PROBIOTICS USED IN SHRIMP AQUACULTURE

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INTRODUCTION

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.

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:

The probiotic should not be harmful to the host it is desired for,

It should be accepted by the host, e.g., through ingestion and potential colonization and replication within the host,

It should reach the location where the effect is required to take place,

It should actually work in vivo as opposed to in vitro findings,

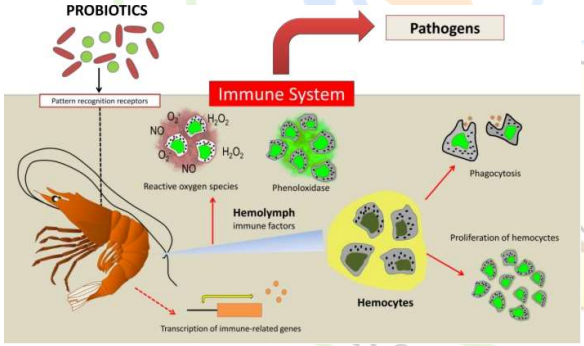
It should preferably not contain virulence resistance genes or AB resistance genes.

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.



Mechanisms of probiotic actions in shrimp

GUT PROBIOTICS:

Gut probiotics are applied in shrimp aquaculture to improve the shrimp intestinal microbial balance. Gut probiotics incorporate bacteria strains in the feed pellet using many types of binders, such as egg white and fish oil, for more efficient application. Gut probiotics usually contain *Lactobacillus* or *Saccharomyces cerevisiae,* nitrifying bacteria, *Streptococci, Roseobacter,* and *Bacillus sp.* These bacteria strains work in the shrimp gut by modifying the microbial balance for the benefit of the shrimp. One of the benefits is enhanced immune response. The defense mechanisms of shrimp are less developed than other aquatic organisms, and they rely on a non-specific immune system. Probiotics support this immune system by enhancing the immune response to pathogen assault. Not only that, probiotics can also prevent pathogen proliferation in the gut, as well as inhibiting pathogenic actions, providing better protection against possible infections and diseases.

Probiotics also have the ability to modify the metabolism of microbiota in the shrimp gut to produce short-chain fatty acids for improved digestion. This leads to increased appetite, better growth and improved FCRs – which in turn reduces the amount of waste from uneaten feed and therefore improves water quality. Overall, feed probiotics provide farmers with better growth, healthier shrimp and less water pollution.

PROBIOTICS IN BIOFLOC SYSTEMS:

Biofloc technology is an emerging system in shrimp aquaculture which offers a more robust means of disease management and a reduced environmental impact by utilising beneficial bacteria for waste recycling and implementing low or zero water exchange. Biofloc technology works by limiting or eliminating water exchange, adding external carbon sources – such as molasses, sugar cane or wheat bran – to the pond and providing high oxygenation.

These three main procedures create an environment with a balanced carbon-to-nitrogen (C/N) ratio that stimulates the growth of microbial communities in the water, especially heterotrophic bacteria. The desired C/N ratio varies between ponds, but usually it is 1:5. Under the right conditions, microorganisms, such as bacteria, algae and phytoplankton, and organic matter – like

feed, faeces and old shells – will join together into "flocs".

These flocs can help improve water quality by converting ammonia and other organic wastes into bacterial biomass. They also recycle wastes into nutritious food for the shrimp by transforming additional carbon sources into microbial protein. In this way, the proliferation of pathogens can be prevented and shrimp growth enhanced. The low or zero water exchange in these systems can also further reduce the spread of pathogens within the farm and water pollution outside it.

In the biofloc system, probiotics are usually used as the bacteria starter. In common practice, probiotics are not administered in the biofloc ponds since the bacteria in them already have a "natural probiotic" effect. However, a recent study shows that the addition of probiotics to the biofloc can further enhance the system, by improving growth, digestion, metabolism, disease-resistance and water quality, as well as reducing the vibrio load. Although still in an early experimental stage, the combination of probiotics and biofloc shows great potential for increasing the sustainability of shrimp farming.

PROBIOTICS IN SEMI-FLOC SYSTEMS

Biofloc technology requires a high degree of technical knowledge to make sure all the inner workings of the system operate smoothly. A simple problem, such as a power cut, would make the pond environment very toxic, because aeration stops, significantly reducing the dissolved oxygen (DO) level. This leads to the emergence of a hybrid system called semi-floc which combines traditional and biofloc approaches for a more affordable, doable and environmentally sustainable aquaculture system.

Both biofloc and semi-floc have the same purpose – to manipulate the microbial interaction in the pond to create flocs. However, if biofloc relies mostly on heterotrophic bacteria, semi-floc combines a balance of autotrophic and heterotrophic organisms. Because of this, semi-floc requires regular siphoning to take out excess nitrogen, as opposed to relying on microbial decomposition by heterotrophic bacteria. Water exchange is also permissible, if required, but should be kept to a minimum.

In a semi-floc system, the bio-microfloc formation is driven by additional chemicals and microbial products or probiotics. In contrast with biofloc technology, semi-floc needs probiotic administration during culture in order to maintain a good FCR. The coating of Bacillus probiotics can help improve feed digestibility and absorption.

SYNBIOTICS

Innovations in modern shrimp aquaculture continue to push forward. As probiotics have become mainstream, a new holistic approach has emerged, combining probiotics with the traditional technique of prebiotics. Together they are called synbiotics. While probiotics are live organisms beneficial for shrimp intestinal microbiota as well as the water, prebiotics are non-digestible feed additives which provide beneficial effects by stimulating the growth and activity of beneficial bacteria in the shrimp gut. Prebiotics have been applied by shrimp farmers for a long time. Now, the advocates of synbiotics claim their approach as a low-cost technique with more productivity and efficiency.

Applying synbiotics in farms is quite simple. Prebiotics, usually in the form of rice bran, and probiotics are fermented in sterile freshwater, together with enzymes and buffers. After fermentation is done, the substance can be administered directly into the pond or mixed with the feed. It is shown that the application of synbiotics can stimulate growth, lower the FCR, and reduce pH fluctuations.

PROBIOTICS IN ACTIVATION OF SHRIMP IMMUNEDEFENCES

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.

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	*Penaeusmonodon*	Better water quality parameters, increased length and weight of the animal
Bacillus subtilis E20	*Litopenaeu svannamei*	Enhance humoral immune response

CONCLUSIONS:

The perennial problem of shrimp disease has driven the shrimp aquaculture industry to find viable solutions which are safe and sustainable – both economically and environmentally. Probiotics in various forms have been giving farmers substantial help in managing disease, decreasing environmental impact and enhancing production. As more research and development are currently underway, we can only expect probiotics to become better and more efficient.

Among the various small millets, finger millet (*Eleusine coracana* L. Gaertn.) cultivated over an area of 1.19 million hectares with a production of 1.98 million tonne and productivity.