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PROBIOTICS AND ITS APPLICATION IN MARICULTURE

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Introduction

Aquaculture is a multidisciplinary activity, more complex than agriculture due to the multidimensional aquatic medium. The aquaculture boom and increased socio-economic benefits together with increase in extent and intensity of aquaculture are alleged to have created several problems, particularly those of deteriorating water and soil quality and outbreak of diseases (Kutty, 1999). For example, the global production of farmed shrimp has doubled in the past 15 years. India has also witnessed such spectacular growth in shrimp farming until the mid nineties, after which it became beset with disease and environmental problems. Overstocking, overfeeding and excessive use of antibiotics during farming are some of the reasons attributed to the outbreak of shrimp diseases. Even by the year 2001, a total solution to the problem has evaded the scientists and policy makers. But what has been recognised now by scientists and policy makers is that aquaculture must be environmentally friendly so that it can sustain itself without seriously affecting the coastal ecosystem. In other words, the ecological footprint of aquaculture must be sufficiently small so as to ensure sustainability.

In the recent past, the farm animal production industry in the west was also plagued with disease outbreaks resulting in excessive use of antibiotics. The resulting residual antibiotics in meat products lead to curbing of its usage in animal rearing and production. The farmers then turned to an age-old practice of using beneficial bacteria to quell infectious diseases. Thus, Parker (1974) introduced the modern concept of probiotics more than 25 years ago. Aquatic animals are quite different from land animals for which the probiotic concept was developed, and therefore, the probiotic usage in aquaculture, especially in shrimp culture has taken a different meaning. The high risk of losing their crop to disease attack has prompted many shrimp farmers all over the world, and especially in India, to use probiotics during their culture operations.

In this paper, an attempt is made to review the state of probiotic usage in aquaculture, particularly shrimp farming. The concept of probiotics as used in animals and humans and the manner in which it is differently employed in the aquatic environment will be examined in detail. A pointer to the future research needs in this direction will also be attempted.

What are probiotics?

The origin of the term probiotic is attributed to Parker (1974) who defined them as organisms and substances, which contribute to intestinal microbial balance. However, the concept of microbial manipulation was first appreciated by Metchnikoff during the early 1900s when he viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. Although evidence for a link between longevity and ingestion of fermented milk products has not been proven yet, some workers have claimed that its therapeutic value is related to viable bacteria, in particular *Lactobacillus* sp. Although a strict definition of probiotics is difficult to come by, Tannock (1997) proposed it as "living microbial cells administered as dietary supplements with the aim of improving health". Gatesoupe (1999) reviewed the state of probiotic usage in aquaculture and stated that the first application of probiotics in aquaculture is relatively recent, but the interest in such environmentally friendly treatments is increasing rapidly.

There now exists a growing number of scientific papers, which deal specifically with use of probiotics in aquatic animals. Yet, more questions have been raised as to whether probiotics have any relevance in the aquatic environment (Gatesoupe, 1999). Aquatic animals are quite different from land animals for which the probiotic concept was developed. Live-bearing endotherms undergo embryonic development within an amnion, whereas the larval forms of most fish and shellfish are released into the external medium at an early ontogenetic stage. Thus the latter are exposed to all types of microflora available in the medium, while the former develop a particular type (obligate or facultative anaerobes) of gastrointestinal microbiota. Most identified probiotics belong to the dominant or sub-dominant genera of *Bifidobacterium*, *Lactobacillus* and *Streptococcus*. On the other hand environmental microbes like *Vibrio* and *Pseudomonas* are the most common genera in crustaceans (Moriarty, 1990), marine fish (Sakata, 1990) and bivalves (Prieur et al., 1990).

As compared to terrestrial livestock where resident microbes benefit from a fairly constant gastrointestinal habitat, the intestinal tracts of aquatic animals have microbes that are transient (Moriarty, 1990). Aquatic animals being poikilotherms, their gut-associated microbiota varies with temperature (Liesel, 1990) and salinity (Ringo and Strom, 1994). The continuous water flow increases the influence of the surrounding medium, in much the same way as the effect of water flow observed in filter feeders like bivalves, shrimp larvae and live food organisms (Gatesoupe, 1999). The environment and the food eaten play a key role and thus, in bivalves (Sugita et al., 1981; Prieur et al., 1990) and in penaeids (Moriarty, 1990) the associated microbiota is very similar to those found in seawater and sediment. In larval and juvenile fish, the influence of food on gut microflora has been clearly demonstrated (Ringo et al., 1995; Gatesoupe, 1999). Similar conclusions on crustaceans, especially penaeids are yet to be made although the influence of bacteria brought through live food organisms is well known.

Types of Aquatic Probiotics

Recognising the conceptual difference of terrestrial and aquatic probiotics, Gatesoupe (1999) suggested a modification in the definition of probiotics as used in aquaculture. He defined probiotics as - *microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health*. He further classified (Fig.1) the microbial preparations used in aquaculture into 3 types – biocontrol agents, probiotics and bioremediation agents. Biocontrol agents are those methods of treatment using the antagonism among microbes to kill or reduce the number of pathogens in the aquaculture environment (Maeda et al., 1997). Those bacterial treatments which improve the water quality and thus indirectly the production were termed as bioremediation agents.

The bioremediation agents have also been termed as bioaugmentation agents or water additives (Moriarty, 1998) and probiotics. Porubcan (1991a) reported the increase in yield and survival of *Penaeus monodon* by using floating biofilters pre-inoculated with nitrifying bacteria. These helped to decrease the amount of ammonia and nitrite in the rearing water. He (Porubcan, 1991b) further reported that the introduction of *Bacillus* spp. in proximity to pond aerators reduced the chemical oxygen demand and increased the yields. Recently, several commercial products have sought to exploit the idea that bacteria, which improve water quality, may be beneficial to animal health. Among shrimp farmers in India, these products are known as *water-probiotics* and most of them contain nitrifying bacteria and/or *Bacillus* spp. The nitrifying bacteria have strict ecological niches, and they have not been detected in the gastrointestinal tract of animals (Gatesoupe, 1999). *Bacillus* spp. are not autochthonous in the gastrointestinal tract, but they have been isolated from fish (Kennedy et al., 1998; Sugita et al., 1998), crustaceans (Austin and Allen, 1982; Sharmila et al., 1996), bivalves (Sugita et al., 1981) and shrimp larval rearing medium (Mohamed, 1996; Rengpipat et al., 1998). Many of these *Bacillus* spp. strains have antibiotic properties and may be active during intestinal transit.

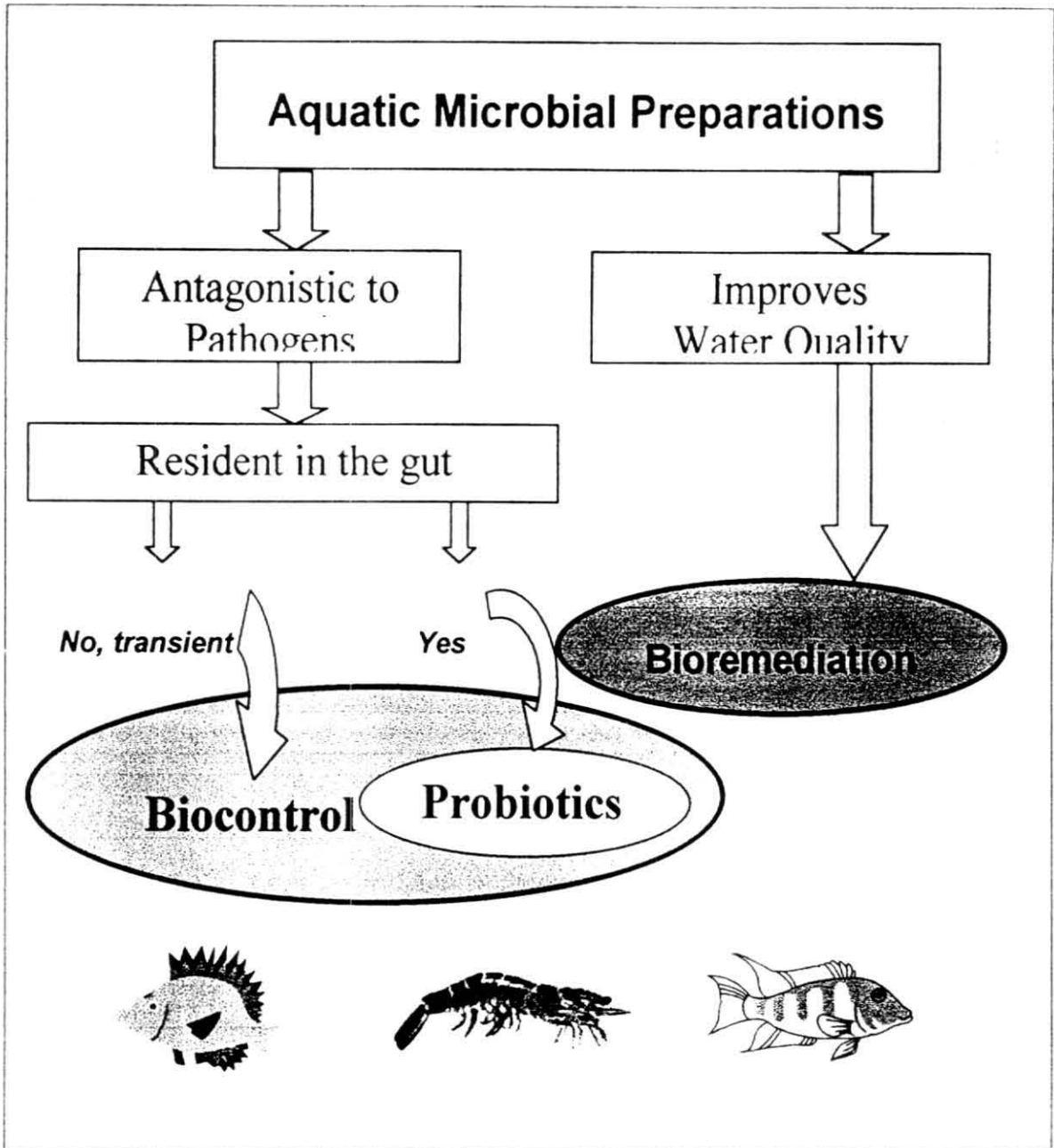


Fig. 1. Classification of microbial treatments used in aquaculture according to current terminology modified from Gatesoupe (1999).

It is important to clear the concepts and definitions with regard to the term probiotics. At present the definitions and classifications brought forth by Gatesoupe (1999) serve the purpose and can be applied without confusion in shrimp culture. The commercial availability of probiotics and bioremediation agents in shrimp culture and its widespread usage in India has spawned separate terminologies among shrimp farmers. The strict probiotic agents are known as gut-probiotics and the bioremediation agents are known as water-probiotics.

Modes of Action

Probiotics

Several mechanisms have been investigated whereby bacteria could function as a probiotic. These include adhesion to digestive tract wall to prevent colonisation of pathogens (competitive attachment), neutralisation of toxins, bacteriocidal activity and increased immune competence. The experimental introduction of lactic acid bacteria (LAB) into the intestine of fish has already been reviewed (Ringo and Gatesoupe, 1998). Several studies have shown that it is possible to maintain artificially the LAB population at high levels by regular intake through feed especially in cod, salmon and turbot. Such studies on tropical fish species and crustaceans are lacking. Adhesion is acknowledged as the first step of a microorganism in the process of colonisation and the intestinal mucous plays a vital role in this process. There are reports in endothermic animals that there is a certain degree of host-specificity in the adhesion process. Adhesion to intestinal mucous has also been assayed in vitro in fish (Joborn et al., 1997). Intestinal bacteria of turbot adhere specifically to intestinal mucous than to any control surface. Specific adhesins have been demonstrated in the adhesion of yeasts to intestinal cell walls of rainbow trout (Vazquez-Juarez et al., 1997). Similar studies on crustaceans in general and shrimps in particular are wanting.

Production of lactic acid by lactic acid bacteria reduces the pH of the stomach contents in endothermic animals. Studies in vitro have shown that an acid condition of less than pH 4.5 prevents the growth of many bacteria including coliforms, but still allows the growth of some strains of lactobacilli (Sissons, 1989). LAB are also known to produce hydrogen peroxide which has bacteriocidal actions in vitro and produces a metabolite thought to neutralise the effect of enterotoxin released from coliforms. Clearly LAB is the best studied among all probiotics, even so, the effect of its use in crustaceans has not been studied. Although LAB is not the dominant gut microflora in marine fish as compared to endothermic animals, researchers have been able to introduce LAB into larval and juvenile fish with pronounced protection against *Vibrio* infections. Uma et al. (1999) reported that the growth and survival of *P. indicus* juveniles were significantly improved by the addition of Lacto-sacc™ (a commercial livestock probiotic feed supplement composed of *Saccharomyces* sp., *Lactobacillus acidophilus* and *Streptococcus faecium*) at levels ranging from 2.5 to 7.5 g/kg basal feed. A challenge with *Vibrio alginolyticus* resulted in low mortality rate in Lacto-sacc™ fed animals than in control group. However the presence of these organisms in the gut of the shrimp after feeding was not ascertained. In a more recent study, Sridhar and Raj (2001) introduced strains of *Bacillus* and *Micrococcus* isolated from shrimp guts by coating them on compounded diets into *P. indicus* post-larvae. They observed significantly higher specific growth rates and survival in treatment groups than control. Upto 10^6 cfu/shrimp of probiotic organisms was detected in the gut of the post-larvae. Similar introductions are yet to be made with other marine shrimps, yet, it must be borne in mind that Lactobacilli have never been isolated from shrimp guts.

Enhanced immunity by probiotic treatment has been well demonstrated in endothermic animals. For example, raised activities of macrophages and lymphocytes in mice following oral inoculation with LAB was observed implying an immunopotentiating role for LAB in the gut. Itami et al. (1998) reported the enhancement of disease resistance of *Penaeus japonicus* after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. They reported that the phagocytic index and survival after challenge with *Vibrio penaeicida* and white spot syndrome baculovirus of shrimps fed with peptidoglycan was significantly higher than that of control. The immune effects of probiotics in crustaceans is an area in which there is little work and needs immediate attention.

Biocontrol Agents

Antagonism to pathogens is a characteristic of a good aquatic biocontrol agent. Antagonism may be mediated not only by antibiotics, but also by many other inhibitory substances like organic acids, hydrogen peroxide and siderophores (Gatesoupe, 1999). These compounds produced by the biocontrol agents are highly dependent on experimental conditions that are different in vitro and in vivo conditions. It was Maeda and Liao (1992) who first isolated a strain "PM-4" (subsequently identified as *Thalassobacter utilis*) from the rearing water of larval *Penaeus monodon* for use as a biocontrol agent. This strain increased the survival rate of the larvae of *P. monodon* and the swimming crab *Portunus trituberculatus* and repressed the growth of *Vibrio anguillarum* (Maeda et al., 1997; Nogami et al., 1997). Mohamed (1996) used several strains of heterotrophic bacteria as feed for *P. monodon* larvae and found that a strain of *Pseudomonas* increased the percentage survival and a strain of *Micrococcus* increased the metamorphic rate to PL-1 stage. Haryanti et al. (1998) reported the increased survival of *P. monodon* larvae on rearing with a strain "BY-9" which also inhibited the growth of *V. harveyi*.

In Ecuadorian shrimp hatcheries Griffith (1995) reported the control of *Vibrio parahaemolyticus* associated outbreak of vibriosis through the artificially increasing the proportion of *V. alginolyticus* in the rearing medium. Hatchery down time was reduced from approximately 7 days per month to less than 21 days annually, while production volumes increased by 35% and overall antibiotic use was decreased by 94% between 1991 and 1994. Further, Griffith (1995) found that the survival, production, feed conversion and growth rates in the farm were not negatively affected by the use probiotic fed larvae, on the contrary, they were even improved by their application. Table 3 lists the various antagonist agents tried in crustaceans, its source and effect. It can be seen that some bacteria are even antagonistic to viruses and they may even work as a biocontrol agent for viral diseases.

Table 3. Antagonism of aquatic microbes isolated from crustaceans and its effect (modified from Gatesoupe, 1999)

Biocontrol Agent	Source	Tested Against	Effect	Reference
<i>Alteromonas</i> sp	<i>Palaemon macrodactylus</i>	<i>Lagenidium</i> (fungus)	Protection of crustacean embryos from fungal infection	Gil-Tunes et al., 1989
<i>Alteromonas</i> -like	Shrimp hatchery	<i>Vibrio</i> sp.	Protection from vibriosis	Tanasomwang et al., 1998
<i>Pseudoalteromonas undina</i>	Seawater	<i>V. anguillarum</i> IHNV	Increase in growth and survival of larvae	Maeda et al., 1997
<i>Thalassobacter utilis</i>	<i>P. monodon</i>	<i>Haliphthoros</i> sp. (fungus)	Increase in survival of larvae	Nogami et al., 1997
<i>T. utilis</i>	<i>P. monodon</i>	<i>V. anguillarum</i>	Increase in survival of crab larvae	Nogami and Maeda, 1992
<i>V. alginolyticus</i>	<i>P. monodon</i>	<i>V. harveyi</i>	Inhibitory effect	Ruangpan et al., 1998
<i>V. alginolyticus</i>	Shrimp hatchery	<i>Aeromonas salmonicida</i> , <i>V. anguillarum</i> , <i>V. ordalli</i> , <i>Yersina ruckeri</i>	Increased resistance of salmon against experimental infections	Austin et al., 1995
<i>Vibrio</i> sp.	Shrimp hatchery	IHNV, OMV	Effect against fish viruses	Direkbusarakom et al., 1988
<i>V. alginolyticus</i>	Shrimp hatchery	<i>V. parahaemolyticus</i>	Increase in shrimp larval survival	Griffith (1995)
<i>Pseudomonas</i> sp., <i>Micrococcus</i> sp.	Shrimp hatchery	Not determined	Increase in shrimp larval survival and metamorphic rate	Mohamed (1996)

In another recent study by Rengpipat et al.(1998) *Bacillus* S11 bacterium isolated from tiger shrimp habitats in Thailand was added to shrimp feed in three forms: fresh cells, fresh cells in normal saline and a lyophilized form. After a 100-day feeding trial with probiotic supplemented and non-supplemented (control) feeds, *P. monodon* (from PL 30) exhibited significant differences ($p < 0.05$) in growth, survival and external appearance

between probiotic and control groups. There were no significant differences among the three treatment forms. After challenging the shrimps with a shrimp pathogen, *Vibrio harveyi*, by immersion for 10 days, all probiotic treatment groups had 100% survival, whereas the control group had only 26% survival. The main bacterial flora in control group shrimp guts was *Vibrio* spp., while those in all treatment groups were mostly *Bacillus* S11. This kind of bacterial species replacement was also observed in the rearing medium and faeces. However, whether the *Bacillus* S11 was able to colonise the gut even after stopping the probiotic feeding was not investigated.

Bioremediation Agents

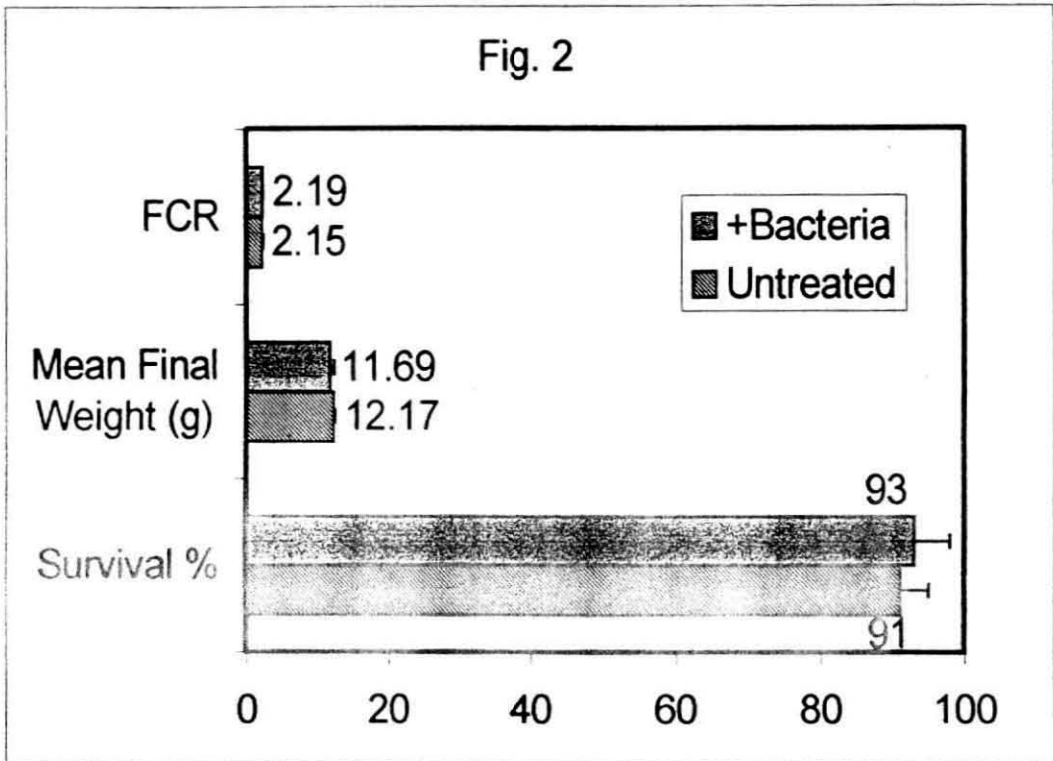
The importance of microbial communities in aquaculture systems and pond productivity cannot be over stressed. Bioremediation agents serve to modify or manipulate the microbial communities in water and sediment such that they reduce or eliminate selected pathogenic microbes and generally improve growth and survival of the targeted species. There are various ways through which bioremediation agents could act in aquaculture systems. These include competitive exclusion of pathogens, enhancing digestion through the supply of essential enzymes, moderating and promoting the direct uptake of dissolved organic materials, active promotion of pathogen inhibiting substances and other possible mechanisms (Jory, 1998). According to Bratwold et al. (1997) the specific ecological applications of microbial ecology management in shrimp ponds include the following: optimising nitrification rates to keep low ammonia concentrations, optimising denitrification rates to eliminate excess nitrogen from ponds as nitrogen gas, maximising carbon mineralisation to carbon dioxide to minimise sludge accumulation, maximising primary productivity that stimulate shrimp production and also secondary crops and maintaining a diverse and stable pond community where undesirable species do not become dominant.

In spite of these theoretical advantages, published results on bioremediation agents, particularly its use in aquaculture ponds, are contradictory. Boyd (1995) and Boyd and Gross (1998) found that bacterial (*Bacillus* sp.) additions in the pond did not improve the water quality as expected. He however observed higher survival of fish in ponds treated frequently with 3 species of live *Bacillus*. The mode of action was unknown because water quality was not measurably improved. Pond studies also showed that applications of an enzyme preparation tended to enhance microbial mineralisation of organic matter, but no effect on fish production was observed. Boyd and Gross (1998) concluded that too little is known about the modes of action these bioremediation agents, the conditions under which they may be effective, their application rates and methods for general recommendation of their use. Nevertheless, the products are safe to humans and the environment, and their use poses no hazards. Therefore, commercial producers should conduct trails with these products, and researchers should conduct experiments with them.

Only a few properly controlled, well documented and peer reviewed studies on probiotic (bioremediation agents) usage in fish and shrimp rearing have appeared in literature (McIntosh et al., 2000). Most of such studies show that the addition of

probiotics has no effect on the water quality of cultured shrimps (Samocho et al., 1998) and cultured channel catfish (Queiroz and Boyd, 1998). McIntosh et al. (2000) hypothesised that the outcome of microbial supplement addition may not be profound in aquaculture facilities where wastewater is flushed daily out of the system. Hence they conducted a study to evaluate if routine use of a commercially produced bacterial supplement could improve water and sludge quality, and *Litopenaeus vannamei* under zero water discharge with a low protein diet and high stocking density. Fig. 2 shows their results in brief. There was no significant difference in the values of survival, mean final weight and FCR of *L. vannamei* between treated and untreated tanks. Besides, there was no significant differences in water and sludge parameters between the untreated shrimp tanks and those that were treated with the bacterial supplement. This study suggests that producing shrimp with 'zero water discharge' does not have any detrimental effect on the survival, mean final weight, FCR and water quality during the grow-out period. While the application of a bacterial supplement did not damage the animals or water quality, it did nothing to improve these parameters.

Fig. 2. Shrimp survival, mean final weight and FCR of shrimp with or without the addition of a bacterial supplement. There is no statistical difference ($P>0.05$) between treatments. Modified from McIntosh et al. (2000)



There are only few reports (Suhendra et al., 1997; Moriarty, 1998; Prabhu et al., 1999) of bioremediation agents working well in shrimp aquaculture systems. In an experiment conducted in Indonesia, Moriarty (1998) compared luminescent *Vibrio* sp. counts and shrimp production in ponds in which a *Bacillus* sp. based bioremediation agent (PondPro-VC™) was used and those in which it was not used. The value of adding selected strains of *Bacillus* as bioremediation agent to control the *Vibrio* populations in

farms using the same water sources, which contained luminous *Vibrio* strains, was demonstrated. The farms that did not use the *Bacillus* cultures experienced almost complete failures in all ponds, with luminescent *Vibrio* disease killing shrimps before 80 days of culture were reached. In contrast, a farm using *Bacillus* cultures in abundance (10^4 - 10^5 / ml) was culturing shrimps for over 160 days without problems. *Vibrio* numbers, especially luminous *Vibrio* numbers were low in treatment pond water and nil in sediment (Table 4). Moriarty (1998) concluded that bioremediation agents are a significant management tool in shrimp culture practice, but their efficacy depends on understanding the nature of competition between species or strains of bacteria. Further, they rely on the same concepts that are used successfully in soil bioremediation. Suhendra et al. (1997) also reported similar results on using selected strains of *Bacillus* strains in shrimp ponds in West Java (Indonesia).

Table 4. Total and luminous *Vibrio* counts in pond water and sediment in control ponds and those using *Bacillus* sp. Modified from Moriarty (1998).

Mean values from 6 ponds	Mean Days of Culture	Water (no./ml)		Sediment (no./ml)	
		<i>Vibrio</i>	Luminous <i>Vibrio</i>	<i>Vibrio</i>	Luminous <i>Vibrio</i>
Control ponds	45	3300	180	5672	26082
<i>Bacillus</i> treated	79	3224	25	4.15	0

There are approximately 15 species of *Bacillus*, which are the main components of commercial probiotic (bioremediation) products for pond aquaculture (Jory et al., 1998). According to Jory (1998) there are several characteristics that make *Bacillus* an ideal bioremediation agent in aquaculture (see Table 5). The pond environmental conditions must be efficiently managed so that the addition of bioremediation agents can have a significant beneficial effect (Moriarty, 1998).

Table 5. General characteristics of *Bacillus* sp. which make it an ideal microbe for use as bioremediation agent or probiotic. From Jory (1998).

Advantages of using *Bacillus* sp as Bioremediation Agents

- ⊙ *Bacillus* can easily move around (motile) because they have a whip like flagella.
- ⊙ *Bacillus* form endospores, which are useful under stressful conditions.
Endospores allow *Bacillus* to reproduce when conditions are favourable
- ⊙ *Bacillus* produce antibiotics of which bacitracin, polymixin, trycodin, gramicidin and circulin are examples
- ⊙ *Bacillus* produce special compounds (enzymes) that can break down

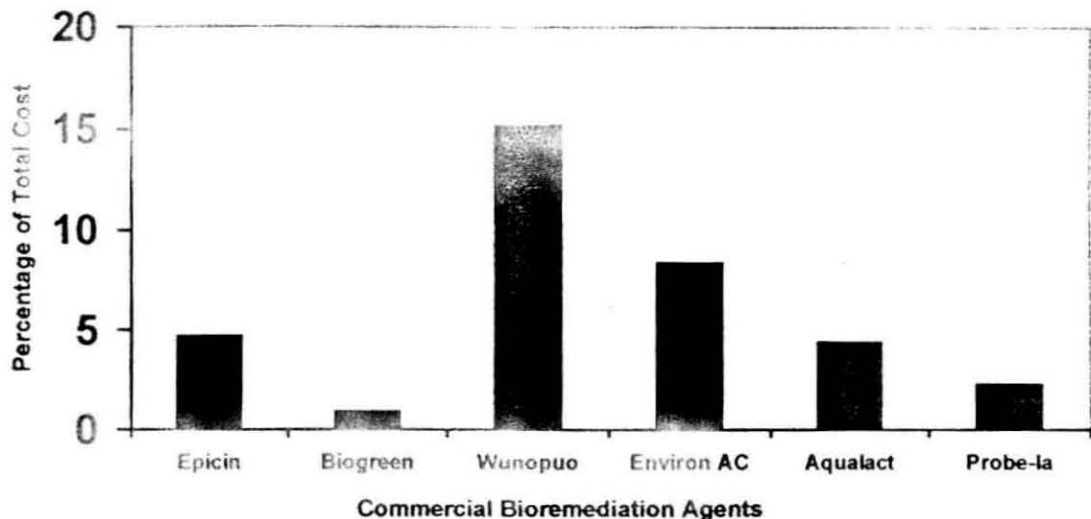
- polysaccharides, nucleic acids and lipids
- ⊙ Bacillus are easily transformable (free DNA is easily incorporated to change its genetic make-up). This is very useful in making 'designer' bacteria
- ⊙ Bacillus are thermophilic, growing at high temperatures (50-70 °C)
- ⊙ Bacillus are easy to isolate from soil or air. They grow well on synthetic media. Ammonium can be its sole nitrogen source. Few isolates require vitamin additions

Adequate oxygen levels (e.g., supplemental aeration) are often a key component for bacterial amendments to work efficiently. Decrease in ammonia levels and increase in nutrient levels and total heterotrophic bacterial counts in ponds where *P. monodon* was cultured for 120 days with application of a commercial bioremediation agent (NS Series Super SPO™) was reported by Prabhu et al. (1999). This also resulted in increased daily growth rates and production of shrimp from the treatment ponds.

Although the advantages of the use of bioremediation agents in shrimp farms is still a point of debate, it is clear that applied research in pond microbial ecology can provide important breakthroughs to improve the environmental sustainability of shrimp culture, particularly in view of the recent negative publicity regarding the environmental impact of shrimp farms.

Notwithstanding these controversies, shrimp farmers in India use a wide variety of commercial probiotics (bioremediation agents) during the grow-out period. In a cost analysis conducted recently on shrimp farms in the state of Andhra Pradesh Anikumari et al. (2001) reported that when probiotics are used the cost of production increases by 0.9-15.2%, for an average production of 1.3 t/ha. Out of the 6 probiotics studied, water probiotics (bioremediation agents) after fermentation and feed probiotics showed lower cost per kg production (Fig.3). The production cost is higher

Fig.3. Cost of bioremediation agents as percentage of total cost in shrimp farms in AP (from Anikumari et al., 2001)



for bioremediation agents that are applied directly. In spite of the high costs, farmers are willing to try untested bioremediation agents in their desperation to save their crop. The survey results (Anikumari et al., 2001) show that the farmers are using both water and feed probiotics. Water probiotics are applied either directly or after fermentation and contains multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. licheniformis*, *Nitrobacter* sp., *Nitrosomonas* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Lactobacillus* sp., *Cellulomonas* sp., *Aerobacter* sp., and the yeast *Saccharomyces cerevisiae*. Testing these commercial bioremediation agents for its cost effectiveness in local farm conditions and setting out the correct protocol for its use is imperative before such products are marketed.

Probiotics in live feeds – Bio-encapsulation

Marine larval rearing involves feeding the hatched larvae with suitable live feeds (diatoms, rotifers, copepods, nematodes, *Artemia* nauplii and metanauplii, mysids etc). Most often live feeds are the primary source of bacterial contamination in rearing systems. By virtue of their size and feeding habits, most live feeds are size specific filter feeders. Therefore, it is possible to incorporate into the live feed particles (say an antibiotic or therapeutic drug like Romet-30 or a probiotic organism) of the appropriate size. This process called bio-encapsulation is thus an innovative means of delivering drugs and probiotic organisms to the larvae (Mohamed, 2001). Indeed, for fish larvae that are active sight feeders, it is the only effective means of drug delivery and several studies have been made on this aspect.

Enrichment of *Artemia* nauplii with a known probiotic yeast *Saccharomyces boulardii* and its role in enhancing resistance against the pathogen *Vibrio harveyi* was investigated by Patra and Mohamed (2003). *S. boulardii* (SB) was cultured, then fed to Instar II *Artemia* nauplii in three different treatments; 10^2 (T1), 10^3 (T2) and 10^4 (T3) colony forming units (CFU) per ml in triplicate. The algae *Nanochloropsis* sp. was used as control diet. Survival and total count of CFU nauplii⁻¹ was observed on different media (Sabouraud, for enumerating yeasts, Thiosulphate Citrate Bile salts Sucrose, for enumerating *Vibrio* and Seawater Agar, for enumerating total aerobic flora) for each replication. Enhanced survival of nauplii was observed in treatments as compared to control. Results indicated that enrichment of *S. boulardii* in *Artemia* nauplii proceeded in a linear fashion, and up to 3500 CFU of *S. boulardii* could be detected in one nauplii at 10^4 CFU ml⁻¹ treatment. No conclusive trend could be observed in the count of *Vibrio* and total aerobic flora due to treatment. Enriched nauplii were then challenged with the pathogen *V. harveyi* for 24 h and 48 h at a concentration of 6.1×10^6 CFU ml⁻¹. The survival counts at 48 h showed that the resistance of the nauplii was significantly ($P < 0.01$) improved in those fed with 10^4 CFU ml⁻¹ *S. boulardii* (90% survival rate after 48 h of challenge versus less than 40% for the infected control group without SB and treatments T1 and T2). This study shows that *S. boulardii*, which has been used for the first time in an aquatic live feed organism, has a profound beneficial effect on the nauplii by increasing its resistance to a pathogenic *Vibrio* infection.

Microalgal cultures are a virtual storehouse of various microorganisms, and therefore, by feeding marine microalgae to marine larvae, we transfer many potentially pathogenic microorganisms to the culture medium. The consequences are low survival and poor quality larvae, besides failure of microalgal culture due to over-growth of microorganisms. Probiotic organisms have been incorporated into microalgal cultures with remarkable benefits. In a recent study Rajiv (2003) found that the addition of the probiotic yeast *S. boulardii* as a single addition to *Chaetoceros* culture resulted in significantly ($P < 0.01$) improved (162% increase in maximum algal density) algal growth rates with prolonged stationary period when compared to the control. It also helped in keeping the total aerobic bacterial counts and total *Vibrio* counts on TCBS in the medium to very low levels.

Perspectives and Conclusions

It is well known that microorganisms cannot be avoided in aquaculture operations (Ringo and Birbeck, 1999). The key to successful management of aquaculture operations lies in the manipulation of these microbes through innovative means such as use of probiotics. The state of the art of aquatic probiotics has not reached to the level found in land animals. The application of probiotics for fish and shrimps, either as a biocontrol or as a bioremedial measure shows promise, but much more research efforts are needed to come to a complete understanding. Gatesoupe (1999) stated that the first question that remains unanswered in most cases is the fate of the probiotic organism in the rearing medium or in the gut. More investigations using molecular and immunological approaches may yield better results.

Even without much research backing, a vast number of commercial probiotic products are being used by shrimp farmers, mostly under pressure from marketing agents and peers. It is essential that proper testing of these products under local environmental conditions be done before they are marketed. Government research laboratories therefore, have to equip themselves for carrying out tests of these products and ascertain the factual in the claims. At the same time, the search for new and better probiotics should continue. Unlike endothermic animals, the ubiquitous environmental microbe *Vibrio* dominates the gut microflora of fishes and shrimps. It is very likely that non-pathogenic *Vibrios* hold the key to isolating and developing a successful probiont for use in aquaculture.

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