

# Probiotics in aquaculture

By D. J. W. Moriarty, O. Decamp and P. Lavens

Many products now being sold to the aquaculture industry are not in fact true probiotics and may even be deleterious. The concepts of probiotics and their applications to aquaculture are discussed below.

Probiotics, the natural, beneficial bacteria are now well accepted and widely used in shrimp aquaculture. Potentially, they may have one or more beneficial functions for aquaculture producers:

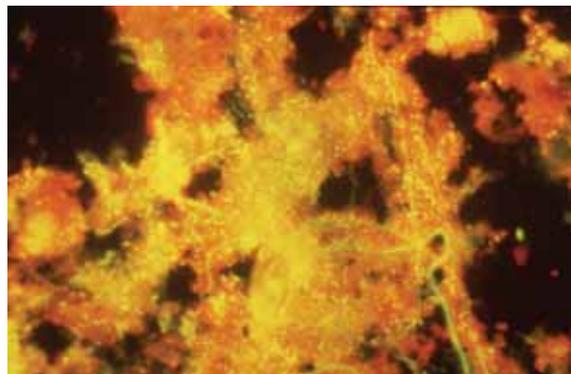
- Water and pond bottom sediment quality are improved, leading to less stress on shrimp and thus improved health.
- Effluent water is cleaner, thus environmental impact is low.
- Pathogenic bacteria and their virulence can be controlled, and the overall microbial ecosystem can be managed.
- Antibiotics are not used. This stops the increase in virulence and pathogenicity in aquatic bacterial pathogens due to antibiotics. It will also minimise the risk of multiple antibiotic resistance.
- Stimulation of the shrimp immune system.
- Improved gut flora and hence lower disease incidence and increased food assimilation

Hence, productivity and profits are increased when selected probiotics are used.

## Some concepts in probiotic bacteria

The term *probiotic* has been defined as “a mono- or mixed culture of live microorganisms that when applied to animals or man, affect beneficially the host by improving the properties of the indigenous microflora”. Moriarty (1996a, 1998) extended the definition for aquaculture to include the addition of natural bacteria to tanks and ponds in which the animals live.

Probiotic bacteria improve the health of shrimp or fish by controlling pathogens and improving water quality by modifying the microbial community composition of the water and sediment. Probiotic bacteria enter the gut or attach to external surfaces of the animals either directly



*Bacterial communities in water and sediment are complex with many different species interacting at close quarters. True probiotics are designed to enhance beneficial species and inhibit deleterious ones.*

from the water or via attachment first to food or other ingested particles. Thus, they are used in aquaculture both as water and sediment quality conditioners and as feed supplements.

When we started work with probiotics in commercial shrimp farms, the products that were available had a low number of the important genus: bacteria *Bacillus*. Before use they had to be brewed by the farmer with a nutrient medium to produce a high enough number to be added to a pond to be beneficial (e.g. see Moriarty 1996 a, 1996 b, 1998). Now, we can produce pure strains of *Bacillus* at low cost and market these as powdered mixtures of spores with a long shelf life. The powders are simple for the farmer to use.

***“The changing of a bacterial community takes time. It is an ongoing process that requires addition of the beneficial strains of bacteria throughout the culture period”.***

Many shrimp and fish farmers often think of probiotics as medicines like antibiotics. They expect a quick and decisive effect. They are then discouraged from using probiotics when the results are not immediate or dramatic. The changing of a bacterial community takes time. It is an ongoing process that requires addition of the beneficial strains of bacteria throughout the culture period. The bacteria that are added must be selected for specific functions, added at a high enough population density and under the right environmental conditions to be effective.

## Bacillus – the true probiotics for shrimp aquaculture

Gram positive *Bacillus* species are spore formers and produce a wide range of antagonistic compounds. They are suitable as commercial probiotics in aquaculture. Species such as *B. subtilis* and *B. licheniformis* occur naturally in fresh and sea water environments and are found naturally in the intestinal tracts of prawns. They are considered true probiotics for shrimp aquaculture (Moriarty 1998, 1999; Decamp and Moriarty, 2005).

Ineffective products that are sold as probiotics have caused farmers to question the probiotic concept, rather than the nature or mode of action or number of the bacteria in the product. Some contain inappropriate species of bacteria, or population densities that are too low to be effective for aquaculture.

For example, products for crustaceans containing *Lactobacillus* species that were produced for human or land animals are not appropriate for shrimp where these bacteria do not occur naturally. Some products in Asia have labels indicating *Clostridium* species, *Pseudomonas putida*, *P. aeruginosa*, *Enterococcus faecium* and *E. faecalis*, which are human and/or fish pathogens. Some products contain purple sulphur bacteria, which require light and hydrogen sulphide under anaerobic conditions. Such conditions would be lethal to shrimp.

The microflora of the sediment and water in which the cultured shrimp or fish live is influenced by the microbes released from faeces of all the animals in their environment. If a pathogen is present, its population density can be magnified through interactions in the intestinal tracts of the animals and in the faeces. When food for aquatic animals is added to the water, it adsorbs or absorbs bacteria from the water before it is eaten.

However, when probiotic bacteria are added to ponds or tank water and are adsorbed to feed, they enter the intestinal tract and compete with pathogens. Thus the farmer can manipulate the species composition by seeding large numbers of desirable strains of bacteria or algae; *in other words, by giving chance a helping hand.*

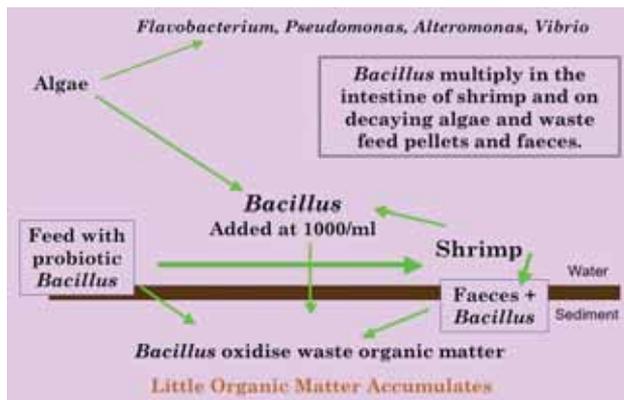
## Pond processes mediated by bacteria

*Vibrio* bacteria often dominate when algal blooms die and shrimp numbers are high. Oxygen diffusion is limited in organic detritus on the pond bottom, especially when the feeding rate is high and thus oxygen is rapidly depleted. Fermenting bacteria, which include *Vibrio*, become active and release organic acids, some of which are toxic to shrimp and which are used by sulphate-reducing bacteria in shrimp ponds.

It is important to ensure that organic detritus and slime do not build up on the pond bottom. All faeces, excess feed and dead algae must be decomposed rapidly. Aeration by itself is not enough. Active bacterial populations must be changed to species that are adapted to rapid degradation of complex organic molecules. The *Bacillus* group produce a wide range of exo-enzymes that are very efficient at breaking down large molecules such as protein and fats.

When selected *Bacillus* strains are added to ponds frequently and at high density, they degrade organic matter faster than in situations where only the natural populations are available. Denitrifying *Bacillus*, which breakdown organic waste and use nitrate when oxygen is depleted, are especially effective on the pond bottom. A product is now available on the market from INVE that contains specially selected bacteria to speed up degradation processes (Figure 1).

**Figure 1: Effect of *Bacillus* at high population density in ponds. *Bacillus* compete with other bacteria in the pond for organic matter from algae, feed and animals. Specially selected *Bacillus* displace pathogenic *Vibrio*.**

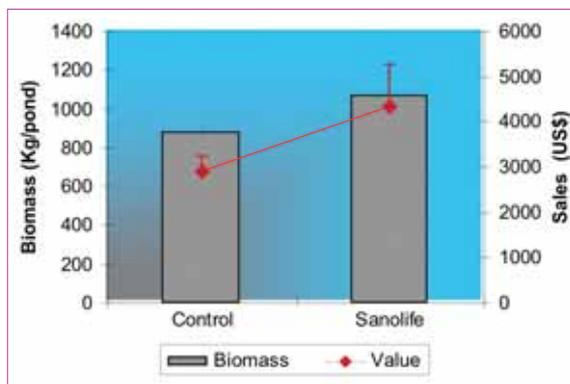


## Results of probiotic use in shrimp grow out

Where appropriate and effective products are used, the results are good. For example, in Indonesia and Philippines, Moriarty (1998 and 1999) showed that production was high and consistent in all ponds where probiotics and sound management technology were used, whereas untreated ponds or ponds with antibiotics often collapsed due to disease.

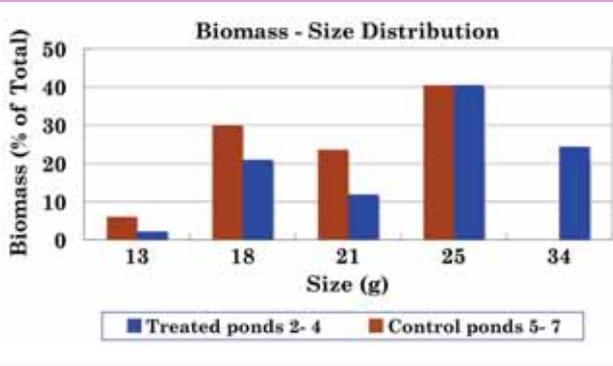
In 2004, at Teknomin farm in Andra Pradesh, India, 25% of black tiger shrimp *Penaeus monodon* reached 34g in the 3 ponds where the probiotics were added to feed (Figure 2). The largest average size of shrimp in the control ponds was only 25g during the same time period (115 days). The net profit was therefore far greater when the probiotics were used (Figure 3). This was achieved by using the respective probiotics

**Figure 2: Biomass and size distribution of shrimp on Teknomin farm. Means are for 3 ponds treated with Sanolife® PRO-W in the water and Sanolife® PRO-1 and PRO-2 in feed of *Penaeus monodon* at Teknomin farm in Andra Pradesh, India, and 3 control ponds. Stocking density 10/m<sup>2</sup>.**



in the water and by incorporating the *Sanolife*<sup>®</sup> probiotics in all feed, so that the intestinal tract of the shrimp was colonised by *Bacillus* species that competed successfully with the pathogenic *Vibrio* species.

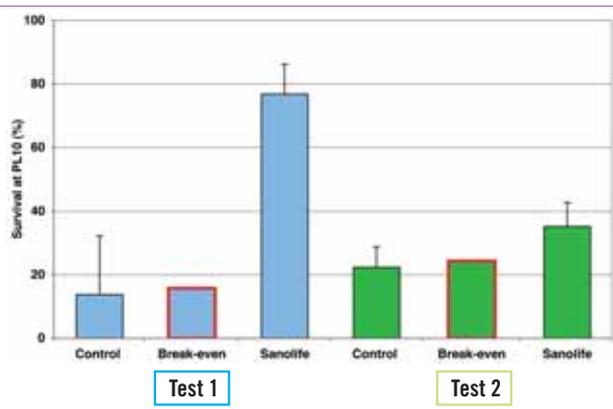
**Figure 3: A combination of SANOLIFE<sup>®</sup> PRO-1, PRO-2 and PRO-W was evaluated in India, with 3 ponds per treatment. Higher survival and harvested shrimp biomass were observed from ponds having been treated with the Sanolife probiotics. Lower FCR was observed in ponds receiving the Sanolife treatments, i.e. 1.52±0.13 compared to 1.6±0.18 for the negative control ponds. Furthermore, bigger sized shrimp were found only in ponds treated with the probiotics. This led to a >10-fold increase in benefits with the Sanolife treatment.**



### Probiotic use in hatcheries

The main function of probiotic products for hatcheries is to control pathogenic bacteria, especially *Vibrio harveyi* and *V. alginolyticus*. However, results are not always good, because *Vibrios* evolve very quickly, and many very resistant strains are present in some areas, partially in response to antibiotic use. Some of these are also resistant to the probiotics, so probiotic bacterial strains need to be selected that have a wide geographical range. Good results have been reported recently in several different countries for the INVE product for hatcheries (Figure 4).

**Figure 4. In 2 separate tests run over a period of 1 year in Thailand, *Penaeus monodon* nauplii were reared until PL10 and fed a combination of *Chaetoceros*, *Artemia* nauplii and Lansy Shrimp (40% live food substitution) INVE larval diet. In these replicated tests, the daily application of SANOLIFE<sup>®</sup> MIC probiotics at a final concentration of 1-5x10<sup>4</sup> cfu/ml of tank water gave results similar to those observed with prophylactic application of antibiotic and the benefits in term of survival. In both tests, a net profit was realized with the Sanolife<sup>®</sup> MIC, whereas survivals in the controls were less than the breakeven number.**



Following germination in sterile water, the product was added daily to the larval tanks at a concentration of 0.5 ppm until the zoea 2 stage and then 1 ppm from zoea 3 stage to harvest. Its daily application gave results similar to those observed with prophylactic application of antibiotics. The improved survival/biomass compared to negative control outweighed the cost of the probiotics in all tests.

Obviously, minimizing the risk of vibriosis demands a multi-disciplinary approach, including good hygiene and sanitation measures to control the input of potential pathogens, as well as a sound farm management. **However, probiotics are only cost-effective when they are properly applied together with a suitable farm management.**

### References

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