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Probiotics in aquaculture

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ABSTRACT

Use of probiotics, the beneficial digestive bacteria, are well documented in human and animal nutrition. Bacteria belonging to the genera Lactobacillus, Enteroccocus, Pediococcus *and* Bacillus, *microscopic fungi and saccharomyces yeast have been widely used as probiotics. In aquaculture, the usage of probiotics is mainly confined to hatcheries of shrimps, bivalves and fishes. However, scientific studies are scanty on the use of probiotics in grow out systems of fish/shrimps and even its benefits are debated. Contrary to the traditional use of probiotics as feed additives, it is used in aquaculture and production systems to modify the microbial population of the environment ultimately leading to better growth and survival of the targetted species. Even non-pathogenic strains of pathogenic bacteria are being used as probiotics in shrimp culture. Use of various brands of commercial probiotics have become a regular farming practice in shrimp culture in India, particularly after the major viral disease outbreak in 1995. The paper reviews the use of probiotics in 'coastal aquaculture systems'.*

Introduction

Rapid stride in coastal aquaculture received a temporary setback due to widespread disease outbreaks in shrimp farms in early 90's. The panic reaction from the farmers resulted in large scale use of chemical and biological preparations to prevent and control disease. Indescriminate use of antibiotics, chemicals and pesticides coupled with unplanned, mushrooming growth of shrimp farms in developing countries created social and environmental problems drawing attention from scientists, environmentalists and legislators. Consequently the emphasis of coastal aquaculture, especially shrimp culture, has shifted from maximising production to sustainable production with minimum damage to environment. The search for safe and permanent solution has led aquascientists to the use of beneficial microorganisms or 'probiotics' for environmental clean up and maintenance of water quality, in addition to their role in nutrition as well as in exclusion of endogenous pathogens. This article reviews the use of probiotics in aquaculture, with special reference to shrimp culture.

What are probiotics

The term probiotics was used originally to describe the organisms and substances that contribute to intestinal microbial balance. Such substances prevented colonization of gut by pathogenic organisms and promoted utilisation of feed. Use of probiotics is well documented in human and animal nutrition. Bacteria belonging to the genera *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Bacillus*, microscopic fungi and Saccharomyces yeast are the main probiotics used in animal nutrition. Contrary to its traditional use as food additives, probiotics are used in aquaculture to mainly modify and manipulate the microbial population of the environment and to reduce or eliminate selected pathogenic species of microorganisms leading to better growth and survival (Jory, 1998). According to Morrarty (1966) microbial food webs are an integral part of aquaculture ponds and have direct impact on productivity.

Probiotics in live feed and fish culture

Probiotics are widely used in livefeed culture of rotifer, *Artemia* and copepods used as feed for larval fish in many hatcheries. Garriques and Arevalo (1995) described the use of spray dried probiotic bacterial preparation to increase production of rotifer and to limit bacterial proliferation in rotifer during enrichment with fish oil. Its use also reduced the proportion of vibrios in the bacterial flora of rotifer. Same authors reported that digestions of micro algae was enhanced in *Artemia*, when it is grown with bacteria (Flexibacter) as food supplement. The growth of Japanese flounder fry was enhanced when it was fed with rotifer fed on Lactic Acid Bacteria (LAB). The total count of bacteria reduced from 99,000 to 54,000 in LAB treated rotifer after enrichment with cuttlefish oil (Gatesoupe *et al.*, 1989). Similar reduction in gram negative bacteria (*Virbio spp.*) was reported into Copepod *Aearlea tousa* by treatment with *Bacillus* spp. isolated from common stort (*Centropomus undecemalis*) larvae (Fernandez *et al.*, 1995). Differential growth rate was obtained when the treated copepod was fed to 7-14 day old tomato clown fish (*Amphiprion frenalus*). The same *Bacillus* spp. increased production and reduced gram-negative vibrios in rotifers (Gianelli *et al.*, 1995) and reduced total bacteria (*Staphylococcus, Monococcus* and *Vibrio* spp.) in *Artemia* (Gensler *et al.*, 1995). High survival and weight gain and enhanced resistance to pathogenic vibrios were reported in turbot (*Scophthalmus maximus*) larvae when fed with probiotic enriched rotifer and bacteria (Garriques and Arevalo, 1995).

Gut flora, which play prominant part in animal nutrition, do not have a major role in digestion in cold water fish but do play a significant role in intestinal fermentation in warm water fishes (Gatessoupe *et al.*, 1989). Besides enzymes, the gut bacteria in fishes may bring in nutrients like vitamin B12. Use of antibiotics have only temporary effects in gut microflora since plasmid mediated resistance will be transferred to pathogens. Lactic acid bacteria (LAB) like *Streptococcus, Lactobacillus* and *Carnobacterium* belong to the normal microflora of the gastrointestinal tract in healthy fish but the population of LAB in digestive tract is affected by nutritional and environmental factors like polyunsaturated fatty acids, chromic oxide, stress, salinity etc. LAB isolates from fish gut can act as probiotics and prevent colonization of gut by pathogenic bacteria (Ringo and Gatesoupe, 1998). Sujita and Shibuya (1996) were successful in isolating intenstinal bacteria, from seven species of fish. that have antibacterial activities against pathogenic bacteria.

A case of 'good vibrio' (which form yellow colonies in TCBS agar) acting as probiotic in fish was reported by Austin *et al.* (1995). A strain of *Vibrio alginolyticus* inhibited growth of fish pathogens like *Vibrio ordalli,*, *V.angullarum, Aeromonas salmonicida* and *Yersinia ruckeri*. When the probiotic was added to Atlantic salmon culture, a reduction in mortality was recorded when challenged with *A. salmonicida*. Smith and Davey (1993) isolated a fluorescent strain of pseudomonid bacteria that could competitively inhibit the growth of fish pathogen *A. salmonicida* in culture media, probably due to competition for iron.

Probiotics used in shellfish larval rearing

Growth and survival of prawn and crab larvae could be enhanced by use of selected bacterial strains that had vibriostatic activity (Maeda and Nogami, 1989). A similar result was obtained in the crab (*Portunus trituberculatus*) larvae by a bacterial strain isolated from crustacean culture pond (Nogami and Maeda, 1992). This bacteria strain, in addition to competitive exclusion of pathogens, also acted as a nutrient source.

Vibrios are among the natural microflora of shrimps (Lightner, 1993) and some strains like *Vibrio harveyi, Vibrio parahaemolyticus* etc. are opportunistic pathogens. As in the case of fish a strain of *Vibrio alginolyticus* is used as probiotic agent for larval rearing of *Penaeus vannamei* in Ecuador. Use of this strain increased survival of *P. vannamei* post-larvae by competitive exclusion of pathogenic virbios and reduced antibiotic prophylaxis in intensive larval culture systems (Griffith 1995). Hong *et al.* (1998), quoting Ecuadorian scientists, reported relationship between zoea syndrome and presence of *V. harveyi* as type 222 in *P. vannamei*. The zoea syndrome was effectively controlled by using a strain of *V. alginolyticus* as probiotic. Use of *V.alginolyticus* has reduced hatchery down time from 7 days per month to less than 21 days annually. The production of *P. vannamei* larvae was increased by 35% and antibiotic use was decreased by 94% between 1991 and 1994. Farm trial also showed that survival, production, feed conversion and growth rates were not negatively affected by hatchery use of probiotics (GomezGil, 1995).

Use of *Lactobacillus* spp. against pathogenic vibrios and white spot disease was reported in *Penaeus monodon*. Inhibitory activity of two *Lactobacillus* spp. against *Vibrio* spp. *Escherischia coli, Staphylococcus* and *Bacillus subtiles* was demonstrated in laboratory culture. Possibility of using two non-pathogenic vibrio strains against pathogenic viruses, is indicated in the results obtained in fish (Hong *et al.*, 1998).

Use of photosynthetic bacteria (*Rhodomonas* spp.) as probiotic improved water quality, reduced shell fouling in larvae, reduced metamorphosis time by one day and improved post larval production in *Penaeus chinensis*. It also acted as auxiliary food (Hong *et al.*, 1998).

Bacterial strain isolated from natural intestinal muciflora of *P.chinensis* increased desease resistance, low salinity tolerance and weight gain in its larvae. The probiotic bacteria produced digestive enzymes which helped to improve digestion of shrimp larvae (Hong *et al.*, 1998).

Probiotics, serving as food supplement that promotes larval growth have been reported in *Penaeus japonicus* and the crab *Portunus trituberculatus* (Maeda and Nogami *et al.*, 1992) in *P.trituberculatus* larvae (Maeda & Liao, 1994) and in *P.monodon* (Sunilkumar, 1996). In India, most of the shrimp hatcheries use commercial probiotics with promising results in reducing vibrio population in culture water and decreasing disease outbreak, leading to higher survival of *P. monodon* larvae. Another hatchery practice followed in India, as in many South East Asian countries, is providing inoculam, (to the tune of 10% of total volume) from the most successful tanks (Jayagopal, Personal communication).

Probiotic bacteria are being used in molluscan hatcheries also for competitive exclusion of pathogens and as food supplement. Enhanced growth rate of Pacific oyster, *Crassosteria gigas* with probiotic bacteria at a rate of 0.1 million cell per ml, improved survival and growth (Douillet and Langdon, 1994). The probiotic provides essential nutrients or improve oyster digestion by supplying digestive enzymes to the larvae and remove metabolic wastes of the larvae.

Use of probiotics in grow-out

Use of a commercial probiotic at a rate of 6 kg/ha every 7 days for a period of 147-154 days in 0.19 ha ponds growing *P. monodon* was found to reduce ammonia levels, increase survival rates and increase production per hactre (Tobbu *et al.*, 1997). Addition of photosynthetic bacteria in food or culture water was found to improve quality of water by eliminating ammonia nitrogen, hydrogen sulphide, organic acids and other harmful materials rapidly and enhance growth rate of the prawn *P. chinensis* (Hong *et al.*, 1998).

Moriarty (1966) and Suhendra *et al.* (1997) have reported farm trials with probiotic *Bacillus* spp. in Indonesian shrimp forms. The use of probiotic considerably reduced luminiscent vibrio, *V. harveyi*, in water column and sediments, reduced outbreaks due to vibrios and viruses, reduced organic mater accumulation and improved water quality, shrimp size and production. However, strict maintenance of water quality and sludge removal were required to get maximum benefit from probiotic use.

Selective addition of nutrients and expansion of habitats and culture addition may cause shifts in microbial community that can result in faster carbon - nitrogen cycling and removal, eventually reducing ammonia and sludge accumulation in shrimp ponds (Browdy and Bratwold, 1997).

Detritus Management System (DMS) bacteria are used in many shrimp farms in Indonesia to improve water quality leading to enhanced production. DMS bacteria act by accelerating the natural process in the pond, reduce detritus by converting it into CO_2 and reduce the total organic content. It makes more oxygen available to fish/shrimp by reducing the oxygen requirements of heterotrophs. It also extracts phosphorus from water and controls the phytoplankton production by competing with it for phosphorus. It also serve as food source when it forms a coating over reduced detritus. DMS consists of bacteria that reproduce slowly but are highly efficient at reducing the amount of detritus in the pond. Hence they are to be added at regular intervals and at sufficient quantity to get best result.

Chandrika (1999) isolated pigmented *Bacillus* from paddy-cum-prawn fields and prennial prawn fields in Narakkal, Kerala that inhibit growth of pathogenic bacteria like vibrios, aeromonas and *E.coli.* Gram-negative slime forming myxobacteria were also inhibited by two of the isolated *Bacillus* spp. These strains have the potential to be used as probiotic through feed or in DMS.

Commercial probiotics are regularly being used in Indian shrimp farms since 1990. The use of probiotics is reported to give stable phytoplankton bloom, pH alkalinity and improve dissolved oxygen. The ammonia and nitrite levels and vibrio counts were also reduced in probiotic treated ponds. Application of 10 kg of probiotic at the cost of Rs.2500 per kg for a stocking rate of $10/m^2$ are being practiced in few corporate shrimp farms in India for *P. monodon* culture. Fermented juice of tea seed cake, groundnut oil cake, yeast, jaggery and toddy are also being used by some shrimp farmers in India.

Composition of probiotics used in aquaculture

Fifteen species of *Bacillus* constitute the generally recommended microorganisms used as probiotics in pond aquaculture and these form the main component of many commercial probiotics. *Bacillus* are motile, produce endospores to tide over unfavourable conditions, produce antibiotics and enzymes. They grow at high temperature and are easily isolated and cultured in synthetic media using sugars, alcohols and organic acids as carbon sources and ammonia as sole nitrogen source. *Bacillus* are the main component of DMS bacteria. *Bacillus, Lactobacillus, Nitrobactor, Nitrosomonas, Pseudomonas, Cellulomonas, Rhodopseudomonas, Enterobacter, Enterococcus, Pseudococcus, Staphylococcus* etc. are the bacterial flora reported to be present in many commercial probiotics (Boyd 1995, Guillot, 1998). Among the vibros, *V. alginolyticus* strain has been successfully used as probiotic in the shrimp hatcheries in Ecuador.

Application of probiotics

Probiotics are marketed in liquid and powder forms. The hatcheries generally use liquid forms which are directly added to hatchery tanks. The liquid can be applied any time of the day in indoor hatchery tanks, while it should be applied either in the morning or in the evening in outdoor tanks. Good aeration in hatchery tank is required to get best results.

The dry probiotics that come in packets have to be brewed at farm site before application. Each kit of dry probiotic contains a packet of dry powder and a packet of enzyme catalist. Brewing has to be done in clean disinfected water after emptying the packets and blending thoroughly. Usually, it is brewed at 27-32°C for 16 to 18 hours with continuous aeration. The finished products must be used within 72 hours. Maximum aeration is required in semi-intensive culture ponds. If aeration is less, the application of probiotic has to be spread to two consecutive days, applying 50% of the dose each time.

Anaerobic probiotics, specially meant for extensive culture systems are also being marketed by few companies recently.

Action of probiotics

The probiotics act in aquaculture systems in the following manner.

1. Competitive exclusion of pathogenic bacteria.
2. Enhancement of digestion through production of exoenzymes.
3. By moderating and promoting direct uptake of dissolved organic materials.

4. By inhibiting growth of pathogenic bacteria through production of antibiotics.

Other possible mechanisms of action of probiotics are :

a. Controlling phytoplankton and blue-green algal bloom.

b. Preventing off-flavour.

c. Reducing toxic metabolites like nitrate, nitrites and ammonia and phosphates.

d. Increasing dissolved oxygen concentration by efficient removal of detritus and thereby heterotrophs.

e. Maximising carbon mineralisation as CO_2 to minimise sludge accumulation.

f. Maximising primary productivity.

g. Maintaining diverse and stable pond community.

h. Eliminating cost of cleaning ponds after harvest.

i. Reducing cost of disease treatment and crop management.

(Jory 1998, Moriarly 1996, Hong *et al.*, 1998).

Microbial activity in a pond is linked to water quality and disease outbreak. Heterotrophic bacteria use oxygen and produce toxic metabolites and since the diffusion of oxygen is minimum at pond bottom, the aerobic bacteria oxidize nitrate producing toxic metabolites and create an oxygen debt leading to an increase in reduced sediments and anoxic conditions. Shrimps exposed to reduced organic matter has less resistance to pathogens. *Bacillus* spp. produce wide range of exoenzymes which reduce available organic matter for other bacteria and reduce organic matter accumulation at the bottom. The species composition of a microbial community in a pond is determined by chance as well as by favourable physiological factors that helps it to grow and dominate. Chance favours the organisms that happens to be at the right place at the right time. Addition of probiotics is thus giving chance a helping hand (Moriarty, 1996).

Bacteria selected for bioremediation must be selected for specific funcitons and added at high enough density and under the right environmental conditions. According to Boyd (1995), in shrimp ponds, the major factors affecting bacterial activity is dissolved oxygen supply which is usually abundant. If activity of described bacteria is low it is due to poor environmental conditions for bacterial activity and application of bacterial amendments or enzymes may not enhance bacterial activity and improve water quality. He could not get the desired beneficial activities by addition of probiotics in culture ponds.

Future of probiotics in aquaculture

Probiotics are widely used in USA. Japan, European countries, Indonesia, Thailand and India in aquaculture. It has been projected as a promising practice to improve the efficiency of aquaculture systems in general and this technology tends to have the potential to be a significant factor in sustainable shrimp culture. Experience of Boyd (1995), however, has demonstrated that simple addition of probiotics will only increase production cost and may not yield the desired results and the right environment has to be provided (Moriarty, 1996) for that. Many vendors of commercial probiotics are not aware of the physiological and ecological requirements of their bacterial products and do not pass the information to the clients (Boyd, 1995). Utmost care should be taken in selection of appropriate probiotic combinations and all necessary environmental safeguards should be provided to get optimum benefits from probiotic use. Exaggerated claims of probiotic combinations are to be scientifically evaluated in different culture conditions. Unfortunately, very few studies have been published on actual field trials. It is desirable to discourage use of imported probiotics, till its beneficiary effects in local conditions are properly evaluated. Another potential social problem in countries like India will be the handling of probiotics at farmsite. The farm hands, mostly, unskilled, should be properly trained in handling microbial preparation while brewing at farm site to avoid accidental infections to them.

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