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Review

Probiotics in aquaculture

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Abstract

Probiotics, which are micro-organisms or their products with health benefit to the host, have found use in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds. A wide range of microalgae (*Tetraselmis*), yeasts (*Debaryomyces*, *Phaffia* and *Saccharomyces*) and Gram-positive (*Bacillus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus* and *Weissella*) and Gram-negative bacteria (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas* and *Vibrio*) has been evaluated. However, the mode of action of the probiotics is rarely investigated, but possibilities include competitive exclusion, i.e. the probiotics actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and/or space, alteration of microbial metabolism, and/or by the stimulation of host immunity. Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible components. There is accumulating evidence that probiotics are effective at inhibiting a wide range of fish pathogens, but the reasons for the inhibitions are often unstated.

Keywords: bacteria, crustacea, disease control, finfish, microalgae, probiotics, yeasts.

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The definition of a probiotic

What is a probiotic? A widely accepted definition is taken from Fuller (1987), who considered that a probiotic is a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal (microbial) balance. The important components of this definition reflect the need for a living micro-organism and application to the host as a feed supplement. However, other workers have broadened the definition. For example, Gram, Melchiorsen, Spanggaard, Huber & Nielsen (1999) proposed that a probiotic is any live microbial supplement, which beneficially affects the host animal by improving its microbial balance. In this example, there is no association with feed. Furthermore, Salminen, Ouwehand, Benno & Lee (1999) considered a probiotic as any microbial (but not necessarily living) preparation or the components of microbial cells with a beneficial effect on the health of the host. Here, the need for live cells in association with feed has been ignored. In short, it is apparent that there are variations in the actual understanding of the term probiotic. Based on the observation that organisms are capable of modifying the bacterial composition of water and sediment, albeit temporarily, Moriarty (1999) suggested that the definition of a probiotic in aquaculture should include the addition of live naturally occurring bacteria to tanks and ponds in which animals live, i.e. the concept of biological control as discussed by Maeda, Nogami, Kanematsu & Hirayama (1997). As a compromise, it would appear that a probiotic is an entire or component(s) of a micro-organism that is beneficial to the health of the host. This

all-embracing concept could impinge on other areas of disease control, particularly vaccinology.

Of course, probiotics must not be harmful to the host (Salminen *et al.* 1999) and they will need to be effective over a range of temperature extremes and variations in salinity (Fuller 1987). Application could be via feed (as implied by the definition of Fuller 1987) or by immersion or injection (as could occur with the definition of Salminen *et al.* 1999). This is where confusion could occur, i.e. what is the distinction between a probiotic applied by injection or immersion, and a vaccine? Any confusion could have legal implications for the registration of probiotics in some countries. Specifically, when licensing/registering probiotics for use in fish should the organisms be considered as feed additives (=probiotic *stricto sensu*) or veterinary products (=vaccines)? Notwithstanding, it is essential to determine whether the benefit of a probiotic is actual or perceived, i.e. could the probiotic really be only a placebo?

It is worth emphasizing that, according to Fuller (1987), a probiotic should provide actual benefit to the host, be able to survive in the digestive tract, be capable of commercialization, i.e. grown on an industrial scale, and should be stable and viable for prolonged storage conditions and in the field.

Probiotics in human and terrestrial animal use

In contrast to aquaculture, probiotics for use in humans and terrestrial animals have centred on use of lactic acid bacteria, particularly representatives of *Bifidobacterium*, *Lactobacillus* and *Streptococcus* (Fuller 1987; Smoragiewicz, Bielecka, Babuchowski, Boutard & Dubeau 1993). Indeed, a common concept of a probiotic is of a beneficial lactic acid bacterium, which is suited for survival in the digestive tract because of tolerance to acidity and bile salts (Fuller 1987; Smoragiewicz *et al.* 1993). Such bacteria may be found in a range of fermented milk products, including buttermilk (e.g. Rodas, Angulo, de la Cruz & Garcia 2002) and yogurt (e.g. Shinohara, Matsumoto, Ushiyama, Wakiguchi, Akasawa & Saito 2002), destined for human consumption. In addition, probiotics are used in poultry (e.g. Fulton, Nersessian & Reed 2002) and cattle (e.g. Khuntia & Chaudhary 2002). In short, probiotics are well established as important for use with humans, poultry and cattle. The involvement of probiotics in aquaculture is comparatively new, but they are quickly becoming recognized as important for disease control.

Probiotics evaluated for use in aquaculture

The range of probiotics examined for use in aquaculture has encompassed both Gram-negative and Gram-positive bacteria, bacteriophages, yeasts and unicellular algae (Table 1). In particular, probiotics have been reported to be successful with a wide range of invertebrates (e.g. Riquelme, Araya, Vergara, Rojas, Guaita & Candia 1997; Araya, Jorquera & Riquelme 1999; Ruiz-Ponte, Samain, Sanchez & Nicolas 1999; Gomez-Gil, Roque & Turnbull 2000; Riquelme, Araya & Escribano 2000) and vertebrates (see Skjermo & Vadstein 1999; Gatesoupe 2000; Makridis, Jon Fjellheim, Skjermo & Vadstein 2000; Verschuere, Rombaut, Sorgeloos & Verstraete 2000; Huys, Dhert, Robles, Ollevier, Sorgeloos & Swings 2001). There is some evidence of host specificity but the significance of the observations awaits further study (Fuller 1992; Salminen, Isolauri & Salminen 1997). To date, probiotics have been used in artificial feed (Robertson, O'Dowd, Burrells, Williams & Austin 2000), live feed, i.e. artemia and rotifers (Gatesoupe 1991; Harzevilli, vanDuffel, Dhert, Swings & Sorgeloos 1998) and in water (Austin, Stuckey, Robertson, Effendi & Griffith 1995; Moriarty 1999; Ringø & Birkbeck 1999).

Gram-positive bacteria

Aerobic Gram-positive endospore-forming bacteria, i.e. *Bacillus* spp., have been evaluated as probiotics, with uses including the improvement of water quality by influencing the composition of waterborne microbial populations and by reducing the number of pathogens in the vicinity of the farmed species (Wang, Ji & Xu 1999). Thus, the bacilli are thought to antagonize potential pathogens in the aquatic environment. This is curious because it is generally accepted that laboratory cultures do not survive well when re-introduced into the natural environment; the cells being often outcompeted/antagonized by the natural microflora (Austin 1988). Nevertheless, a direct benefit to the use of the bacilli was the reduction in the use of chemicals in the aquatic environment and in enhanced growth of the farmed species (Wang *et al.* 1999).

Table 1 Probiotics considered for use in aquaculture

Identity of the probiotic	Source	Used on	Method of application	Reference
Gram-positive bacteria				
Bacillus sp. S11	*Penaeus monodon*	*P. monodon*	Premixed with feed	Rengipat *et al.* (1998)[b]
Bacillus sp. 48	Common snook	*Centropomus undecimalis*	Added to water; reduced salinity	Kennedy *et al.* (1998)[b]
Bacillus sp.	Commercial product	Penaeids	Water	Moriarty (1998)[b,c]
Bacillus sp.	Commercial product	Channel catfish	Spread in pond water	Queiroz Boyd (1998)[b,c]
Bacillus sp.		Water	Added to water	Wayne *et al.* (1999)
Carnobacterium sp. BA211	*Oncorhynchus mykiss* digestive tract	*O. mykiss*	Premix with feed	Irianto & Austin (2002)[a,b]
Carnobacterium inhibens K1	Atlantic salmon intestine	Salmonids	Premix with feed	Jöborn *et al.* (1997)[a,b]
Carnobacterium divergens	Atlantic salmon intestine	*Gadus morhua*	Feed	Gildberg *et al.* (1997)[b]
Enterococcus faecium SF 68	Commercial product	*Anguilla anguilla*	?	Chang & Liu (2002)[b]
Lactobacillus sp.	Tilapia intestine	*Oreochromis niloticus*	Premix with feed	Suyanandana *et al.* (1998)[b]
Lactobacillus helveticus	Turbot larvae	*Scophthalmus maximus*	Indirectly via rotifers	Gatesoupe (1991)[b]
Lactobacillus lactis AR21	Rotifer mass culture	*Brachionus plicatilis*	Feed additive	Harzevili *et al.* (1998)[b]
Lactobacillus plantarum	Turbot larvae	*S. maximus*	Indirectly via rotifers	Gatesoupe (1991)[b]
Lactobacillus rhamnosus ATCC 53103	Culture collection	*O. mykiss*	Mixed with feed	Nikoskelainen *et al.* (2001)[b]
Micrococcus luteus A1-6	*O. mykiss* digestive tract	*O. mykiss*	Premix with feed	Irianto & Austin (2002)[a,b]
Streptococcus thermophilus	Turbot larvae	*S. maximus*	Indirectly via rotifers	Gatesoupe (1991)[b]
Unnamed lactic acid bacteria	Atlantic salmon	*Salmo salar*	Premix with feed	Gildberg *et al.* (1995)[b]
Weissella helenica DS-12	Flounder intestine	*Paralichthys olivaceus*	Premix with feed	Byun *et al.* (1997)[b]
G-probiotic	Commercial product	*O. niloticus*	Premix with feed	Naik *et al.* (1999)[b]
Mixed culture, mostly *Bacillus* spp.	Commercial product	*B. plicatilis*	Mixed with water	Hirata *et al.* (1998)[b]
Gram-negative bacteria				
Aeromonas hydrophila A3-51	*O. mykiss* digestive tract	*O. mykiss*	Premix with feed	Irianto & Austin (2002)[a,b]
Aeromonas media	?	*Crassostrea gigas*	Mixed with water	Gibson *et al.* (1998)[b]
'Alteromonas' CA2	?	*C. gigas*	Mixed with water	Douillet & Langdon (1994)[b]
Photorhodobacterium sp.	?	*Penaeus chinensis*	Mixed in water	Xu, pers. comm.[b]
Pseudomonas fluorescens	*Salmo trutta*	*S. salar*	Bath	Smith & Davey (1993)[b]
Pseudomonas fluorescens	Iced fresh water fish, *Lates niloticus*	*O. mykiss*	Bath for 6 days	Gram *et al.* (1999)[a,b]
Pseudomonas fluorescens AH2	*O. mykiss*	*O. mykiss*	Mixed with water to 10^5 or 10^6 cells mL^{-1}	Gram *et al.* (2001)[a,b]
Pseudomonas sp.	*O. mykiss*	*O. mykiss*	Mixed in water	Spanggaard *et al.* (2001)[a,b]
Roseobacter sp. BS 107	?	Scallop larvae	Mixed in water	Ruiz-Ponte *et al.* (1999)[a,b]
Vibrio alginolyticus	Beach sand	Penaeids, salmonids	Feed, bath for 10 min	Austin *et al.* (1995)[a,b]
Vibrio fluvialis	*O. mykiss* digestive tract	*O. mykiss*	Premix with feed	Irianto & Austin (2002)[a,b]
Bioboost forte (bacteria and yeast)	Commercial product	*Catla catla*	Premix with feed	Mohanty *et al.* (1996)[b]
Bacteriophage				
Representative of (Myoviridae and podoviridae)	?	*Plecoglossus altivelis*	Premix with feed	Park *et al.* (2000)[a,b]
Yeast				
Saccharomyces cerevisiae, S. exiguous, Phaffia rhodozoma	Commercial product	*Litopenaeus vannamei*	Premix with feed	Scholz *et al.* (1999)[b]
Debaryomyces hansenii	*Dicentrarchus labrax* gut	*D. labrax* larvae	Mixed in diet	Tovar *et al.* (2002)[b]
Microalgae				
Tetraselmis suecica	Conmmercial product	Penaeids, *S. salar*	Feed	Austin *et al.* (1992)[a,b,c]

[a] *In vitro* experiments.
[b] *In vivo* studies.
[c] Field experiments.

The use of probiotics has been accompanied by a concomitant reduction in the levels of antimicrobial compounds (particularly antibiotics) used in aquaculture and in improved appetite and/or growth performance of the farmed species. The former is obvious insofar as if the animals are otherwise healthy then there will not be any need to use antimicrobial compounds. However, the inference about improved appetite and growth is more difficult to reconcile. In particular, it is important to determine whether or not the probiotic actually tastes good or does it modify the feed thereby improving digestibility (and taste).

Apart from laboratory preparations of bacteria, some workers have used commercially available products. For example, Queiroz & Boyd (1998) and Moriarty (1998) used commercial preparations containing *Bacillus* spp. in catfish and shrimp ponds, respectively. Hirata, Murata, Yamada, Ishitani & Wachi (1998) used mixed cultures consisting mainly of *Bacillus* spp. to improve the performance of the rotifer *Brachionus plicatilis* in water. Furthermore, Kennedy, Tucker, Neidic, Vermeer, Cooper, Jarrell & Sennett (1998) used *Bacillus* 48 to enhance the quality and viability of common snook, *Centropomus undecimalis* (Bloch). These workers found that *Bacillus* improved the survival of larvae, increased food absorption by enhancing protease levels and gave better growth. Also, the probiotic decreased the number of suspected pathogenic bacteria in the gut. However, there was no evidence of benefit during the 100-day period of an experiment involving the administration of *Bacillus* S11 as wet, i.e. freshly grown, or lyophilized cells, or saline suspensions to penaeids in feed (Rengpipat, Phianphak, Piyatiratitivorakul & Menasveta 1998). It is noteworthy that Chang & Liu (2002) used *Bacillus toyoi* and *Enterococcus faecium* SF 68 from commercial products to reduce edwardsiellosis in European eel, *Anguilla anguilla* (L). The study indicated that *E. faecium* SF68, but not *B. toyoi*, reduced mortalities in eels, and suppressed the growth of *E. tarda in vitro*. It is relevant to note that *E. faecium* has long been known as a probiotic for humans, whereas *B. toyoi* has been used with terrestrial animals. *E. faecium* has also been useful at improving growth when fed to sheat fish, *Silurus glanis* L. Thus, after feeding for 58 days with a dose equivalent to 2×10^8 bacteria g^{-1} of food, the experimental fish achieved better growth (~11%) compared with the controls (Bogut, Milakovic, Brkic, Novoselic & Bukvic 2000). Also, the enterococci influenced the microflora of the intestine, reducing the incidence of *Escherichia coli*, *Staphylococcus aureus* and *Clostridium* spp.

DS-12, which was assigned to *Weissella hellenica* by DNA:DNA hybridization (Cai, Benno, Nakase & Oh 1998), was one of 199 cultures recovered from the intestinal contents of farmed flounder, *Paralichthys olivaceus* (Temminck & Schlegel), in South Korea, and was antagonistic to some bacterial fish pathogens and is regarded to have potential as a probiotic (Byun, Park, Benno & Oh 1997).

Also, an isolate of *Micrococcus luteus* was found to have potential in combating *A. salmonicida* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Irianto & Austin 2002).

The lactic acid producing bacteria, i.e. putative lactobacillli (e.g. Gildberg & Mikkelsen 1998) have been the focus of much interest. As a topical example, Gatesoupe (1991) reported the benefit of using *Lactobacillus plantarum* and *Lactobacillus helveticus* in turbot, *Scophthalmus maximus* (L.), leading to enhanced growth. The human probiotic, *Lactobacillus rhamnosus* ATCC (American Type Culture Collection, Rockville, MD, USA) 53101, was administered at a dose or 10^9 and 10^{12} cells g^{-1} of feed to rainbow trout for 51 days, and reduced mortalities from 52.6 to 18.9% (10^9 cells g^{-1} of feed) and to 46.3% (10^{12} cells g^{-1} of feed) following challenge with *Aeromonas salmonicida* (Nikoskelainen, Ouwehand, Salminen & Bylund 2001). It is apparent that increased dosage is not necessarily reflective of superior protection. In this example, 10^9 cells g^{-1} gave more convincing protection than the comparatively massive dose of 10^{12} cells g^{-1}. It is speculative what might have happened if even lesser numbers of cells were evaluated. In another example, Gildberg, Mikkelsen, Sandaker & Ringø (1997) reported that the administration of *Carnobacterium divergens* to Atlantic cod, *Gadus morhua* L., fry resulted in resistance to *Vibrio anguillarum*. Moreover, Harzevili *et al.* (1998) used *Lactococcus lactis* AR21, which stimulated the growth of rotifers and inhibited *V. anguillarum*. Similarly, encouraging data were obtained by Byun *et al.* (1997) and Suyanandana, Budhaka, Sassanarakkit, Saman, Disayaboot, Cai & Benno (1998) using *Lactobacillus* as feed additives for flounder and tilapia, respectively. Conversely, Gildberg, Johansen & Boegwald (1995) did not find any improvement in using lactic acid bacteria, isolated from salmon intestine, with Atlantic salmon, *Salmo salar* L., fry challenged with *A. salmonicida*.

Jöborn, Olsson, Westerdahl, Conway & Kjelleberg (1997) determined that *Carnobacterium inhibens* K1 (Jöborn, Dorsch, Christer, Westerdahl & Kjelleberg 1999), which was isolated from the gastrointestinal tract of Atlantic salmon, produced inhibitory substances active against bacterial fish pathogens *in vitro*. The results of *in vivo* experiments demonstrated that the bacteria were metabolically active in both intestinal mucus and the faeces of salmonids. Moreover, there was no evidence of any detrimental effect on the host (Robertson *et al.* 2000). The value of *Carnobacterium* K was verified by Robertson *et al.* (2000), who demonstrated antagonism against a wide range of fish pathogens and confirmed efficacy at reducing mortalities in salmonids caused by *A. salmonicida*, *V. ordalii* and *Yersinia ruckeri*. Moreover, it was apparent that recipient fish showed enhanced appetite and fared better compared with the controls, i.e. there was less evidence of minor health problems such as fin and tail rot (Robertson *et al.* 2000).

Aerobic heterotrophic bacteria from the digestive tract of Atlantic salmon, rainbow trout and turbot were examined for inhibitory activity against *A. salmonicida* (Irianto & Austin 2002) using a cross-streaking method, which produced zones of clearing in and overgrowth of *A. salmonicida* (Robertson *et al.* 2000). Inhibitory cultures were checked for purity and applied to rainbow trout feed to achieve a dosage of 10^7 cells g^{-1} of food (Robertson *et al.* 2000). Groups of rainbow trout were fed on demand with modified diets for 7 and 14 days, after which the fish were challenged with a virulent culture of *A. salmonicida*. An isolate identified as *Carnobacterium* sp. did not reveal any harmful effects when injected intramuscularly and intraperitoneally at 10^7 cells $fish^{-1}$ into groups of rainbow trout. Moreover, within 1 day of application in food, fish demonstrated enhanced feeding activity. Indeed, there was a virtual feeding frenzy. Challenge with *A. salmonicida* led to a marked reduction in mortalities compared with controls (Irianto & Austin 2002). Subsequent work pointed to the success of this organism at controlling *A. salmonicida* infections in rainbow trout fry and fingerlings (Irianto & Austin 2002).

Gram-negative bacteria

Pseudomonas fluorescens has been reported to inhibit *Saprolegnia* sp. and *A. salmonicida* in finfish culture (Smith & Davey 1993; Bly, Quiniou, Lawson & Clem 1997), and *Pseudomonas* I-2 antagonized shrimp pathogenic *V. harveyi*, *V. fluvialis*, *V. parahaemolyticus*, *V. vulnificus* and *Photobacterium damselae* by means of low molecular weight inhibitors (Chythanya, Karunasagar & Karunasagar 2002). Moreover, Gram *et al.* (1999) determined that bathing rainbow trout for 6 days in *P. fluorescens* AH2, which was isolated from *Lates niloticus* (L.), reduced mortality from 47 to 32% following challenge with *V. anguillarum*. In a large-scale investigation, Spanggaard, Huber, Nielsen, Sick, Pipper, Martinussen, Slierendrecht & Gram (2001) recovered 1018 bacterial and yeast isolates from the skin, gills and intestine of rainbow trout. Of these, 45 isolates were inhibitory to *V. anguillarum* in a disc diffusion assay. The dominant antagonist was *Pseudomonas*, which improved the survival of rainbow trout against vibriosis following the addition of cultures to water. Yet, *P. fluorescens* AH2, which was regarded as an effective probiotic for rainbow trout conferring protection against vibriosis, did not protect Atlantic salmon against infection with *A. salmonicida* despite *in vitro* methods indicating inhibition of the pathogen (Gram, Lovold, Nielsen, Melchiorsen & Spanggaard 2001). Here, inhibition was enhanced in iron-depleted conditions. Thus, in the case of Atlantic salmon, there was a lack of correlation between the results of *in vitro* and *in vivo* studies.

Other bacteria have improved the culture of larval crab, Pacific oyster and turbot (Nogami & Maeda 1992; Douillet & Langdon 1994; Gatesoupe 1994). Thus, *V. proteolyticus* improved protein digestion in juvenile turbot when administered by oral intubation (DeSchrijver & Ollevier 2000). Also, Douillet & Langdon (1994) showed that strain CA2, which was probably an *Alteromonas*, increased the survival of Pacific oyster, *Crassostrea gigas*, when administered in water. Moreover, Gibson, Woodworth & George (1998) and Gibson (1999) noted that *A. media* A199 controlled infection by *V. tubiashii* in Pacific oyster larvae. The culture produced bacteriocin-like inhibitory substances against several pathogenic bacteria in culture media. Irianto & Austin (2002) reported that cultures of *A. hydrophila* and *V. fluvialis* were effective at controlling infections by *A. salmonicida* in rainbow trout. In addition, Ruiz-Ponte *et al.* (1999) found that *Roseobacter* (BS 107) in co-culture with *V. anguillarum*, was inhibitory to *Vibrio*, with cell extracts (of BS107) enhancing the survival of larval scallop (Ruiz-Ponte *et al.* 1999).

Since 1995, there has been success with the use of probiotics in the Ecuadorian shrimp industry, specifically to control the high incidence of larval diseases. A beneficial outcome has been a reported reduction in the use of antibiotics during larval rearing (Garriques & Arevalo 1995; Garriques, personal communication, 1995). It would appear that the probiotics have been isolated from sea water on plates of thiosulphate citrate bile salt sucrose agar (=cholera agar), with preliminary studies indicating that useful isolates produced round yellow colonies of 3–5 mm in diameter. These colonies were identified as *V. alginolyticus* by the API 20E rapid identification system (Bio-Mériéux) (Garriques, personal communication, 1995). Indeed, Vandenberghe, Verdonck, Robles-Arozarena, Rivera, Bolland, Balladares, Gomez-Gil, Calderon, Sorgeloos & Swings (1999) reported that 23 isolates corresponded with the definition of *V. alginolyticus*, with others being *Vibrio* spp. Austin *et al.* (1995) found that one of these isolates of *V. alginolyticus* inhibited a range of bacterial fish pathogens, including *V. ordalii*, *V. anguillarum*, *A. salmonicida* and *Y. ruckeri*. Moreover, the probiotic protected Atlantic salmon following challenge with *A. salmonicida* and to a lesser extent *V. anguillarum* and *V. ordalii*. More recently, San Miguel, Zherdmant, Serrano, Donoso, Mendoza, Motte, Carrera, Morales & Mialhe (unpublished observation) suggested that probiotics may be most effective when applied to penaeid larval rearing tanks containing naupliar stages, when the larvae have not yet started feeding and so lack an established microflora. It is conceivable that at this stage the larvae would become colonized by the probiotic, therefore allowing some control of the gut microflora. The origin for the use of *V. alginolyticus* in Ecuadorian aquaculture is unclear.

Although there have been no published reports of the use of probiotics in Chinese aquaculture, some researchers have studied the potential benefit of using phototrophic bacteria of the genus *Photorhodobacterium* in grow out ponds culturing *Penaeus chinensis* (Xu, personal communication, 1997).

Bacteriophages

It is debatable whether or not bacteriophages constitute *bona fide* probiotics. Nevertheless, information will be included here for completeness. Park, Shimamura, Fukunaga, Mori & Nakai (2000) worked with two cultures of bacteriophages, which were derived from diseased ayu, *Plecoglossus altivelis* (Temminck & Schlegel), and represented the families Myoviridae and Podoviridae. By oral administration (in feed), the bacteriophages protected against infection by *P. plecoglossicida*, which is a pathogen of cultured ayu. The workers monitored the effects of bacteriophages on *P. plecoglossicida* populations and concluded that there was a rapid decline in the number of bacterial cells in the kidneys and in water.

Yeasts

Catla, *Catla catla* (Hamilton), has been used to evaluate the potential of both bacteria and yeasts as probiotics, with data indicating that successful candidates led to increased survival and body weight (Mohanty, Swain & Tripathi 1996). Naik, Murthy & Ramesha (1999) used a commercial premix, G-probiotic, in tilapia, *Oreochromis mossambicus* (Peters), feed, and determined that food conversion and protein efficiency was best at a dose of 7.5 g of G-probiotic kg^{-1} of diet. It is noteworthy that cells and β -glucan of *Saccharomyces cerevisiae*, an isolate of *S. exiguous* containing xeaxanthin (HPPR1) and *Phaffia rhodozyma* improved resistance of juvenile penaeids to vibriosis (Scholz, Garcia Diaz, Ricque, Cruz Suarez, Vargas Albores & Latchford 1999). Here, the data revealed that the diets containing *P. rhodozyma* led to a great improvement of larval survival. Also, *Debaryomyces hansenii*, a polyamine (spermine and spermidine) producing yeast recovered from the digestive tract of fish, improved the survival but led to reduced growth of larval sea bass, *Dicentrarchus labrax* (L.), following incorporation into the diet (Tovar, Zambonino, Cahu, Gatesoupe, Vazquez-Juarez & Lesel 2002). The presence of the yeast, which was capable of adherence to the gut, led to enhanced amylase secretion and a stimulation of brush border membrane enzymes in the 27-day-old larvae.

Microalgae

A heterotrophically grown, spray-dried unicellular alga, *Tetraselmis suecica*, has been used as a feed for penaeids and as a feed-additive for salmonids with data revealing a reduction in the level of bacterial diseases (Austin & Day 1990; Austin, Baudet & Stobie 1992). It was suggested that the mode of action may have reflected the presence of unspecified antimicrobial compounds in the algal cells.

Immunostimulants

Given that some definitions of probiotics include components of microbial cells (Salminen *et al.* 1999), it is appropriate to discuss products that have hitherto been regarded as immunostimulants. Certainly, many studies have utilized whole or components of microbial cells as immunostimulants, specifically to stimulate the immune system against pathogens. Lipopolysaccharides (LPS) from Gram-negative bacteria, vibrio vaccines, *Clostridium butyricum* spores, and glucan from yeast cell walls have been evaluated for use in aquaculture (Sakai 1998). It is recognized that immunostimulants enhance the host defence system against pathogens by increasing phagocytosis, antibody production, increasing the chemiluminescent response and by superoxide anion production (Sakai 1998).

The assessment of the potential of candidates for use as probiotics

Most studies concerned with the effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or, conversely, increased survival (Moriarty 1998; Skjermo & Vadstein 1999; Chang & Liu 2002; Irianto & Austin 2002), the improved resistance against disease (Gatesoupe 1994), the ability to adhere to and colonize the gut (Jöborn *et al.* 1997), the ability to antagonize other organisms notably putative pathogens (Jöborn *et al.* 1997), the ability to reduce the number of bacterial cells in kidneys (Park *et al.* 2000), the production of polyamines and digestive enzyme activity (Tovar *et al.* 2002), and the development of the non-specific immune system by means of cellular systems, e.g. increased phagocytic and lysozyme activities (Irianto & Austin 2002). There has been a tendency to emphasize laboratory rather than field studies. Moreover, the approaches used have been narrow rather than broad-based. Consequently, the information content of the resultant publications is often restricted, with limited value for application to the problems of aquaculture.

Mode of action of probiotics

The most likely modes of action as reported by Fuller (1987) include:

- stimulation of humoral and/or cellular immune response;
- alteration of microbial metabolism by the increase or decrease of relevant enzyme levels;
- competitive exclusion by which the probiotic antagonizes the potential pathogen by the production of inhibitory compounds or by competition for nutrients, space (=adhesion sites in the digestive tract) or oxygen.

It should also be emphasized that a placebo effect cannot always be ruled out. Unfortunately, information regarding the mode of action of probiotics used in aquaculture is incomplete, with authors rarely considering this important aspect. Benefits to the host have been reported to include the improvement in nutrition by the detoxification of potentially harmful compounds in feeds, the denaturing of potentially indigestible components in the diet by hydrolytic enzymes including amylases and proteases, the production of vitamins, such as biotin and vitamin B_{12} (Fuller & Turvy 1971; Parker 1974; Roach & Tannock 1980; Sugita, Miyajima & Deguchi 1991; Fuller 1992; Sugita, Takahashi & Deguchi 1992; Smoragiewicz *et al.* 1993; Sugita, Kawasaki, Kumazawa & Deguchi 1996; Hoshino, Ishizaki, Sakamoto, Kumeta, Yumoto, Matsuyama & Ohgiya 1997), the production of inhibitory compounds (Spanggaard *et al.* 2001) and the stimulation of host immunity (Fuller 1997; Gibson, Saavendra, MacFarlane & MacFarlane 1997). Obviously, a given probiotic could elicit more than one protective response by the host. Also, different organisms could lead to distinct and separate effects on the host.

In the case of *C. inhibens* K, it was realized that the organism produced weak antimicrobial activity and cells were capable of remaining in the digestive tract during feeding regimes (Robertson *et al.* 2000). Of course, the longevity of probiotics in the digestive tract may reflect the age and health status of the fish. For example, administration of probiotics to juvenile/first-feeding fish or to older animals immediately after antibiotic treatment may lead to prolonged colonization (by the probiotic). The comparative study of Irianto & Austin (2002) revealed that feeding with Gram-positive and Gram-negative probiotics at 10^7 cells g^{-1} of feed led to a stimulation of cellular rather than humoral (serum or mucus antibodies) immunity. Notably, there was an increase in the number of erythrocytes, macrophages, lymphocytes and enhanced lysozyme activity within 2 weeks of feeding with probiotics. In this case, the probiotics were behaving almost like oral vaccines.

Conclusions

There is confusion over the precise meaning of the term, probiotic, with definitions overlapping with oral vaccines and immunostimulants. Nevertheless, it is apparent that a diverse range of bacteria has been examined as probiotics for possible use in aquaculture. Clearly, some cultures are beneficial to the host in terms of enhanced growth and reduced incidences of disease. One indirect benefit may well be a reduction in the use of pharmaceutical compounds. However, the precise mechanism of action of probiotics is largely unknown. Care must be exercised in the choice of probiotic, because it is essential to ensure that the organism is harmless to the host. Concern must be expressed over the use of probiotics from taxa considered to be pathogenic for aquatic animals – *V. alginolyticus* is a case in point; and the lack of appropriate *in vivo* challenge trials. There is a concern that apparently harmless organisms may regain virulence, or be pathogenic to different species to those studied. However, there are market opportunities, and it is perhaps surprising that there is a lack of commercial products for the aquaculture industry.

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