Judicious use of probiotics in shrimp culture systems

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Focal Points at a Glance: *There is an urgent need for strict regulations on the manufacture and use of probiotics to obtain their intended prophylactic effects. So, the aquaculture professionals should understand that probiotics are prophylactics and not therapeutic agents, and they need to be applied judiciously in the culture systems.*

Intensification in shrimp culture practices in India has led to many problems not visualised either by the industry or by the scientific community. In an effort to achieve higher profits, farmers are using various chemicals and biological products without understanding the basic concepts behind their composition and method of application. Probiotics are one such group of products often misused.The term "probiotic" has been defined as: "a mono or mixed culture of live micro-organisms that are applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora".Intensification of shrimp culture operations in India, may increase the cost of commercial probiotics products application which could be up to Rs.15/kg for shrimp. Due to lack of knowledge on these probiotics,in the Indian aquaculture market there are a range of probiotics products which have not been evaluated scientifically.

Farming practices *vs* pond bacterial population: Water exchange is the normal practice in semi-intensive and intensive aquaculture ponds so as to reduce stress in pond ecosystem. The principal reason to apply water exchange is to flush out excess phytoplankton and other organic matter, to eliminate metabolic toxic products, mainly ammonia and to increase the dissolved oxygen concentration. The dilution of the ecosystem has also an impact on the microbial population and processes. Among the microbial populations, nitrifying bacteria, which are usually slow growers need several days to develop and are easily affected at high water exchange rates, thus preventing the consolidation of a nitrifying bacteria population. On the other hand, disease concerns and lack of quality water availability from the source has prevented the regular water exchange and is forcing the farmers to practice zero water exchange. Under these prevailing conditions, bacterial decomposition of organic matter from the sediment and water column can reduce the necessity of water exchange. Thus advantage of reducing water exchange rates, even up to the level of zero-exchange, has synergistic effects between algae and bacteria, leading to more stable water quality and improved pond environment.

Traditionally, shrimp farmers have been applying the indigenous preparation of fermentation filtrate into the culture ponds for better pond environment and shrimp growth. However, in order to address water quality and other concerns due to increasing densities of culture, addition of bacterial products (probiotics) has become necessary. The probiotic products are a fairly recent addition to the Indian market. It is claimed that these improve the water quality parameters in the culture ponds.

Mechanisms of probiotics action: Probiotics are known to act in more than one way in aquaculture. It is generally believed by the farming community and industry that probiotics often help in improving the quality of pond environment and shrimp growth leading to higher production though precise scientific information on underlying mechanism which is limited. Scientifically, the possible mode of action for probiotics could be (a) production of inhibitory compounds (b) competition for chemicals or available energy (c) competition for adhesion sites (d) enhancement of the immune response (e) improvement of water and soil quality (f) interaction with phytoplankton and (g) enzymatic contribution to digestion. Thus different types of probiotic microbe scan exhibit one or more of the above mentioned action.

Types of probiotics and principles of their application: Probiotics applied in aquaculture can be classified, broadly into (a) gut acting (b) water probiotics and (c) soil probiotics.

Gut probiotics generally colonise in the shrimp gut and competitively inhibit the pathogenic bacteria in addition to possible release of some bactericidal molecules. Hence, these types of probiotics need to be applied on regular basis throughout the culture period including at larval stages of seed in hatcheries. Since, the site of action is gut; it is scientifically prudent to apply these products through feed top dressing so as to reach the site of action without any loss in the pond environment. Water probiotics generally contain bacteria associated with nitrogen recycling and photosynthesis which get established in the pond environment. Since growth of these bacteria depends largely on the availability of suitable metabolites, they need to be applied during the second half of the grow-out period when

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accumulation of nitrogenous waste increase in the pond bottom.

Excess accumulation of feed material and creation of anaerobic conditions in the pond bottom are generally expected to occur during the later period in the grow-out culture.Soil probiotics which generally contain bacteria associated with sulphur recycling establish in the pond bottom and help in converting toxic hydrogen sulphide into non-toxic sulphur and sulphur related compounds. Since probiotic products with sulphur recycling bacterial consortia need sulphur-containing metabolites for their growth, application of such products in the early period of culture may not be of much help for their establishment in the pond environment and are only beneficial during the later stages of culture. Important types of probiotics available in the market for shrimp farming and their microbial composition are given in Table 1.

Beneficial effects of probiotic microbes

***Bacillus* spp.:** Probiotic activity of Bacillus spp, *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus megaterium* have been well documented. These bacteria are known to inhibit the growth and pathogenicity of *Vibrio harveyi*. Recently studied *Bacillus pumilus* has been reported to secrete a novel compound 4-phenyl butanoic acid which inhibits the formation of antibiotic resistant biofilms. Since, formation of biofilm is a major problem in shrimp hatcheries, probiotic with this bacterium will be more effective in larval rearing systems.

***Nitrosomonas* spp. and *Nitrobacter* spp.:** These are the bacteria involved in nitrogen cycle and convert the toxic ammonia excreted by cultured shrimp through the process of nitrification. *Nitrosomonas* spp. is an ammonia oxidising bacteria, which oxidises ammonia to nitrite, while *Nitrobacter* spp. is nitrite-oxidising bacteria converting nitrite to nitrate.This accumulated nitrate is utilised by the phytoplankton in the aquaculture pond and improves the natural productivity.

***Paracoccus* spp.:** *Paracoccus pantotuophus* when applied during the pond preparation helps in reducing the hydrogen sulphide. Another multifunctional species *Paracoccus denitrificans* functions in converting nitrate in the system to nitrogen gas and release it into the atmosphere. Additionally these bacteria remove phosphorous from the systems which help in reduction of algal bloom.

***Rhodococcus* spp.:** Ability of these bacteria in the degradation of organic pollutants has been well documented. However, studies on their usefulness in aquaculture are limited in immunity stimulating effects of cellular extract of these bacteria in rainbow trout.

Photosynthetic bacteria*:** These are the specialised group of bacteria existing widely in nature having a number of functional factors and has great potential as probiotics for aquaculture applications. Commercially ***Rhodopseudomonas palustris and***Rhodobacter sphaeroides*** are widely used as probiotics in shrimp farming. These bacteria are known for their ability to switch between four different modes of metabolism. They can function as photoautotrophic, photo-heterotrophic, chemoautotrophic and chemoheterotrophic. This effectively means that these bacteria, a) can grow with or without oxygen, b) use light, inorganic compounds, or organic compounds as energy source, c) acquire carbon from either carbon dioxide fixation or green plant-derived compounds and d) also fix nitrogen. These bacteria are also known to inhibit the growth of Vibrio in fish culture ponds due to their efficient nutrient scavenging ability, though no information is available regarding the similar effects in shrimp culture.

***Thiobacillus denitrificans*:** These are unusual probiotic bacteria which are widely distributed in environment that can synthesise its own food from inorganic substances. This bacterium, are useful specially due to its ability to couple two reactions: denitrification (converting nitrite/nitrate to nitrogen gas which is released in to the atmosphere) and oxidation of sulphur compounds. However, denitrification efficacy of these bacteria are useful if the ammonia oxidising bacteria (*Nitrosomonas* spp) are available in sufficient quantity in pond environment or applied externally to convert the toxic ammonia to nitrite in the system.

***Cellolomonas* spp**: These occur as a group of bacteria involved in degradation of cellulose and enhance the digestibility of the feed.

Rationale for probiotic application

Environmental conditions required for multiplication of the probiotic bacteria in the pond and in animals depends on the concentration and schedule of application, combination of probiotics bacteria and mode of application which needs to be considered by farmers for judicious use of probiotics in shrimp aquaculture.

Environmental conditions required for multiplication

One needs to understand that probiotic product is a single or consortia of live bacteria which have to be multiplied in the pond environment in sufficient quantities to effectively conduct the intended function. These bacteria are very sensitive to parameters like pH, temperature, salinity, DO, concentration of nitrite, ammonia etc. The levels of multiplication and their efficiency of metabolic function depends largely on the pond environmental parameters and availability of specific nutrient. Hence it is not necessary that a probiotic product works similarly in all seasons, regions and stage of grow-out culture. Hence there is a need for scientific evaluation of the identified product's effectiveness under the varying culture conditions. Some of the commercial probiotic products tested in our laboratory containing one to 10 billion bacterial cells have shown to contain only 1 to 5 million. Hence, the effectiveness of the product not only depends on the periodicity and environmental conditions in the pond but also on the bacterial concentration.

Dose and schedule

For any micro-organism to function in a specified manner under the given environmental condition they need to be

Table 1.Types of probiotics available in Indian market and their microbial composition

Gut acting probiotics	*Bacillus pumilus, B. megaterum, Bacillus subtilis, B. polymyxa, B. licheniformis, Cellolomonas* spp
Water probiotics	*Nitrosomonas* spp, *Nitrobacter* spp
Soil probiotics	*Paracoccus* spp, *Rhodococcus* spp, *Rhodobacter* spp, *Thiobacillus denitrificans*

maintained at some minimum numbers. These microbes need to be colonised in the environment before they can effectively exhibit the beneficial functions. Hence, it is essential to apply the selected probiotic product at the recommended dosage and schedule. Many farmers are applying high doses and more frequently than the recommended one, expecting to have more benefit in one go, which ultimately leads to high cost of production. One of our recent studies in Gujarat has indicated that higher levels of probiotic application does not improve the survival, growth rate, average body weight, FCR and production (kg/ha) in comparison to ponds with moderate applications. Hence, there is an ample scope for reducing the quantum of probiotics applied without compromising the economic benefits in shrimp farming. Generally, if the pond has been properly prepared, the water quality is not of much concern during the initial period of culture, since the biomass and metabolic rates are low. As the culture progresses, the shrimp biomass increases and water quality deteriorates, mainly as a result of accumulation of metabolite waste, unutilised, and decay of biotic material, and the pond ecosystem comes under stress. Thus, the application of probiotics in the first quarter needs some consideration due to low quantum of raw material for microbial growth. However, with the increasing biomass in the later part, and with the microbial growth being minimal or almost not available, leads to accumulation of wastage (organic load). So application of the probiotics becomes essential.

Unscientific combination of probiotics

Many of the probiotics products in the market have different combinations of gut, water and soil bacterial consortia. The consortia of probiotics products contain the combination of bacteria involved in nitrogen cycle (mainly functioning in the water column), inhibition of pathogenic bacteria (mainly functioning in the gut) and sulphur cycle (mainly functioning at pond bottom).Though different bacterial populations have different mechanism and site of action, the product is being applied to the pond directly through broadcasting. On the other hand, different groups of bacteria function synergistically if applied in suitable combinations. Since *Thiobacillus denitrificans* has both sulphur oxidising and denitrifying ability, the practice of application of ammonia oxidising bacteria, before *T.dentrificans* helps in controlling the high concentration of ammonia. However, commercial probiotics products containing *T.denitrificans* do not contain the *Nitromonas* spp of bacteria.

Mode of application

Under shrimp culture operations, probiotics such as gut probiotics are applied by coating on the feed or broadcast in case of the soil or water probiotics. For the selected probiotic product to exhibit its intended benefit, method of application to deliver it to site of action is very crucial which is often neglected. As the name of probiotics suggest, a gut acting probiotics should reach the shrimp gut and similarly the water and soil probiotics to the pond water and bottom respectively. Since these three types of probiotics have different site specific microbes with specific action and so they are to be applied accordingly. However, these principles are overlooked when the commercial probiotic products are packaged and released to market. It is very common to see in the market, products with mixture of gut acting and soil acting probiotic bacteria with suggested method of application as broadcast, which reaches the pond bottom. Scientifically it is difficult to understand how gut acting probiotic bacteria reach the shrimp gut to control pathogenic vibrio when broadcast. Hence, it is important to understand the type of probiotics and its mechanism of action to decide the appropriate method of application. Claims on the effectiveness of these combinations, need to be supported with scientific studies.

Labelling of probiotic products

As per the Drug Controller Act, every medicinal product including probiotics used in human and veterinary practice need to be labelled with contents, concentration, dose and indications. However, most of the products do not follow such regulations in Indian aquaculture. Thus the market is full of products without proper labelling and some manufacturers claim effectiveness of the product in removing ammonia, cleaning the pond bottom and improving the growth and survival of shrimp. So, farmers should be aware of such products and avoid their applications without scientific backing.

Thus, there is an urgent need to strictly regulate the manufacture and use of these probiotics to obtain their intended prophylactic effects. So, the aquaculture professionals need to understand that probiotics are prophylactic but not therapeutic agents, So there is a need for judicious application of probiotics in the culture period with the understanding that they are slow in action but with the intended effect.

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Remains of 325 million year old shark found

Scientists are reported to have announced the discovery of an impeccably preserved fossilised remains of a shark that lived 325 million years ago in what is now Arkansas in the USA, complete with a series of cartilage arches that supported its gills and jaws, a report says.

Exceptional discovery: Because shark skeletons are made of soft cartilage, not hard bone, finding anything more than scrappy fossilised remains of teeth and vertebrae is rare. Finding a fossil shark in an almost three-dimensional state of preservation which showed important skeletal structures, is exceptional.

This primitive shark, named *Ozarcus mapesae,* may lead scientists to rethink shark evolution, erasing the notion that these fishes of the deep have remained little changed since they first appeared at least 420 million years ago.

"These fishes have been very successful, among the top predators in the Earth's oceans, for over 400 million years," said paleontologist John Maisey of the American Museum of Natural History in New York, one of the researchers, is reported to have said.

He is reported to have further said: "The best analogy I can come up with is this: It is like comparing a Model T Ford with a modern automobile. They are both recognisably the same kind of thing. But they are completely different under the hood. We found the Model T of sharks," whose research was published in the journal *Nature.*