Probiotic Bacteria - Are They Beneficial?

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ABSTRACT

Probiotic bacteria are now widely used as prophylactics in poultry, swine, and other terrestrial animals for protection against pathogenic microorganisms. More recently, probiotics have found application in aquaculture. There are substantial advantages for probiotic use in shrimp and fish culture. These benefits include protection against bacterial pathogens resistant to antimicrobials and absence of residues that can taint animals intended for human consumption. In laboratory studies, we demonstrated improved weight gain and better survival in *Penaeus monodon* after being fed a probiont as a feed supplement. Furthermore, we observed better protection against luminous vibriosis and better immune response compared with control shrimp not fed the probiotic bacterium. Transfer of probiont via *Artemia* by bioencapsulation to postlarval *P. monodon* in a hatchery gave significant benefits. Assessment of our probiont carried out in earthen pond settings simulated commercial growout ponds of *P. monodon*. Survival and growth of shrimp fed with the probiotic supplemented feeds were significantly greater than the control group receiving non probiotic supplemented feed. Challenge tests with a bacterial pathogen demonstrated that probiotic feed could delay disease onset and also reduce its severity.

INTRODUCTION - DEFINITION OF PROBIOTICS

"Probiotics" typically include bacteria and yeasts which benefit the health of the host after consumption. They may be added to food as live microorganisms and help to reconstruct a balanced indigenous microflora in the gastrointestinal tract (Fuller 1989, 1992, 1997; Tannock, 1999). Mono- or mixed-cultures of live microorganisms can be used (Havenaar and Huis in't Veld, 1992). Ideally the administered microbes survive in the host gastrointestinal tract. Lactic acid bacteria are some of the most studied probionts and their usefulness in the treatment of dysfunctions which disturb intestinal microflora and abnormal gut permeability in humans (Conway *et al*., 1987; Fernandes *et al*., 1987), swine (Barrow *et al*., 1980; Tannock *et al*., 1999), and chickens (Nurmi and Rantala, 1973; Berchieri and Barrow, 1990; Garriga *et al*., 1998) is well known. In aquaculture, however, *Vibrio* spp., *Bacillus* spp., lactic acid bacteria and microalgae are mainly utilized as probiotics for growth and survival enhancement, and reduction of pathogens (Austin *et al*., 1992, 1995; Douillet and Langdon, 1994; Gildberg *et al*., 1995, 1997; Rengpipat *et al*., 1998a; Phianphak *et al*., 1999). The altered definition of probiotics as applied to aquaculture (Moriarty, 1998), includes bacteria which improve water quality on addition to water, and/or inhibit pathogens in the

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water. In my opinion, the objective of probiotic use lies in the interaction between beneficial microorganisms and the host's gastrointestinal tract. The definition proposed by Gatesoupe (1999) which states that probiotics are, "microbial cells that are administered in such a way as to enter the gastrointestinal tract and be kept alive" seems reasonable. Fuller's (1989) definition of a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" is also well accepted. To date the mechanism of probiotic action on the host has not been clearly understood. The mechanism may include competitive exclusion of pathogens by interfering with the adhesion sites on the surface of gastrointestinal tract cells, increase of nutrients, production of inhibitory substances against pathogens, and their acting as immunogens and stimulating the host defense response.

PROBIOTIC MICROBES IN FEED SUPPLEMENTS FOR AQUACULTURE

Beneficial microbes defined as probiotics (Fuller, 1989, 1992, 1997), have been used successfully for raising farm animals like swine (Baird, 1977; Pollman *et al*., 1980) and chickens (Dilworth and Day, 1978; Miles *et al*., 1981) by enhancing production and promoting animal health. They have gained acceptance as being more effective than administering antibiotics or chemical substances. More recently beneficial microbes for aquaculture have been isolated from seawater, sediments and gastrointestinal tracts of aquatic animals that have the capability to produce substances that inhibit pathogens (Dopazo *et al*., 1988; Austin and Day, 1990; Austin and Billaud, 1990; Westerdahl *et al*., 1991; Munro *et al*., 1995). However, one must exercise caution in the use of live organisms as probiotics due to their indirect effects on ecosystem cycles and food chains. Table 1 summarizes previous work that used probiotic-feed supplements to increase production and prevent disease.

Bacillus S11, previously isolated from the gastrointestinal tract of *P. monodon* broodstock caught in the gulf of Thailand in our laboratory, demonstrated effective probiotic protection with *P. monodon* (Rengpipat *et al*., 1998a). Using a recirculating-closed system of water in concrete microcosms (each measuring 80 x 74 x 87 cm), we showed the effects of a probiotic bacterium on growth and survival of black tiger shrimp *P. monodon*. After a 100 day feeding trial with probiotic supplemented and non-supplemented (control) feeds, *P. monodon* (from PL30 onwards) exhibited significant differences ($p < 0.05$) in growth, survival and external appearance between the two groups. Figure 1 shows a scanning electron micrograph of the adhesion site of *Bacillus* S11 on the surface of the intestine of a shrimp that had a diet regularly supplemented with *Bacillus* S11. Live *Bacillus* S11 was isolated from the intestine of the shrimp (Figure 2). After challenging shrimps with a shrimp pathogen, *Vibrio harveyi*, by immersion for 10 days, all probiotic treated groups had 100% survival; whereas the control group had only 26% survival. In addition, the control group had a pale hepatopancreas and looked unhealthy, while the probiotic treated group appeared healthy and normal. During the challenge test, reduction of *V. harveyi* D331 in the GI tract of probiotic fed shrimp was noticed as compared to the control groups which suggested competitive exclusion by probiotic *Bacillus* S11 (Figure 3).

Table 1. Probiotics and feed supplements used in aquaculture (modified from Rengpipat *et al*., 1998b).

Aquatic animal	Probiotic strain	Challenge test with	Results	References
Salmon	*Tetraselmis suecica* (unicellular algae)	*A. salmonicida* *A. hydrophila* *Lactobacillus spp.* *S. liquefaciens* *V. anguillarum* *V. salmonicida* *Yersinia ruckeri* typeI	good control of diseases by Prophylaxis	Austin *et al*., 1992
Oyster (larval culture)	CA2	Not done	better yield	Douillet and Langdon, 1994
Salmon	*V. alginolyticus*	*A. salmonicida* *V. anguillarum* *V. ordalii*	good control of disease	Austin *et al*., 1995
Salmon	*Carnobacterium divergens* (lyophilized form could inhibit *V. anguillarum*)	*A. salmonicida*	*Carnobacterium* colonized intestinal wall; could not control disease	Gildberg *et al*., 1995
Cod	*Carnobacterium divergens* (lyophilized form)	*V. anguillarum*	good control of disease	Gildberg *et al*., 1997
Scallop (larval stage)	*Vibrio spp.* *Pseudomonas* sp.	*V. anguillarum* related (VAR)	good control of disease	Riquelme *et al*., 1997
Black tiger shrimp	*Bacillus* strain S11	*V. harveyi*	better yield; good control of disease	Rengpipat *et al*., 1998a
Black tiger shrimp	*Lactobacillus* spp.	*V. harveyi*	better yield; good control of disease	Phianphak *et al*., 1999
Black tiger shrimp	*Bacillus* strain S11	*V. harveyi*	better yield; good control of disease; immunity enhancement	Rengpipat *et al*., 2000

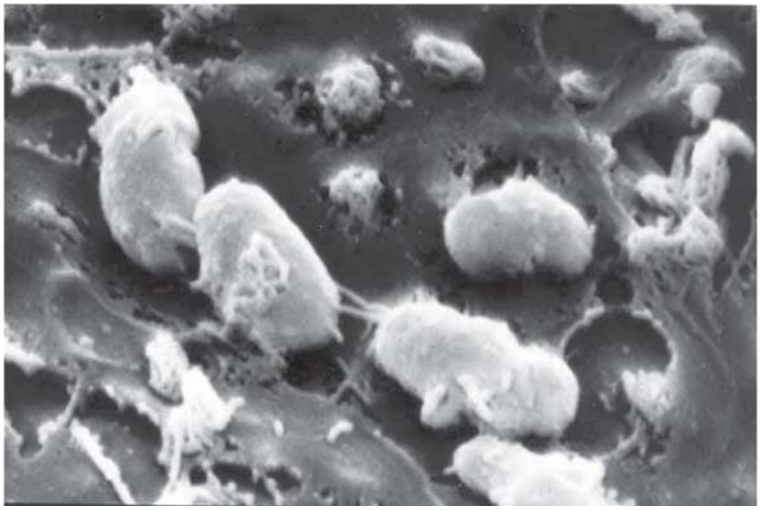

Figure 1. Scanning electron microscopic micrograph of gastrointestinal tract surface of *Penaeus monodon* showing the adhesion site of *Bacillus* S11 probiont.

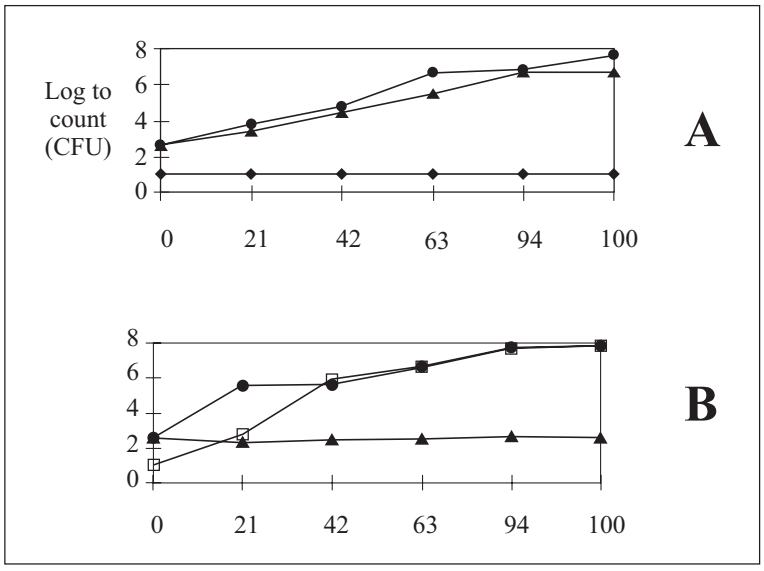

Figure 2. Bacterial counts in shrimp gastrointestinal tract during 100 days of feeding with:
A. regular diet; B. regular diet supplemented with *Bacillus* S11. All values are means of three replicates per treatment.(modified from Rengpipat et al. 1998a). Total bacteria (-●-), *Bacillus* spp. (-◆-), *Bacillus* S11 (-■-), and *Vibrio* spp. (-▲-).

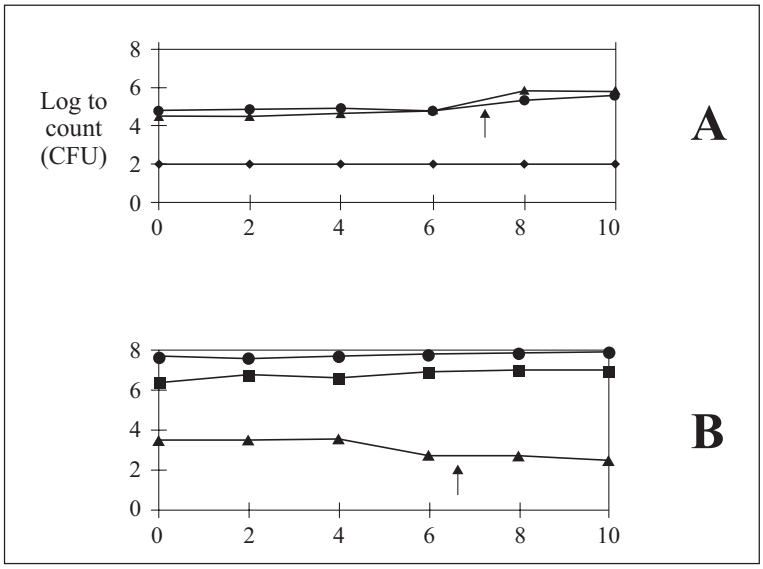

Figure 3. Bacterial counts in shrimp gastrointestinal tract of probiotic treatments and control during 10 days challenge with *Vibrio harveyi* D331. A. regular diet; and B. regular diet supplemented with *Bacillus* S11 (second booster of *V. harveyi* D331 10^7 CFU ml^{-1} by immersion). All values are means of three replicates per treatment (modified from Rengpipat *et al*., 1998a). Total bacteria (-●-), *Bacillus* spp. (-◆-), *Bacillus* S11 (-■-), and *Vibrio* spp. (-▲-).

In another experiment (Rengpipat *et al*., 2000), survival and growth of *P. monodon* fed with the probiont *Bacillus* S11 in two 90 day culture trials increased compared with the non-treated shrimp. *Bacillus* S11 also efficiently activated phagocytosis and increased the phagocytic index (PI) in the hemolymph. Phenoloxidase and antibacterial activities increased with age in all shrimp, and were further enhanced by probiotic treatment. Following the second trial, after 90 days' culture with and without *Bacillus* S11 feed additives, shrimp were exposed to pathogenic, luminescent bacteria (*V. harveyi*). After 10 days exposure to *V. harveyi*, probiotic-treated shrimp had significantly greater ($p < 0.05$) survival (54.35%) compared with non-treated shrimp (35.5%). Immune response were substantial in both treatment groups following the 10-day challenge, but were more pronounced with the probiotic-treated shrimp. The PI was significantly greater with probiotic-treated shrimp (2.7 ± 0.8) compared with controls (0.6 ± 0.3) (Table 2). Thus *Bacillus* S11 evidently provided disease protection by activating both cellular and humoral immune defense responses. Some dead *Bacillus* S11 or their spores might act as a "bacterin", and behave as an immunogen while residing in the shrimp gut (Sung *et al*., 1991) and elicit non specific immune response against bacterial pathogens. From our studies (Rengpipat *et al*., 1998a, 2000) we can surmise that the use of *Bacillus* S11 in shrimp feeds can reduce *P. monodon* mortality during culture by mechanisms such as competitive exclusion or immune enhancement or both activities for good health.

Table 2. Mean immunity index values of control and probiotic treated Penaeus monodon before and after 10 days challenge with Vibrio harveyi 1526.

Immunity indexes	Means (SD)			
	Before		After	
	Control	Probiotic	Control	Probiotic
Total hemocytes [1] (1x107 cell ml^{-1})	1.4 ± 0.6 [b*]	2.6 ± 0.7 [a]	1.1 ± 0.5 [b]	1.1 ± 0.2 [b]
Phagocytic activity [1] % phagocytosis Phagocytic index ABPC	1.0 ± 0.5 [c] 0.0 ± 0.0 [c] 1.6 ± 0.5 [a]	2.2 ± 1.0 [c] 0.1 ± 0.1 [c] 2.0 ± 0.4 [a]	6.0 ± 1.8 [b] 0.6 ± 0.3 [b] 1.7 ± 0.3 [a]	10.5 ± 1.8 [a] 2.7 ± 0.8 [a] 2.5 ± 0.3 [a]
Phenoloxidase [2] (units/min/mg Protein)	10.3 ± 9.0 [b]	41.0 ± 10.1 [a]	7.7 ± 1.0 [b]	24.7 ± 12.6 [ab]
Antibacterial activity [3] (% inhibition)	17.9 ± 28.1 [c]	32.4 ± 29.1 [bc]	70.5 ± 15 [ab]	87.4 ± 9.3 [a]

*Means not sharing a common superscript letter between row values differ significantly ($p < 0.05$).
[1] n = 3; [2] n = 5; [3] n = 6.
ABPC = The average numbers of beads ingested per cell.

BIOENCAPSULATION VIA ARTEMIA

Bacillus strain IP5832 spores (Paciflor 9) were supplemented into a rotifer diet and fed to turbot larvae *Scophthalmus maximus* (Gatesoupe, 1991). After culture, the wet weight of turbot larvae increased compared with controls. *Bacillus* spores improved the nutritional value of rotifers. After challenge with an opportunistic Vibrionaceae species, mortality of turbot decreased. *Carnobacterium* sp. isolated from rotifers (*Brachionus plicatilis*) was used to enrich rotifers before feeding to turbot larvae by Gatesoupe (1994). Again a decrease in mortality of larvae was observed after challenge with a pathogenic *Vibrio* sp. Antagonism and/or improved nutritional value of the rotifers was suggested to be the mode of action. *Bacillus* S11, was used as a probiotic bacterium by passage through *Artemia* sp. fed to black tiger shrimp, *P. monodon* (Rengpipat *et al*., 1998b). It was found that black tiger shrimp larvae reared using the *Bacillus*-fortified *Artemia* probiotic as a feed had significantly shorter development times and fewer disease problems than larvae reared without the probiotic (Table 3). *Bacillus* S11 encapulated within *Artemia* provides a model of bioencapsulation and shows the possibility of probionts passing through *Artemia* that could be routinely used to feed shrimp larvae. This method may prove beneficial for hatchery postlarvae or for improvement of survival during the initial stages of earthen pond culture.

Table 3. Average live weight and length of *Penaeus monodon* cultured for 2 weeks in two feed treatments

Parameters	Control	Probiotic
Weight (mg)	26.0*	43.8*
Length (cm)	1.71[b] ± 0.20	1.83[a] ± 0.31

Control: shrimp with artemia; Probiotic: shrimp with *Bacillus* S11-fed artemia; *Total weight divided by a number of shrimp (43 shrimp); b,a Different superscripts in the same row significantly different. *P. monodon* (PL-10) after acclimatization for 5 days, uniform-size post larvae, were selected for testing.

PROBIOTIC APPLICATION IN OUTDOOR, EARTHEN PONDS FOR SHRIMP CULTURE

Moriarty (1998) added bacteria directly into pond water and proposed microbial water additives as one direction for probiotic application. The addition of *Bacillus* spp. into a shrimp pond showed inhibitory activity against luminous *Vibrio* sp. However there was no clear evidence that *Bacillus* spp. affected shrimp health or improved water quality. Higher survival of shrimp from bacterial treated ponds was detected. In my opinion this management should be catagorized under bioaugmentation or biocontrol which improves the microbial ecology of water and sediment. Improvement may be by the degradation of organic matter after digestion by extracellular enzymes secreted by *Bacillus* spp. or other microorganisms in the pond.

Bacillus S11 is considered a saprophytic strain which is environmently friendly and has been proven before as a probiont for black tiger shrimp (Rengpipat *et al*., 1998a) when mixed with shrimp feed. After 100-day culture of shrimp during field trials in 2-m^2 net cages located in outdoor, earthen ponds at different season were performed (Rengpipat *et al*., 2003). Shrimp fed probiotic feed (PF) averaged 25.4 and 22.0 g, compared with 18.6 and 18.3 g for shrimp not fed with probiotics (NF) in the two trials, respectively. PF fed shrimp survival was 76.6% and 86.8% during these two trials, compared with 65% and 62.5% for NF fed shrimp (Table 4). Projected yields on an annual basis (two 100 day crops) were 59% greater with probiotic fed shrimp. Thus it seems likely that the probiotic bacterium will be useful in *P. monodon* culture on a commercial scale.

Table 4. *Penaeus monodon* survival after 100 days culture in net cages in an earthen pond and either fed a probiotic fortified feed (Bacillus S11 Probiont) or feed without probiotic (Control). Each treatment group mean includes 12 cages in Trial I and 6 cages in Trial II.

Treatment	Survival (%)	
	Trial I	Trial II
Control	65 ± 11.5	62.5 ± 7.5
Probiotic	76.6 ± 6.2*	86.8 ± 5.0*

*Significantly ($P<0.05$) different between groups in same trial.
Trial I: Hatchery reared *P. monodon* of 0.6-0.9 g, 80 shrimp/cage were fed in earthen pond, with water salinity 8%, during June-September, 2000.
Trial II: Conditions nearly identical to Trial I were experimented during November-February, 2000.

FUTURE TRENDS OF PROBIOTIC USE IN AQUACULTURE

Identification and selection of the right strain of probiotic bacterium in aquaculture require extensive studies, starting from which specimen should be selected. Criteria for probiotic properties should be established for directing the experimental trials from laboratory to industrial scale. The decision to use a probiont for commercial purposes needs supporting evidence from *in vitro* and *in vivo* data. This is especially important for probiotics in aquaculture as they will eventually disperse into the water and sediment in the pond and later will act as a big soup bowl for microbial diversity. An imbalance of microbes in the pond could be created if the right probiont is not used. Since most female marine animals

release their eggs outside their bodies, the addition of water or feed with probionts might create good transient or residential flora for larvae and later develop as permanent residential flora in the GI tract of older stages. Regular probiotic feeding of marine animals during cultivation is an appropriate strategy to maintain the amount of microbial attachment to the GI tract and interfere with the attachment of pathogens. Conducting the search for novel probiotics to raise marine animals should be undertaken since they will serve as alternatives to antibiotics and chemicals. Concerning residues, if one can control pathogens in the raw materials and avoid chemicals, then the food will be considered safe for human consumption as required by Codex Alimentarius.

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