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PROBIOTICS USED IN SHRIMP AQUACULTURE

M. Petchimuthu

Email

mpetchimuthumfsc@gmail.com

Department of Fish Pathology and Health Management, Dr. MGR Fisheries College and Research Institute, Thalainayeru, Nagapattinam, Tamil Nadu, India

The term, probiotic, simply means “for life”, originating from the Greek words “pro” and “bios”. Probioticis defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance”. 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 minimize the risk of multiple antibiotic resistances.
- Stimulation of the shrimp immune system.
- Improved gut flora and hence lower disease incidence and increased food assimilation.

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 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. 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.

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.

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.

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.

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

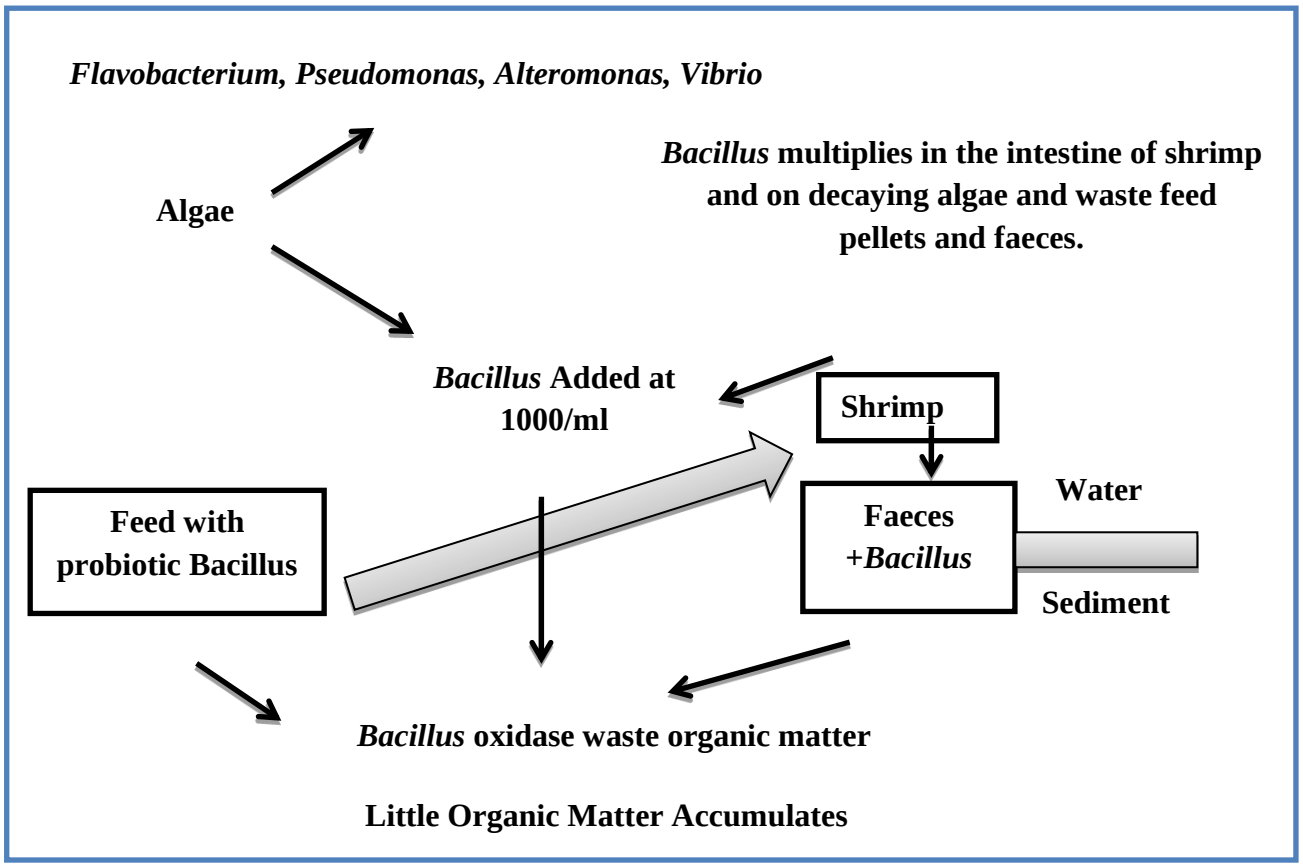

Fig 1: Effect of Bacillus at high population density in ponds. Bacillus competes with other bacteria in the pond for organic matter from algae, feed and animals. Specially selected Bacillus displaces pathogenic Vibrio.

Probiotics in Shrimp Aquaculture

1. Criteria for Selection of Probiotics for Shrimp Aquaculture.

It has been widely published that a probiotic must possess certain properties. 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.

2. Evaluation of Probiotic Potential of Microbial Strains Other Than Animal Origin

Some of the probiotic strains are isolated from fermented foods, pond sediments, soil, water, and so forth. The procedure for evaluation of probiotic potential of microbial strains other than animal origin. The experimental conditions of the probiotic potential tests vary according to the target host and the further application of probiotics. After the above evaluation process, the strain is further tested for economic evaluation.

3. Application of Probiotics in Shrimp Aquaculture

Probiotic activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration and route, and frequency of delivery. Some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses, actively participating in the fight against infections, whereas others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules, or modulate the inflammatory/ immune response. Several studies have demonstrated that pattern recognition receptors (PRPs), such as toll-like receptors

(TLRs) signaling pathways, immune responses, and the secretion of antimicrobial peptides such as defense's and chemokine's by the epithelium play important roles in these mechanisms.

Probiotics in Activation of Shrimp Immune Defences

Probiotics were successfully reported for their beneficial effects in warm-blooded animals. Experiments indicate that probiotic bacteria administered orally may induce increased resistance to enteric infections. As mentioned earlier, shrimp has a poorly developed immune system and probiotics were known to play an important role in the enhancement of immune response in shrimp.

The probiotic bacteria *Lactobacillus plantarum* was reported to enhance the immune responses and gene expression in white shrimp, *Litopenaeus vannamei*,when given in diet. The bacteria influenced both the cellular and humoral immune defences in the shrimp. *L. plantarum* was known to enhance the phenoloxidase (PO) activity, prophenoloxidase (ProPO) activity, respiratory bursts, superoxide dismutase (SOD) activity and clearance efficiency of *Vibrio alginolyticus*, peroxinectin mRNA transcription, and survival rate after challenge with *V. alginolyticus*.

These effects the immune defenses also maintain the defence levels in the shrimp offering a prolonged protection. Probiotics strains *Vibrio* P62, *Vibrio* P63, and *Bacillus* P64 were isolated from hepatopancreas of healthy wild shrimp *Penaeus vannamei,* and their immunostimulatory effect was studied.

Among the three, P64 showed a significantly higher immunity index and showed immune response similar to that of *V. alginolyticus* whereas the other two only showed good probiotic properties. Here, the P64 gave the immune alert with a significant increase in the hyaline cell population.

Table 1: Benefits of Probiotics in aquaculture

Probiotic strain	Used on	Effect of probiotic strain
Bacillus S11	*Penaeu smonodon*	Protection against *Vibrio harveyi* by stimulation of cellular and humoral immune defenses
Bacillus subtilis UTM 126	*Litopenaeus vannamei*	Control vibriosis by producing bacitracin, gramicidin, polymyxin, tyrotricidin, and competitive exclusion
Streptomyces	*Penaeus monodon*	Better water quality parameters, increased length and weight of the animal
Bacillus subtilis E20	*Litopenaeus vannamei*	Enhance humoral immune response

Conclusions

The probiotics in aquatic environment is still a controversial concept due to lack of authentic evidence or real environment demonstrations on the successful use of probiotics and their mechanisms of action. Probiotics is an alternative to antibiotics and chemicals in aquaculture which provide better health benefits, higher growth rate, increased survival rates and produce safe organic fish products to meet the protein requirements of future generations.

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