and a similar increase in the search for alternatives to antibiotics. The field of probiotics intended for aquacultured animals is now attracting considerable attention and a number of commercial products are available, particularly directed at shrimp larval culture. However, the advent of new probiotic screening techniques that incorporate an initial *in vivo* component will allow for a wider range of bacteria to be identified as probiotics. The successful acquisition of such novel probiotics might also depend on obtaining a better understanding of the microbial ecology of a cultured species as well as restricting the probiotic screens to the bacterial species that share the immediate environment with the cultured species. Probiotic strains that are already adapted, through natural processes, to the dynamics of an aquaculture production system will probably lessen any farm management environmental manipulation practices required to achieve the desired probiotic effect in the final product. Introducing such specifically intended probiotics is bound to favour an increase in the application of probiotics, particularly in mollusc production.

References

Aarestrup, F.M., 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. International Journal of Antimicrobial Agents 12, 279–285.

Acar, J., Casewell, M., Freeman, J., Friis, C., Goossens, H., 2000. Avoparcin and virginiamycin as animal growth promoters: a plea fro science in decision-making. Clinical Microbiology and Infection 6 (9), 477–482.

Akinbowale, O.L., Peng, H., Barton, M.D., 2006. Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. Journal of Applied Microbiology 100, 1103–1113.

Alavandi, S.V., Vijayan, K.K., Santiago, T.C., Poornima, M., Jithendran, K.P., Ali, S.A., Rajan, J.J.S., 2004. Evaluation of *Pseudomonas* sp. PM 11 and

Vibrio fluvalis PM 17 on immune indices of tiger shrimp, *Penaeus monodon*. Fish and Shellfish Immunology 17, 115–120.
Alcaide, E., Blasco, M.D., Esteve, C., 2005. Occurrence of drug-resistant bacteria in two European eel farms. Applied and Environmental Microbiology 71 (6), 3348–3350.
Aubin, J., Gatesoupe, F.-J., Labbé, L., Lebrun, L., 2005. Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research 36, 758–767.
Avendaño, R.E., Riquelme, C.E., 1999. Establishment of mixed probiotics and microalgae as food for bivalve larvae. Aquaculture Research 30, 893–900.
Bairagi, A., Sakar Ghosh, K., Sen, S.K., Ray, A.K., 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International 10, 109–121.
Bairagi, A., Sarkar Ghosh, K., Sen, S.K., Ray, A.K., 2004. Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. Aquaculture Research 35, 436–446.
Barbosa, T.M., Serra, C.R., La Ragione, R.M., Woodward, M.J., Henriques, A.O., 2005. Screening for *Bacillus isolatres* in the broiler gastrointestinal tract. Applied and Environmental Microbiology 71 (2), 968–978.
Barker, K.H., Herson, D.S., 1978. Interactions between the diatom *Thallasiosira pseudonanna* and an associated *Pseudomonad* in a mariculture system. Applied and Environmental Microbiology 35 (4), 791–796.
Bergh, O., Naas, K.E., Harboe, T., 1994. Shift in intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. Canadian Journal of Fisheries and Aquatic Science 51, 1899–1903.
Bhavsar, A.P., Brown, E.D., 2006. The worm turns for antimicrobial discovery. Nature Biotechnology 24 (9), 1098–1100.
Bly, J.E., Quiniou, S.M.-A., Lawson, L.A., Clem, L.W., 1997. Inhibition of *Saprolegnia* pathogenic for fish by *Pseudomonas fluorescens*. Journal of Fish Diseases 20, 35–40.
Bomba, A., Nemcová, R., Mudroňa, D., Guba, P., 2002. The possibilities of potentiating the efficacy of probiotics. Trends in Food Science and Technology 13, 121–126.
Brinkhoff, T., Bach, G., Heidorn, T., Liang, L., Schlingloff, A., Simon, M., 2004. Antibiotic production by a *Roseobacter* clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. Applied and Environmental Microbiology 70 (4), 2560–2565.
Brunt, J., Austin, B., 2005. Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 28, 693–701.
Burgess, J.G., Jordan, E.M., Bregu, M., Mearns-Spragg, A., Boyd, K.G., 1999. Microbial antagonism: a neglected avenue of natural products research. Journal of Biotechnology 70, 27–32.
Carraturo, A., Raieta, K., Ottaviani, D., Russo, G.L., 2006. Inhibition of *Vibrio parahaemolyticus* by a bacteriocin-like inhibitory substance (BLIS) produced by *Vibrio mediterranei* 1. Journal of Applied Microbiology 101, 234–241.
Castagliuolo, I., Riegler, M.F., Valenick, L., LaMont, J.T., Pothoulakis, C., 1999. *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. Infection and Immunity 67 (1), 302–307.
Castro, D., Pujalte, M.J., Lopez-Cortes, L., Garay, E., Borrego, J.J., 2002. Vibrios isolated from the cultured manila clam (*Ruditapes philippinarum*): numerical taxonomy and antibacterial activities. Journal of Applied Microbiology 93, 438–447.
Chabrillón, M., Rico, R.M., Arijo, S., Díaz-Rosales, P., Balebonz, M.C., Moriñigo, M.A., 2005. Interactions of microorganisms isolated from gilthead sea bream, *Sparus aurata* L., on *Vibrio harveyi*, a pathogen of farmed Senegalese sole, *Solea senegalensis* (Kaup). Journal of Fish Diseases 28, 531–537.
Chabrillón, M., Arijo, S., Díaz-Rosales, P., Balebonz, M.C., Moriñigo, M.A., 2006. Interference of *Listonella anguillarum* with potential probiotic microorganisms isolated from farmed gilthead seabream (*Sparus aurata*, L.). Aquaculture Research 37, 78–86.
Chang, C.-I., Liu, W.-Y., 2002. An evaluation of two probiotic bacterial strains, *Enterococcus faecium* SF68 and *Bacillus toyoi*, for reducing edwardsiellosis in cultured European eel, *Anguilla anguilla* L. Journal of Fish Diseases 25, 311–315.
Chelossi, E., Vezzulli, L., Milano, A., Branzoni, M., Fabiano, M., Riccardi, G., Banat, I.M., 2003. Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. Aquaculture 219, 83–97.
Cherif, A., Ouzari, H., Daffonchio, D., Cherif, H., Ben Slama, K., Hassen, A., Jaoua, S., Boudabous, A., 2001. Thuricin 7: a novel bacteriocin produced by *Bacillus thuringiensis* BMG1.7, a new strain isolated from soil. Letters in Applied Microbiology 32, 243–247.
Chythanya, R., Karunasagar, I., Karunasagar, I., 2002. Inhibition of shrimp pathogenic vibrios by a marine *Pseudomonas* I-2 strain. Aquaculture 208, 1–10.
Cladera-Olivera, F., Caron, G.R., Brandelli, A., 2004. Bacteriocin-like substance production by *Bacillus licheniformis* strain P40. Letters in Applied Microbiology 38, 251–256.
Corcoran, B.M., Ross, R.P., Fitzgerald, G.F., Stanton, C., 2004. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. Journal of Applied Microbiology 96, 1024–1039.
Dahan, S., Dalmasso, G., Imbert, V., Peyron, J.-F., Rampal, P., Czerucka, D., 2003. *Saccharomyces boulardii* interferes with enterohemorrhagic *Escherichia coli*-induced signaling pathways in T84 cells. Infection and immunity 71 (2), 766–773.
Das, B.K., Samal, S.K., Samantaray, B.R., Sethi, S., Pattnaik, P., Misha, B.K., 2006. Antagonistic activity of cellular components of *Pseudomonas* species against *Aeromonas hydrophila*. Aquaculture 253, 17–24.
Decamp, O., Moriarty, D.J.W., 2006. Probiotics as alternative to antimicrobials: limitations and potential. World Aquaculture 37 (4), 60–62.
Delsol, A.A., Randall, L., Cooles, S., Woodward, M.J., Sunderland, J., Roe, J.M., 2005. Effect of the growth promoter avilamycin on emergence and persistence of antimicrobial resistance in enteric bacteria in the pig. Journal of Applied Microbiology 98, 564–571.
Direkbusarakom, S., Yoshimizu, M., Ezura, Y., Ruangpan, L., Danayadol, Y., 1998. *Vibrio* spp., the dominant flora in shrimp hatchery against some fish pathogenic viruses. Journal of Marine Biotechnology 6, 266–267.
Dopazo, C.P., Lemos, M.L., Lodeiros, C., Bolinches, J., Barja, J.L., Toranzo, A.E., 1988. Inhibitory acitivy of antibiotic-producing marine bacteria against fish pathogens. Journal of Applied Bacteriology 65, 97–101.
Douillet, P.A., 2000a. Bacterial additives that consistently enhance rotifer growth under synxenic culture conditions 1. Evaluation of commercial products and pure isolates. Aquaculture 182, 249–260.
Douillet, P.A., 2000b. Bacterial additives that consistently enhance rotifer growth under synxenic culture conditions 2. Use of single and multiple bacterial probiotics. Aquaculture 182, 241–248.
Douillet, P., Langdon, C.J., 1993. Effects of marine bacteria on the culture of axenic oyster *Crassostrea gigas* (Thunberg) larvae. The Biological Bulletin 184, 36–51.
Douillet, P.A., Langdon, C.J., 1994. Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture 119, 25–40.
Duc, L.H., Hong, H.A., Barbosa, T.M., Henriques, A.O., Cutting, S.M., 2004. Characterization of *Bacillus* probiotics available for human use. Applied and Environmental Microbiology 70 (4), 2161–2171.
Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., Morrissey, D., Thornton, G., Fitzgerald, G., Shanahan, F., Collins, J.K., 1999. Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie van Leeuwenhoek 76, 279–292.
Elston, R., Humphrey, K., Gee, A., Cheney, D., Davis, J., 2004. Progress in the development of effective probiotic bacteria for bivalve shellfish hatcheries and nurseries. Journal of Shellfish Research (Abstracts) 23 (2), 654.
FAO Fishery Information, Data and Statistics Unit (FIDI) c2002, 2007. Fishery Statistical Collections. FIGIS Data Collection. FAO-, Rome. Updated March 2007. Available via FIGIS from: http://www.fao.org/figis/servlet/static?dom=collection&xml=global-aquaculture-production.xml.
Fooks, L.J., Fuller, R., Gibson, G.R., 1999. Prebiotics, probiotics and human gut microbiology. International Dairy Journal 9, 53–61.
Fukami, K., Nishijima, T., Hata, Y., 1992. Availability of deep seawater and effects of bacteria isolated from deep seawater on the mass culture of food microalga *Chaetoceros ceratosporum*. Nippon Suisan Gakkaishi 58 (5), 931–936.
Fukami, K., Nishijima, T., Ishida, Y., 1997. Stimulative and inhibitory effects of bacteria on the growth of microalgae. Hydrobiologia 358, 185–191.

Fuller, R., 1989. Probiotics in man and animals. Journal of Applied Bacteriology 66, 365–378.

Gatesoupe, F.-J., 1991. The effect of three strains of lactic 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., 2002. Probiotic and formaldehyde treatments of *Artemia* nauplii as food for larval pollack, *Pollachius pollachius*. Aquaculture 212, 347–360.

Garcia, T., Otto, K., Kjelleberg, S., Nelson, D.R., 1997. Growth of *Vibrio anguillarum* in salmon intestinal mucus. Applied and Environmental Microbiology 63 (3), 1034–1039.

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

Gildberg, A., Johansen, A., Bøgwald, 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., Mikkelsen, H., Sandaker, E., Ringø, 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.

Gil-Turnes, M.S., Hay, M.E., Fenical, W., 1989. Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science 246, 116–118.

Gismondo, M.R., Drago, L., Lombardi, A., 1999. Review of probiotics available to modify gastrointestinal flora. International Journal of Antimicrobial Agents 12, 287–292.

Gomez-Gil, B., Rogue, A., Velasco-Blanco, G., 2002. Culture of *Vibrio alginolyticus* C7b, a potential probiotic bacterium, with the microalga *Chaetoceros muelleri*. Aquaculture 211, 43–48.

Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I., Nielsen, T.F., 1999. Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. Applied and Environmental Microbiology 65 (3), 969–973.

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

Gullian, M., Thompson, F., Rodriguez, J., 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture 233, 1–14.

Hagi, T., Tanaka, D., Iwamura, Y., Hoshino, T., 2004. Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. Aquaculture 234, 335–346.

Haines, K.C., Guillard, R.R.L., 1974. Growth of vitamin B_{12} requiring diatoms in mixed laboratory cultures with vitamin B_{12} producing marine bacteria. Journal of Phycology 10, 245–252.

Hansen, G.H., Olafsen, J.A., 1999. Bacterial interactions in early life stages of marine cold water fish. Microbial Ecology 38, 1–26.

Heckman, R., 2004. What else can happen? Other problems for fish production. Aquaculture Magazine 30 (3), 27–40.

Hirata, H., Murata, O., Yamada, S., Ishitani, H., Wachi, M., 1998. Probiotic culture of the rotifer *Brachionus plicatilis*. Hydrobiologia 387/388, 495–498.

Hjelm, M., Bergh, Ø., Riaza, A., Nielsen, J., Melchiorsen, J., Jensen, S., Duncan, H., Ahrens, P., Birbeck, H., Gram, L., 2004a. Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. Systematic and Applied Microbiology 27, 360–371.

Hjelm, M., Riaza, A., Formoso, F., Melchiorsen, J., Gram, L., 2004b. Seasonal incidence of autochthonous antagonistic *Roseobacter* spp. and *Vibrionaceae* strains in a turbot larva (*Scophthalmus maximus*) rearing system. Applied and Environmental Microbiology 70 (12), 7288–7294.

Hong, H.A., Duc, L.H., Cutting, S.M., 2005. The use of bacterial spore formers as probiotics. FEMS Microbiology Reviews 29, 813–835.

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

Irianto, A., Austin, B., 2002a. Probiotics in aquaculture. Journal of Fish Diseases 25, 633–642.

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

Irianto, A., Austin, B., 2003. Use of dead probiotic cells to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 26, 59–62.

Irianto, A., Robertson, P.A.W., Austin, B., 2003. Oral administration of formalin-inactivated cells of *Aeromonas hydrophila* A3-51 controls infection by atypical *A. salmonicida* in goldfish, *Carassius auratus* (L.). Journal of Fish Diseases 26, 117–120.

Isolauri, E., Salminen, S., Ouwehand, A.C., 2004. Probiotics. Best Practice and Research in Clinical Gastroenterology 18 (2), 299–313.

Itami, T., Asano, M., Tokushige, K., Kubono, K., Nakagawa, A., Noboru, T., Nishimura, H., Maeda, M., Kondo, M., Takahashi, Y., 1998. Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. Aquaculture 164, 277–288.

Ivanova, E.P., Nicolau, D.V., Yumoto, N., Taguchi, T., Okamoto, K., Tatsu, Y., Yoshikawa, S., 1998. Impact of conditions of cultivation and adsorption on antimicrobial activity of marine bacteria. Marine Biology 130, 545–551.

Jöborn, A., Olsson, J.C., Westerdahl, A., Conway, P.L., 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. Journal of Fish Diseases 20, 383–392.

Jorquera, M.A., Riquelme, C.E., Loyola, L.A., Muñoz, L.F., 1999. Production of bactericidal substances by a marine vibrio isolated from cultures of the scallop *Argopecten purpuratus*. Aquaculture International 7, 433–448.

Jorquera, M.A., Silva, F.R., Riquelme, C.E., 2001. Bacteria in the culture of the scallop *Argopecten purpuratus* (Lamarck, 1819). Aquaculture International 9, 285–303.

Kamei, Y., Yoshimizu, M., Ezura, Y., Kimura, T., 1988. Screening of bacteria with antiviral activity from fresh water salmonid hatcheries. Microbiology and Immunology 32 (1), 67–73.

Karunasagar, I., Pai, R., Malathi, G.R., Karunasagar, I., 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture 128, 203–209.

Kawano, Y., Nagawa, Y., Nakanishi, H., Nakajima, H., Matsuo, M., Higashihara, T., 1997. Production of thiotropocin by a marine bacterium, *Caulobacter* sp. and its antimicroalgal activites. Journal of Marine Biotechnology 5, 225–229.

Klewicki, R., Klewicka, E., 2004. Antagonistic activity of lactic acid bacteria as probiotics against selected bacteria of the Enterobaceriacae family in the presence of polyols and their galactosyl derivatives. Biotechnology Letters 26, 317–320.

Krovacek, K., Faris, A., Ahne, W., Månsson, I., 1987. Adhesion of *Aeromonas hydrophila* and *Vibrio anguillarum* to fish cells and to mucus-coated glass slides. FEMS Microbiology Letters 42, 85–89.

Kumar, R., Mukherjee, S., Prasad, K.P., Pal, A.K., 2006. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). Aquaculture Research 37, 1215–1221.

Lara-Flores, M., Olvera-Novoa, M.A., Guzmán-Méndez, B.E., López-Madrid, W., 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216, 193–201.

Lategan, M.J., Gibson, L.F., 2003. Antagonistic activity of *Aeromonas* media strain A199 against *Saprolegnia* sp., an opportunistic pathogen of the eel, *Anguilla australis* Richardson. Journal of Fish Diseases 26, 147–153.

Lategan, M.J., Torpy, F.R., Gibson, L.F., 2004a. Biocontrol of saprolegniosis in silver perch *Bidyanus bidyanus* (Mitchell) by *Aeromonas* media strain A199. Aquaculture 235, 77–88.

Lategan, M.J., Torpy, F.R., Gibson, L.F., 2004b. Control of saprolegniosis in the eel *Anguilla australis* Richardson, by *Aeromonas* media strain A199. Aquaculture 240, 19–27.

Lategan, M.J., Booth, W., Shimmon, R., Gibson, L.F., 2006. An inhibitory substance produced by *Aeromonas* media A199, an aquatic probiotic. Aquaculture 254, 115–124.

Lemos, M.L., Dopazo, C.P., Toranzo, A.E., Barja, J.L., 1991. Competitive dominance of antibiotic-producing marine bacteria in mixed cultures. Journal of Applied Bacteriology 71, 228–232.

Li, J., Tan, B., Mai, K., Ai, Q., Zhang, W., Xu, W., Liufu, Z., Ma, H., 2006. Comparative study between probiotic bacterium Arthrobacter XE-7 and chloramphenicol on protection of *Penaeus chinensis* post-larvae from pathogenic vibrios. Aquaculture 253, 140–147.

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

Macey, B.M., Coyne, V.E., 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. Aquaculture 245, 249–261.

Maeda, M., Nogami, K., Kanematsu, M., Hirayama, K., 1997. The concept of biological control methods in aquaculture. Hydrobiologia 358, 285–290.

Makridis, P., Fjellheim, A.J., Skjermo, J., Vadstein, O., 2000. Colonization of the gut in first feeding turbot by bacterial strains added to the water or bioencapsulated in rotifers. Aquaculture International 8, 367–380.

Makridis, P., Martins, S., Vercauteren, T., Van Driessche, K., Decamp, O., Dinis, M.T., 2005. Evaluation of candidate probiotic strains for gilthead sea bream larvae (*Sparus aurata*) using an *in vivo* approach. Letter in Applied Microbiology 40, 274–277.

Maroni, K., 2000. Monitoring and regulation of marine aquaculture in Norway. Journal of Applied Ichthyology 16, 192–195.

Martin, A., 2005. Groups Ask FDA to Keep Some Antibiotics out of Animal Feed. Chicago Tribune, 8/4/2005, p. 21.

McIntosh, D., Samocha, T.M., Jones, E.R., Lawrence, A.L., McKee, D.A., Horowitz, S., Horowitz, A., 2000. The effect of a commercial bacterial supplement on the high-density culturing of *Litopenaeus vannamei* with a low-protein diet in an outdoor tank system and no water exchange. Aquaculture Engineering 21, 215–227.

Metchnikoff, E., 1907. The Prolongation of Life. Optimistic Studies. William Heinemann, London.

Meunpol, O., Lopinyosiri, K., Menasveta, P., 2003. The effects of ozone and probiotics on the survival of black tiger shrimp (*Penaeus monodon*). Aquaculture 220, 437–448.

Molina-Aja, A., Garcia-Gasca, A., Abreu-Grobois, A., Bolan-Mejia, C., Roque, A., Gomez-Gil, B., 2002. Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. FEMS Microbiology Letters 213, 7–12.

Mombelli, B., Gismondo, M.R., 2000. The use of probiotics in medicinal practice. International Journal of Antimicrobial Agents 16, 531–536.

Moriarty, D.J.W., 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164, 351–358.

Moriarty, D.J.W., 1999. Disease control in shrimp aquaculture with probiotic bacteria. In: Bell, C.R., Brylinsky, M., Johnson-Green, P. (Eds.), Microbial Biosystems: New Frontiers: Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

Naviner, M., Berge, J.-P., Durand, P., Le Bris, H., 1999. Antibacterial activity of the marine diatom *Skeletonema costatum* against aquacultural pathogens. Aquaculture 174, 15–24.

Nawaz, M.S., Erickson, B.D., Khan, A.A., Khan, S.A., Pothuluri, J.V., Rafii, F., Sutherland, J.B., Wagner, R.D., Cerniglia, C.E., 2001. Human health impact and regulatory issues involving antimicrobial resistance in the food animal production environment. Regulatory Research Perspectives 1 (1), 1–10.

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

Nikoskelainen, S., Salminen, S., Bylund, G., Ouwehand, A.C., 2001b. Characterization of the properties of human- and dairy-derived probiotics for the prevention of infectious diseases in fish. Applied and Environmental Microbiology 67 (6), 2430–2435.

Nikoskelainen, S., Ouwehand, A.C., Bylund, G., Salminen, S., Lilius, E.-M., 2003. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish and Shellfish Immunology 15, 443–452.

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

Olsson, J.C., Westerdahl, A., Conway, P.L., Kjelleberg, S., 1992. Intestinal colonization potential of turbot (*Scophthalmus maximus*) and dab (*Limanda limanda*)-associated bacteria with inhibitory effects against *Vibrio anguillarum*. Applied and Environmental Microbiology 58 (2), 551–556.

Orozco-Medina, C., Maeda-Martínez, M., López-Cortés, A., 2002. Effect of aerobic Gram-positive heterotrophic bacteria associated with *Artemia Franciscana* cysts on the survival and development of its larvae. Aquaculture 213, 15–29.

Ouwehand, A.C., Salminen, S., Isolauri, E., 2002. Probiotics: an overview of beneficial effects. Antonie van Leewenhoek 82, 279–289.

Panigrahi, A., Kiron, V., Kobayashi, T., Puangkaew, J., Satoh, S., Sugita, H., 2004. Immune response in the rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Veterinary Immunology and Immunopathology 102, 379–388.

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

Patra, S.K., Mohamed, K.S., 2003. Enrichment of *Artemia* nauplii with the probiotic yeast *Saccharomyces boulardii* and its resistance against a pathogenic *Vibrio*. Aquaculture International 11, 505–514.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R., Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy 53, 28–52.

Planas, M., Vázquez, J.A., Marqués, J., Pérez-Lomba, R., González, M.P., Murado, M., 2004. Enhancement of rotifer (*Branchionus plicatilis*) growth by using terrestrial lactic acid bacteria. Aquaculture 240, 313–329.

Raida, M.K., Larsen, J.L., Nielsen, M.E., Buchmann, K., 2003. Enhanced resistance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against *Yersinia ruckeri* challenge following oral administration of *Bacillus subtilis* and *B. licheniformis* (BioPlus2B). Journal of Fish Diseases 26, 495–498.

Ramirez, R.F., Dixon, B.A., 2003. Enzyme production by obligate intestinal anaerobic bacteria isolated from Oscars (*Astronotus ocellatus*), angelfish (*Pterophyllum scalare*) and southern flounder (*Paralichthys lethostigma*). Aquaculture 227, 417–426.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S., Menasveta, P., 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture 167, 301–313.

Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S., Menasaveta, P., 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquaculture 191, 271–288.

Rico-Mora, R., Voltolina, D., Villaescusa-Celaya, J.A., 1998. Biological control of *Vibrio alginolyticus* in *Skeletonema costatum* (Bacillariophyceae) cultures. Aquacultural Engineering 19, 1–6.

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

Riquelme, C., Hayashida, G., Araya, R., Uchida, A., Satomi, M., Ishida, Y., 1996. Isolation of a native bacterial strain from the scallop *Argopecten purpuratus* with inhibitory effects against pathogenic vibrios. Journal of Shellfish Research 15 (2), 369–374.

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

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

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

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

Ross, R.P., Desmond, C., Fitzgerald, G.F., Stanton, C., 2005. Overcoming the technological hurdles in the development of probiotic foods. Journal of Applied Microbiology 98, 1410–1417.

Ruiz-Ponte, C., Samain, J.F., Sánchez, J.L., Nicolas, J.L., 1999. The benefit of a *Roseobacter* species on the survival of scallop larvae. Marine Biotechnology 1, 52–59.

Sahul Hameed, A.S., Rahaman, K.H., Alagan, A., Yoganandhan, K., 2003. Antibiotic resistance in bacteria isolated from hatchery-reared larvae and post-larvae of *Macrobranchium rosenbergii*. Aquaculture 217, 39–48.

Salminen, S., Ouwehand, A., Benno, Y., Lee, Y.K., 1999. Probiotics: how should they be defined. Trends in Food Science and Technology 10, 107–110.

Schwarz, S., Kehrenberg, C., Walsh, T.R., 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International Journal of Antimicrobial Agents 17, 431–437.

Senok, A.C., Ismaeel, A.Y., Botta, G.A., 2005. Probiotics: facts and myths. Clinical Microbiology and Infection 11 (12), 958–966.

Shiri Harzevili, A.R., Van Duffel, H., Dhert, P., Swings, J., Sorgeloos, P., 1998. Use of a potential probiotic *Lactococcus lactis* AR21 strain for the enhancement of growth in the rotifer *Brachionus plicatilis* (Müller). Aquaculture Research 29, 411–417.

Smith, V.J., Brown, J.H., Hauton, C., 2003. Immunostimulation in crustaceans: does it really protect against infection? Fish and Shellfish Immunology 15, 71–90.

Son, R., Rusul, G., Sahilah, A.M., Zainuri, A., Raha, A.R., Salmah, I., 1997. Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Telapia (*Telapia mossambica*). Letters in Applied Microbiology 24, 479–482.

Spanggaard, B., Huber, I., Nielsen, J., Sick, E.B., Pipper, C.B., Martinussen, T., Slierendrecht, W.J., Gram, L., 2001. The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. Environmental Microbiology 3 (12), 755–765.

Stoffels, G., Nes, I.F., Guðmundsdóttir, Á., 1992. Isolation and properties of a bacteriocin-producing *Carnobacterium piscicola* isolated from fish. Journal of Applied Bacteriology 73, 309–316.

Sugita, H., Matsuo, N., Shibuya, K., Deguchi, Y., 1996a. Production of antibacterial substances by intestinal bacteria isolated from coastal crab and fish species. Journal of Marine Biotechnology 4, 220–223.

Sugita, H., Shibuya, K., Shimooka, H., Deguchi, Y., 1996b. Antibacterial abilities of intestinal bacteria in freshwater cultured fish. Aquaculture 145, 195–203.

Sugita, H., Matsuo, N., Hirose, Y., Iwato, M., Deguchi, Y., 1997a. *Vibrio* sp. strain NM 10, isolated from the intestine of a Japanese coastal fish, has an inhibitory effect against *Pasteurella piscicida*. Applied and Environmental Microbiology 63 (12), 4986–4989.

Sugita, H., Shibuya, K., Hanada, H., Deguchi, Y., 1997b. Antibacterial abilities of intestinal microflora of the river fish. Fisheries Science 63 (3), 379–383.

Sugita, H., Hirose, Y., Matsuo, N., Deguchi, Y., 1998. Production of the antibacterial substance by *Bacillus* sp. strain NM 12, an intestinal bacterium of Japanese coastal fish. Aquaculture 165, 269–280.

Sugita, H., Okano, R., Suzuki, Y., Iwai, D., Mizukami, M., Akiyama, N., Matsuura, S., 2002. Antibacterial abilities of intestinal bacteria from larval and juvenile Japanese flounder against fish pathogens. Fisheries Science 68, 1004–1011.

Sullivan, A., Nord, C.E., 2002. The place of probiotics in human intestinal infections. International Journal of Antimicrobial Agents 20, 313–319.

Suminto Hirayama, K., 1996. Effects of bacterial coexistence on the growth of a marine diatom *Chaetoceros gracilis*. Fisheries Science 62 (1), 40–43.

Suminto Hirayama, K., 1997. Application of a growth-promoting bacteria for stable mass culture of three marine microalgae. Hydrobiologia 358, 223–230.

Taoka, Y., Maeda, H., Jo, J.-Y., Kim, S.-M., Park, S.-I., Yoshikawa, T., Sakata, T., 2006. Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. Fisheries Science 72, 755–766.

Timmerman, H.M., Koning, C.J.M., Mulder, L., Rombouts, F.M., Beynen, A.C., 2004. Monostrain, multistrain and multispecies probiotics — a comparison of functionality and efficacy. International Journal of Food Microbiology 96, 219–233.

Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F.J., Vázquez-Juárez, R., Lésel, R., 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 204, 113–123.

Turnidge, J., 2004. Antibiotic use in animals — prejudices, perceptions and realities. Journal of Antimicrobial Chemotherapy 53, 26–27.

Ukeles, R., Bishop, J., 1975. Enhancement of phytoplankton growth by marine bacteria. Journal of Phycology 11, 142–149.

Van den Bogaard, A.E., Stobberingh, E.E., 2000. Epidemiology of resistance to antibiotics: links between animals and humans. International Journal of Antimicrobial Agents 14, 327–335.

Van der Aa Kühle, A., Skovgaard, K., Jespersen, L., 2005. *In vitro* screening of probiotic properties of *Saccharomyces cerevisiae* var. boulardii and food-borne *Saccharomyces cerevisiae* strains. International Journal of Food Microbiology 101, 29–39.

Vaughan, E.E., de Vries, M.C., Zoetendal, E.G., Ben-Amor, K., Akkermans, A.D.L., de Vos, W.M., 2002. The intestinal LABs. Antonie van Leeuwenhoek 82, 341–352.

Vaseeharan, B., Ramasamy, P., 2003. Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. Letters in Applied Microbiology 36, 83–87.

Vaseeharan, B., Lin, J., Ramasamy, P., 2004. Effect of probiotics, antibiotic sensitivity, pathogenicity, and plasmid profiles of *Listonella anguillarum*-like bacteria isolated from *Penaeus monodon* culture systems. Aquaculture 241, 77–91.

Venkat, H.K., Sahu, N.P., Jain, K.K., 2004. Effect of feeding *Lactobacillus*-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobranchium rosenbergii* (de Man). Aquaculture Research 35, 501–507.

Verschuere, L., Rombaut, G., Huys, G., Dhont, J., Sorgeloos, P., Verstraete, W., 1999. Microbial control of the culture of *Artemia* juveniles through pre-emptive colonization by selected bacterial strains. Applied and Environmental Microbiology 65, 2527–2533.

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., 2000a. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Review 64, 655–671.

Verschuere, L., Heang, H., Criel, G., Sorgeloos, P., Verstraete, W., 2000b. Selected bacterial strains protect *Artemia* spp. from the pathogenic effects of *Vibrio proteolyticus* CW8T2. Applied and Environmental Microbiology 66 (3), 1139–1146.

Vijayan, K.K., Singh, I.S.B., Jayaprakash, N.S., Alavandi, S.V., Pai, S.S., Preetha, R., Rajan, J.J.S., Santiago, T.C., 2006. A brackishwater isolate of *Pseudomonas* PS-102, a potential antagonistic bacterium against pathogenic vibrios in penaeid and non-penaeid rearin systems. Aquaculture 251, 192–200.

Villamil, L., Figueras, A., Planas, M., Novoa, B., 2003. Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. Aquaculture 219, 43–56.

Vine, N.G., Leukes, W.D., Kaiser, H., Daya, S., Baxter, J., Hecht, T., 2004a. Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. Journal of Fish Diseases 27, 319–326.

Vine, N.G., Leukes, W.D., Kaiser, H., 2004b. *In vitro* growth characteristics of five candidate aquaculture probiotics and two fish pathogens grown in fish intestinal mucus. FEMS Microbiology Letters 231, 145–152.

Westerdahl, A., Olsson, J.C., Kjelleberg, S., Conway, P.L., 1991. Isolation and characterization of turbot (*Scophtalmus maximus*)-associated bacteria with inhibitory effects against *Vibrio anguillarum*. Applied and Environmental Microbiology 57 (8), 2223–2228.

Wierup, M., 2001. The experience of reducing antibiotics used in animal production in the Nordic countries. International Journal of Antimicrobial Agents 18, 287–290.

Witte, W., 2000. Selective pressure by antibiotic use in livestock. International Journal of Antimicrobial Agents 16, S19–S24.

Yan, L., Boyd, K.G., Burgess, J.G., 2002. Surface attachment induced production of antimicrobial compounds by marine epiphytic bacteria using modified roller bottle cultivation. Marine Biotechnology 4, 356–366.

Ziemer, C.J., Gibson, G.R., 1998. An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. International Dairy Journal 8, 473–479.