COMMERCIAL PROBIOTICS IN TANK REARED NURSERY PHASE OF FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879)

By

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THESIS

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PANANGAD, COCHIN

Dedicated To

My
Grand Mothers
&
Parents

DECLARATION

I hereby declare that this thesis entitled **"COMMERCIAL PROBIOTICS IN TANK REARED NURSERY PHASE OF FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879)"** is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or society.

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CERTIFICATE

Certified that this thesis entitled **"COMMERCIAL PROBIOTICS IN TANK REARED NURSERY PHASE OF FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879)"** is a record of research work done independently by **Ms. E.B.BERYL** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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Introduction

1. INTRODUCTION

Aquaculture is one of the fastest growing food production sectors, which has emerged as an industry capable of supplying protein rich food to the starving millions of the world. Aquaculture management aims to get sustainable, maximum production per unit of waterspread area in a commercially viable manner.

Loss due to diseases is one of the major constraints in the development of aquaculture. It is widely accepted that in disease management, prevention and control are always better than cure. Prevention and control of diseases are vital in the sustainable development of aquaculture. Various therapeutic and prophylactic measures are used for prevention and treatment of diseases in aquaculture systems. In therapeutics, antibiotics are used widely to cure certain diseases while in prophylactics immunostimulants, probiotics, and vaccines are generally used. Prophylactic chemicals/agents serve to prevent diseases unlike therapeutics, which cure diseases. Prophylactic measures are more effective when applied before the onset of disease and in the early larval or postlarval stage when the internal organs, especially the digestive system, are in the developmental stage.

Antibiotics are used both as prophylactic and therapeutic agents against several diseases, or as growth promoters. They are a heterogenous group of substances produced by microorganisms that act on bacteria either by inhibiting their growth or destroying them (Ghosh and Ray, 2003), and are administered either through feed or by direct addition to the rearing water. Use of antibiotic therapy for systemic bacterial infections began in the late 1930's and was followed by the introduction of sulfamerazine in 1948. The most widely used antibiotics in aquaculture are Oxytetracycline, Oxolinic acid, Chloramphenicol, Streptomycin, Virginiamycin etc. Antibiotics act by inhibiting the synthesis of cell wall protein, nucleic acid

precursor, DNA and RNA and/or interfering with enzyme systems, mycolic acid synthesis and /or blocking cytoplasmic membrane activities.

Despite their advantages and utility in treating many diseases, the use of antibiotics has always been a matter of debate. Antibiotics and chemotherapeutants have several shortcomings like the problems of drug residues in the treated animal, the risk of generating resistant pathogens, and the impact of environmental pollution. The possible consequences of antibiotic misuse in aquaculture include the development of antibiotic resistant microbes, multiple antibiotic resistance, resistance transfer to pathogenic bacteria, and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens (Frappaolo and Guest, 1986). The continuous use of antibiotics may lead to the development of resistant strains that gain increased tolerance to antibiotics. The drug residues remain in the product even after it is processed. This residual effect may thus be transferred to human beings consuming these products. Antibiotics often persist for a long period in both animal tissues and sediments causing undefined health implications to humans and animals. Certain antibiotics are known to be carcinogenic, allergenic and/or capable of causing antibiotic resistance and hormone imbalances in consumers (Jameson, 2003). The farm effluent may also cause environmental pollution.

Of late there has been an increased global awareness of the ill effects due to the overuse of antibiotics. In 1969, the Swann Committee had restricted the use of antibiotics in aquaculture. The USFDA has banned certain antibiotics used in food fishes. In India too, only selected antibiotics are approved for use in aquaculture. Modern techniques to detect even a minute quantity of antibiotic residues in the exported marine products led to the rejection of the export consignments from India by many buying nations, which has affected the export market of farmed products adversely. Many farmers still use antibiotics, as they have no other efficient alternative. However, their use could be effectively regulated only if a successful alternative is suggested. Due to the constraints faced in the use

of antibiotics to control diseases in culture systems, an alternative was proposed in feeding the animals with probiotics.

Probiotics (*Pros* = for; *Bios* = life) are used as a means to biologically control the growth of harmful microbes. In recent years, there has been a growing interest to understand the relevance of microbial communities in aquaculture systems and their importance for pond productivity (Darryl, 1998). The beneficial effects of probiotics are competition with pathogens for nutrients or for adhesion sites and stimulation of the immune system (Gatesoupe, 1999). Kumar and Sharma (2001) recommended probiotics, the beneficial bacterial strains as a suitable alternative to the use of antibiotics. This is also referred to as microbial biotechnology.

Probiotics were first used to treat terrestrial animals and their application in aquaculture seems relatively recent (Kozasa, 1986). Probiotics given through feed are referred to as feed probiotics and the same microbes administered through water are water probiotics or bioremediators. Irrespective of the mode of administration, the purpose remains the same. The term probiotics is reserved to strains transient or resident in the gastrointestinal tract. The larval forms of most fish and shellfish are released into the external environment at an early ontogenetic stage. They are highly vulnerable to gastrointestinal microbiota-associated disorders, because they start feeding even before the digestive tract is not fully developed (Timmermans, 1987), and when the immune system is still incomplete (Vadstein, 1997). Therefore, it is better to administer probiotics during the larval stages.

Tests of antagonism, adhesion or challenge are essential to select the candidate probiotics. Three main characteristics that have been ascribed to microbes as candidates to improve the health of their host include the antagonism of pathogens, shown *in vitro* in most cases; the colonization potential of the candidate probionts and the capacity of some strains to increase the resistance to disease of their host as confirmed by challenge

tests. *Bacillus, Pseudomonas, Vibrio* and *Lactobacillus* species are generally used as probiotics. Only strains that are antagonistic to pathogens are used.

Beneficial microorganisms when administered into the gut, compete with harmful microorganisms for food, space etc. and eliminate the unwanted harmful microorganisms from the system. Probiotics are able to establish themselves in the digestive tract, prevent colonization of pathogenic organisms by competitive attachment and promote optimal utilization of the feed. Other mechanisms of action are neutralisation of toxins, bactericidal activity and increased immune competence. They also promote growth and survival in aquaculture systems. Hence probiotics are now used widely in aquaculture.

Unlike antibiotics, probiotics are not medicines to cure diseases, but given to prevent disease occurrence. Antibiotics are substances produced by microorganisims, but probiotics are live microorganisms. Immunostimulants also enhance the host defence system against pathogens by increasing phagocytosis, antibody production, increasing the chemiluminescent response and by super oxide anion production (Sakai, 1998). They act inside the organisms by strengthening the immune system and increase resistance to diseases whereas the probiotics can act both inside and outside the organism. Thus probiotics are more versatile when compared to immunostimulants. Probiotics also have a superior mode of action to vaccines. Vaccine is a preparation of non-virulent disease organisms or immunogens, which still retain the capacity to stimulate the production of antibodies or resistance. The negative effects of vaccines are reduced growth, reduced feeding, handling induced injuries, stress-induced metabolic changes and regulatory surveillance of pathogens.

The giant fresh water prawn, *Macrobrachium rosenbergii* (De Man, 1879) is a commercially important species widely cultured throughout the tropics, subtropics and some parts of temperate regions (New, 2000). The information on the application of probiotics in freshwater prawn culture is

scarce. The present study was therefore an attempt to fill this gap so as to standardize the application of probiotics in freshwater prawn culture.

Research has proven that the best time to administer probiotics is the early growth stage of the animal. Nursery rearing is an important step in the culture of freshwater prawns. Postlarvae can be cultured in high densities from metamorphosis to juveniles in nursery systems that enable potential savings in space, labour, feed and cost. In addition, the early mortalities will have already occurred before growout facilities are stocked and strong juveniles are therefore selected (New, 2002).

Although *M. rosenbergii* has long been considered as relatively free of diseases, recent reports have revealed that this prawn is susceptible to diseases that could seriously affect the viability of its culture. Many diseases including the white tail disease affect the larvae and juveniles of this prawn during the hatchery, nursery or grow out periods (Bonami, 2002; Vijayan *et al.*, 2005). Nurseries are particularly prone to diseases in view of the high stocking densities employed. There are several commercial probiotics available in the market, which have potential to be used in freshwater prawn culture, although their efficacy has not yet been tested.

The present study therefore attempts to evaluate the efficiency of two commercial probiotics containing two common probiont bacterial strains, namely *Lactobacillus* and *Pseudomonas* as alternative to antibiotics on the growth and survival of *M. rosenbergii* postlarvae and also in controlling the diseases and increasing the production in nurseries.

The main objectives of the study are:

(i) To study the effect of two commercial probiotics on the chemical and biological characteristics of the water and soil in the nursery rearing of *M. rosenbergii*;

(ii) To study the effect of two commercial probiotics in altering the microbial population in water and soil during nursery rearing; and

(iii) To determine the effect of two commercial probiotics on the growth and survival of *M. rosenbergii* juveniles during the nursery phase.

Review of Literature

2. REVIEW OF LITERATURE

The giant freshwater prawn, *Macrobrachium rosenbergii* has great aquaculture potential due to its large size, tolerance to water quality changes, ability to cope with handling stress, and ability to feed on unconventional feeds (El Sayed, 1997). Freshwater prawns of the genus *Macrobrachium* are distributed throughout the tropical and subtropical regions and more than 100 species are known to exist today (New, 2000). A number of diseases emerged recently in freshwater prawn farming in countries like Thailand, China, India, Vietnam, Brazil etc. that pose a serious threat to the sustainability of prawn farming. The ill effects of antibiotics have long been proved, and the use of probiotics is now believed to be one of the best methods to combat diseases in animal husbandry. This review summarises the available information on the emergence of probiotics, their mode of action, the different strains of microorganisms used and their application in aquaculture.

2.1. EMERGENCE OF PROBIOTICS

Probiotics being proposed as an alternative to antibiotics, selected, concentrated and viable counts of LAB often composed of *L. acidophilus* and *S. faecium* or strains of *Bacilli* were used at a high dose in feed, in order to prevent digestive disorders and/or to increase zoo-technical performances (Vanbelle *et al.*, 1990).

The use of beneficial bacteria (probiotics) to displace pathogens by competitive processes is being used in animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture (Havenaar and Hius in't Veld, 1992). Sorgeloos (1994) recommended that an improved knowledge of the composition and the functional role of the microflora present in the culture tank or added through the food has allowed to improve disinfection protocols, eventually

complemented with a probiotic use of selected microflora in aquaculture practices. Available data show a variable but net positive impact from the use of beneficial bacteria (Austin *et al.*, 1995).

Probiotics are products composed of complementary benign bacteria which are added into feeds or encapsulated into natural food to stimulate the population of 'friendly bacteria' and prevent the colonization of pathogenic microorganisms in the gut (Lavilla-Pitogo and Paner, 1999). A recent development for the disease management in shrimp hatcheries is the incorporation of live microbes into the hatchery tanks. Such a management technique is known as microbial biotechnology and these microbial products are popularly referred to as probiotics (Vasudevan, 2000).

2.2. DEFINITION OF PROBIOTICS

The term probiotics was first used by Lilly and Stillwell (1965) who proposed the definition as substances produced by one protozoan that stimulated the growth of another. Since then several definitions were attributed to probiotics by various authors.

Parker (1974) defined probiotics as organisms and substances, which contribute to intestinal microbial balance, while Fuller (1989) considered them as live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance. A probiotic is a mono-or mixed culture of live microorganisms that when applied to animal or man, affects beneficially the host by improving the properties of the indigenous microflora (Havenaar and Huis in't Veld, 1992). Gibson and Roberfroid (1995) defined probiotic as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health.

Probiotics are described as single or mixed cultures of selected strains of bacteria, which have varied beneficial effects (Rajagopalsamy and

Venkataramani, 1996). Tannock (1997) put forth the definition that probiotics are living microbial cells administrated as dietary supplements with the aim of improving health. Probiotics are defined by Ruiz-Ponte *et al.* (1998) as beneficial microorganisms, which can protect organisms against pathogens or enhance their growth, while Moriarty (1998) proposed to extend the definition of probiotics to microbial "water additives".

Probiotics are cultures (single or mixed) of selected strains of bacteria that are used in culture and production systems to modify or manipulate the microbial communities in water and sediment, reduce or eliminate selected pathogenic species of microorganisms and generally improve growth and survival of the targeted species (Jory, 1998).

Probiotics were also defined as microbial dietary adjuvants that beneficially affect the host physiology by modulating the mucosal and systemic immunity, as well as improving microbial balance by preventing the colonization of undesirable bacteria in the intestinal tract (Naidu *et al.*, 1999). Verschuere *et al.* (2000) gave a more comprehensive definition for aquaculture probiotics as live microbial adjuncts which have a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment.

2.3. MODE OF ACTION OF PROBIOTICS

The most likely modes of action as reported by Fuller (1989) include

(a) stimulation of humoral and/or cellular immune response;

(b) alteration of microbial metabolism by the increase or decrease of relevant enzyme levels;

(c) competitive exclusion by which the probiotic antagonizes the potential pathogen by the production of inhibitory compounds or by

competition of nutrients, space (=adhesion sites in the digestive tract) or oxygen.

Probiotic bacteria may occupy receptor sites and compete for food, and thus prevent colonisation by detrimental bacteria. Probionts may provide growth factors and inhibit the proliferation of pathogen and stimulate the non-specific immune response (Vanbelle *et al.*, 1990).

The addition of selected bacteria (probiotics) in tanks or ponds may control deleterious forms through (a) competitive exclusion of pathogenic bacteria; (b) enhanced nutrition of larvae by supplying essential enzymes; and (c) production of antibiotic substances that inhibit the growth of undesired cells (Garriques and Arevalo, 1995).

There are various ways through which probiotics may act in aquaculture systems; by competitive exclusion of pathogens; by enhancing digestion through the supply of essential enzymes; by moderating and promoting the direct uptake of dissolved organic materials; by active production of pathogen inhibiting substances; and other possible mechanisms (Jory, 1998). Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible components (Irianto and Austin, 2002). The enzymes secreted by aquaculture probiotic bacteria have a very important role in the degradation of organic matter and thus act to significantly reduce sludge and slime formation. As a result, water quality is improved by reducing the bottom sediments, reducing disease (including *Vibrio* sp., *Aeromonas* sp., and viruses) incidences, enhancing zooplankton numbers, reducing odours and ultimately enhancing aquaculture production (Green and Green, 2003).

2.4. APPLICATION OF PROBIOTICS

The use of probiotics has beneficial effects in the shrimp hatcheries and farms. The possible modes of application of probiotics could be via

feed (Fuller, 1989) or by immersion or injection (Salminen *et al.*, 1998). The probiotic bacteria also have a great ability to work as immunostimulants and inhibit white spot, yellow head and other viruses infecting the shrimp in early larval stages (Vasudevan, 2000). Li Jian *et al.* (2001) administered three kinds of probiotics to *Fenneropenaeus vannamei* and found higher survival rate than the control. The proper identification of pathogens and the application of probiotics may help to control disease outbreaks (Estes *et al.*, 2002) in aquaculture. In order to reduce the pathogenic load caused by live-food organisms, probiotics could be of use (Indulkar and Belsare, 2003).

2.4.1. Feed Probiotics

Griffith (1995) and Gatesoupe (1999) found that the survival, production, feed conversion and growth rates in the farm were improved by the use of probiotic fed larvae. Shankar (1996) evaluated the role of probiotics as an efficient feed and also as a tool for the prevention of the viral attack. Better growth performances and improved immunity status by incorporation of an appropriate dose of probiotic with the diet have been reported by Suralikar (1996) and Himabindu (1998). Himabindu (1998) reported that a significant growth rate was recorded when probiotic was fed to *M. rosenbergii* postlarvae either through feed or as bio-encapsulated in *Artemia*. Uma (1999) prepared feed probiotics, and the shrimp fed with probiotics supplemented feed showed a higher growth, survival and better immunity.

2.4.2. Water Probiotics

Rao and Sudha (1996) recommended the use of probiotics as a promising alternative to solve water quality problems in shrimp farming. Probionts proliferate in rearing waters and provide a better environment for

shrimp as they reduce the level of pathogens in the culture water. The addition of live bacteria to tanks and ponds in which the animals live is advantageous because these bacteria modify the bacterial composition of the water and sediments. The health of animals is thus improved by the elimination of pathogens or at least minimizing the effect of pathogens by improving water quality (Moriarty, 1998). The microorganisms added by probiotic application lead to nutrient enrichment by releasing nutrients into the water from waste materials that accumulated in ponds as leftover feed and die-offs. This possibly results in increased plankton production, restricting penetration of light in the water (Ringo and Gatesoupe, 1998).

Ravi *et al.* (1998) studied the influence of probiotics on growth of Indian white prawn, *Fenneropenaeus indicus*. Ammonia levels were found to be less in the experimental tanks while a steady build-up of the same was observed in the control tanks. Nutrients such as nitrate, phosphate and silicate levels were also found to be more in the experimental tanks while they were steadily built-up in the control tanks. The total heterotrophic bacterial level was observed to have increased in the experimental tanks and decreased in the control tanks. Soon *et al.* (1999) studied the effects of probiotic bacteria on water quality and growth rate of the tiger prawn *Penaeus monodon* (Fabricius) cultured in tanks by using the commercial probiotics AQUA 10, Organica Pond Clarifier, Ajibact, N-96 and VZ-5.

2.5. USE OF PROBIOTICS IN FARMS

Zhoujia *et al.* (1997) demonstrated the importance of probiotic bacteria and its use to improve quality of shrimp pond water. Prabhu *et al.* (1999) investigated the use of probiotics in water quality management during the culture of the black tiger shrimp, *Penaeus monodon* in a shrimp farm. A commercial probiotic (NS Series Super SPO) was used. Transparency and ammonia levels were less in the experimental ponds than in the control pond. The oxygen levels and pH values were more in the

experimental ponds than in control. Nutrients such as nitrate, phosphate and silicate levels were also more in the experimental ponds than in the control pond. THB levels increased in both experimental and control ponds in water and sediment. The average daily growth and production of shrimps were more in the experimental ponds than in the control ponds.

Ravichandran and Jalaluddin (2001) studied stress management strategies with probiotics for preventing shrimp diseases. Gullian and Rodriguez (2002) demonstrated that the isolated beneficial bacteria *Vibrio.* P62 and *Bacillus* P64 of the natural microflora were potential competitors of pathogenic bacteria in *Fenneropenaeus vannamei* rearing ponds. This occurs by competitive exclusion or by stimulation of a defence reaction in the shrimp.

Chandran *et al.* (2003) studied the role of probiotics on the environment of shrimp pond and found that the environmental parameters were within the acceptable limits. Production was better in the experimental pond where the probiotics were used. The biological parameters such as the average body weight, food conversion ratio, as well as the total yield were better in the experimental pond than the control pond, all due to congenial environment created by the probiotics. Green and Green (2003) stressed the importance of probiotics as a useful culture management tool for growout and reservoir ponds throughout the farming period. However, the effectiveness of this beneficial bacterial supplementation depends upon the severity of infection and other cumulative stress factors that govern the condition of shrimp.

2.6. USE OF PROBIOTICS IN HATCHERIES

In shrimp larviculture, probiotics have been used in some hatcheries for several years, resulting in a marked reduction in bacterial disease outbreak (Griffith, 1995). Introduction of bacteria with beneficial effects in marine larviculture is an interesting probiotic approach to obtain control

with microbial problems (Skjermo and Vadstein, 1999). Torrento and Torres (1999) studied the isolation and evaluation of indigenous bacteria for the development of probiotics in the biocontrol of luminescent vibriosis in *P. monodon* and found a significantly higher survival rate in the larvae when treated with any of the vibriostatic bacteria compared with that of the control.

Dharma (2000) tested the efficacy of the probiotic Epicin on the growth and survival in larval rearing of giant freshwater prawn, *M. rosenbergii.* The larvae were stocked at 40/l in each tank and kept on a constant and regular diet of *Artemia salina* nauplii. The probiotic was applied at 0.2 mg/l at every seventh day. It was found that the total heterotrophs ranged from 41.66×10^3 to 603.33×10^3 CFU/ml; pH 7.4 to 8.44, NH_3-N 0.0125 to 0.0350 mg/l, NO_2-N 0.0477 to 0.0860 mg/l, and NO_3-N 0.3520 to 3.5530 mg/l. Ammonia and nitrite were at their highest in the control and the lowest in the probiotic set with water exchange. The growth rate was better in the treatment with probiotic coupled with water exchange compared to the other two treatments.

Kumar (2001) tested the efficiency of Epicin in the larval rearing of *M. rosenbergii.* The larval stocking density was 40/l and the normal feeding schedule of *Artemia* nauplii and egg custard was followed. The probiotic dosage was 0.25 mg/l weekly; one set was maintained without water exchange while the other with 25% water exchange on alternate days. The nitrifier stage-1 and stage-2 were found to proliferate in the probiotic treatments, but not in control, while the denitrifiers were found only in the controls. The total plate count was found to be highest in the set enriched with the probiotic coupled with water exchange, followed by the probiotic treated set without water exchange. Ammonia and nitrite levels in the control sets without water exchange exceeded the toxic levels. Mortality was observed in the probiotic treated set without water exchange towards the end of the experiment. Survival and growth of postlarvae in probiotic

treated sets with water exchange was significantly greater than that in the control with water exchange.

Babu *et al.* (2002) advocated the potential value of microbial biotechnology in shrimp larval disease management. Abraham (2004) demonstrated a suppression in the pathogenic activity of *V. harveyi* M3 by allowing *Alteromonas* sp. to colonize on shrimp larvae. This resulted in reduced mortality of *P. monodon* larvae both *in vitro* and *in vivo*. Therefore, larval mortality in shrimp hatcheries could be prevented by the colonization of the above non-pathogenic bacteria not exceeding the threshold level, which would help to reduce proliferation of bacterial pathogens.

2.7. PROBIOTIC ORGANISMS (PROBIONTS)

The principal bacterial groups tested as probionts in the culture of shrimp, crab, oyster and fish have been *Vibrio, Pseudomonas, Bacillus* and several *Lactobacilli* (Gomez-Gil *et al.*, 2000). Bacteria belonging to the genera *Lactobacillus, Enterococcus, Pediococcus* and *Bacillus*, microscopic fungi and *Saccharomyces* yeast have been widely used as probiotics. 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 targeted species (Vijayakumaran, 2001).

The most commonly used microbes as growth promoters in animal nutrition include Lactic acid bacteria (LAB) such as *Lactobacillus acidophilus, L. delbreuck, L. casei, L. lactis L. reuteri, Streptococcus faecinum, S. salivarus, S. faecium, S. lactis, S. cremoris, S. diacefilactis, Pediococcus* sp., selected strains of *Bacillus* sp. and certain strains of yeast belonging to *Saccharomyces* sp. (Indulkar and Belsare, 2003).

2.7.1.*Bacillus* spp.

There are approximately 15 species of *Bacillus*, which are probably the main component of commercial probiotic products for pond aquaculture, and are generally the recommended microorganisms to use as pond probiotics (Jory, 1998). The value of adding selected strains of *Bacillus* as probiotic bacteria to control *Vibrio* spp. was illustrated by comparing farms in Indonesia using the same water sources, which contained luminous *Vibrio* strains. The farms that did not use the *Bacillus* cultures had almost complete failure in all ponds, with luminescent *Vibrio* disease killing prawns before 80 days of culture. In contrast, a farm using the probiotics was culturing prawns for over 160 days without problems, by using *Bacillus* at abundances of about $1x10^4$ to $1x10^5$/ml. Luminous *Vibrio* numbers were low in ponds where a large abundance of specially selected *Bacillus* species was maintained in the water column. *Vibrio* numbers were also low in sediments and no luminous *Vibrio* occurred in sediments where the probiotic *Bacillus* were used (Moriarty, 1998).

Lobsters injected with *Bacillus subtilis* and *Gaffkya homari* exhibited a significant reduction in circulating haemocyte numbers after 15 min, where as those which received the paraffin emulsion or artificial seawater solution produced little reduction. In lobsters which were injected with *Gaffkya homari* the total haemocyte count returned to the base level after 5 h, whereas in lobsters injected with *Bacillus subtilis*, the haemocyte count returned to base level very slowly (Cornick and Stewart, 1968). Cheng and Butler (1979) observed significant increase in acidphosphatase activity in sera at 1,2 and 4 h post challenge with heat killed *Bacillus megaterium* in *Biomphalaria glabarata* with a shell diameter of 10-12 mm.

Porubcan (1991 a & b) reported two attempts of bacterial treatments to improve water quality and yield of *P. monodon*. He observed that the introduction of *Bacillus* sp. in proximity to pond aerators reduced COD and increased shrimp harvest and that floating biofilters pre-inoculated with

nitrifying bacteria decreased the ammonia and nitrite in the rearing water. This treatment increased shrimp survival.

Rengpipat *et al.* (1998 a & b) found that the probiotic bacteria especially *Bacillus* S11 in feed form has been proved to be beneficial to growth and survival of shrimp. Survival and growth of *P. monodon* fed the probiont *Bacillus* S11 in two 90-day culture trials were increased compared with non-treated shrimp. Rengpipat *et al.* (1998 b) added *Bacillus* S11 bacterium isolated from black tiger shrimp habitat to shrimp feed as a probiotic in three forms: fresh cells, fresh cells in normal brine solution, and in a lyophilized form. After challenging shrimps with a pathogen, *Vibrio harveyi*, by immersion for 10 days, all probiotic treatment groups had 100% survival as against 26% in control. *Bacillus* S11 provided disease protection by activating both cellular and humoral immune defences, as well as presumably providing competitive exclusion in the shrimp's gut (Rengpipat *et al.*, 2000).

Suhendra *et al.* (1997) described a management program, which incorporated the routine use of commercial probiotics (selected strains of naturally occurring *Bacillus* spp.) in a shrimp farm in West Java (Indonesia). This reduced the incidence of *Vibrio* and viral outbreaks that had hit shrimp farms in Indonesia. It reduced organic matter accumulation, improved water quality and increased shrimp size and total production. Gram-negative slime-forming myxobacteriaceae, an undesirable organism in aquaculture ponds were also inhibited by two *Bacillus* spp. and could be included in detritus management system of bacteria showing antagonistic activity.

Bacillus spp. can be successfully used as probiotics in feed preparations and for management of detritus in intensive aquaculture operations to control the attack of microbial diseases reducing high shrimp mortality (Chandrika, 1999). *Bacillus cereus* strain SFU-9 has growth-promoting effect on animals and can enhance immunity. SFU-9 can keep activity for a longer time than other organisms such as yeast, lactic acid

bacteria etc. because it should form spores under disadvantageous conditions. So this strain meets the demands of processing in feed industry (Libin *et al.*, 2001).

Moriarty (1998) noted an increase of prawn survival in ponds where some strains of *Bacillus* spp. were introduced. This treatment decreased the proportion of pathogenic luminous *Vibrio* spp. in the sediments, and to a lesser extent, in the water. Sridhar and Raj (2001) introduced strains of *Bacillus* and *Micrococcus* isolated from shrimp gut, by coating them on compound diets, into *Fenneropenaeus indicus* postlarvae. They observed significantly higher specific growth rates and survival in treatment groups than in control group. Probiotic organisms 10^6 cfu/shrimp were observed in the gut of the postlarvae. Vaseeharan and Ramasamy (2003) controlled the pathogenic *Vibrio harveyi* by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp, *P. monodon*. The probiotic effect of *Bacillus* was tested by exposing shrimp to *Bacillus subtilis* BT23 at a density of 10^6-10^8 cfu ml/l for 6 d before a challenge with *Vibrio harveyi* at 10^3-10^4 cfu ml/l for 1 h infection. The combined results of long and short-term probiotic treatment of *B. subtilis* BT23 showed a 90% reduction in accumulated mortality.

2.7.2. Lactic Acid Bacteria (LAB)

Fuller (1992) reported that Lactic acid bacteria (LAB) occupy the prime place among the growth promoters. Among the different genera/species of LAB, there had been an increasing research interest in *Lactobacillus acidophilus* due to its potential bio-therapeutic properties (Harlander, 1993).

Several bacteriocins had been associated with *Lactobacillus acidophilus* such as Lactocidin, Acidophilin, heat stable Lactacin B, Lactacin F, Acidocin J1229 and heat labile Acidophilin, being responsible for their antibacterial activity (Muriana and Luchansky, 1993). The

antibacterial substance produced by *L. acidophilus* CFR 2032 in purified paneer whey medium showed a broad spectrum of inhibitory activity against Gram +ve and Gram -ve bacteria (Varadaraj *et al.*, 1997). During the metabolism, LAB produce a range of ECPs that could inhibit bacterial growth such as lactic acid (Lindgren and Clevstrom, 1978; Alakomi *et al.*, 2000), organic acids (Midolo *et al.*, 1995), hydrogen peroxide, carbon dioxide (Naidu *et al.*, 1999) and bacteriocins (Villamil *et al.*, 2003). It has been reported that some LAB isolated from the gastrointestinal tract of fish can act as probiotics. These candidates are able to colonise the gut, and act antagonistic against Gram-ve fish pathogens. These harmless bacteriocin-producing strains may reduce the need to use antibiotics in future aquaculture (Ringo and Gatesoupe, 1998). Treatments with *L. brevis* and *L. casei* significantly diminish *V. alginolyticus* load in *Artemia* culture. In the case of lactic acid, only the highest dose used was able to reduce bacterial numbers mainly in the culture water (Villamil *et al.*, 2003).

Lactic acid bacteria are a group of Gram+ve, rods and cocci, that are non-sporing, catalase and oxidase negative, and that have some physiological and ecological characteristics in common. They use carbohydrate as an energy source and produce lactic acid either as the sole product of metabolism (homolactic fermentation) or as the major end product (heterolactic fermentation). They are also usually non-motile and do not reduce nitrate. Members of this group contain both rods (*Lactobacilli* and *Carnobacteria*) and cocci (*Streptococci*) (Ringo and Gatesoupe, 1998).

Lactic acid bacteria (*Pediococcus* sp., *Lactobacillus* sp. and *L. acidophilus*) and marine bacteria (*Alteromonas* sp. and *Vibrio alginolyticus*) were mixed with pellet feed and used to feed juvenile shrimps. The results showed that the percentages of mass production and survival rate of the shrimp fed with probiotics were significantly higher than the control group. Treatments fed with LAB gave the highest survival rate (*Pediococcus* sp., 86.4%, *Lactobacillus* sp., 90.5% and *L. acidophilus,* 93.8%), followed by treatments fed with marine bacteria (*Alteromonas* sp., 74.6% and *V.*

alginolyticus, 61.8%), while the control had the lowest survival rate (59%) (Jiravanichpaisal *et al.*, 1999).

Lactic acid bacteria like *Lactobacillus* and *Carnobacterium* genera are regarded as slow growing, demanding in their nutritional requirements, and their growth is usually restricted to habitats in which sugars are present (Brock and Madigan, 1991). Use of *Lactobacillus plantarum* and *L. acidophilus* in the nutritional probiotic application, inhibiting pathogenic Gram+ve and Gram-ve bacteria improved not only nutritional utilisation of feed but also stimulated non-specific immune response (Mohamed, 1995). Some LAB also seems to be antagonistic against Gram-negative bacteria such as *Vibrio anguillarum*, *V. salmonicida* and *Proteus vulgaris* (Storm, 1998; Ringo and Gatesoupe, 1998). Seema *et al.* (1997) observed that LAB were present in fresh and brackish water fishes. They could not detect these bacteria in fresh marine fish. The authors detected 1.37×10^5 Total Plate Count/g in *P. monodon,* out of which 4.14×10^3/g were LAB. Out of 15 LAB cultures, 13 cultures inhibited the growth of *Bacillus cereus.*

Suralikar (1996) obtained better growth performance in *M. rosenbergii* postlarvae fed on LAB *Lactobacillus lactis* subsp. *cremoris* @ 8.7×10^{11} cfu /100 g of feed. Himabindu (1998) reported better growth performance in postlarvae of *M. rosenbergii* when fed with LAB, *Lactobacillus sporogenes* (24×10^7 cfu /100 g) than when fed with *L. acidophilus* (140×10^{11} cfu /100 g). Suralikar and Sahu (2001) studied the viability of probiotic addition (*Lactobacillus cremoris*), its optimum concentration in the diet, and its impact on growth and survival of *M. rosenbergii* postlarvae and found that the maximum viability of *L. cremoris* in the pelleted diet was 18 days at 4°C. Survival was not affected due to feeding of *L. cremoris* in the diet of *M. rosenbergii* postlarvae.

Harzevili *et al.* (1998) used *Lactococcus lactis* AR21, which stimulated the growth of rotifers and inhibited *V. anguillarum*. Ajitha *et al.* (2004) studied the probiotic effects of LAB against *Vibrio alginolyticus* in *Penaeus (Fenneropenaeus) indicus.* They administered orally a moist feed

base containing $5x10^6$ cells per g of the four LAB probionts (*L. acidophilus, Streptococcus cremoris, L. bulgaricus*-56 and *L. bulgaricus*-57) for a period of four weeks and obtained survival rates of 56-72%.

Arul *et al.* (2004) studied the effect of gut probiotic LAB isolated from marine fish on *Penaeus monodon* postlarvae to control the *Vibrio anguillarum* infection. Here, the percentage survival of postlarvae one day after challenging with *V. anguillarum* by immersion was found to be 100% in all the treatments except control (97%). On the third day challenge, 100% survival was observed in all the treatments except *L. acidophilus* (95%) and control (86.3%). On the fifth day challenge, the survival was found to be 100% in the groups treated with *L. plantarum, L. rhamnosus, Lactococcus lactis* and L13 (isolated strain), 95% in *L. acidophilus* and 74.3% in control.

Jain *et al.* (2004) studied the effect of feeding *Lactobacillus* based probiotics on the gut microflora, on the growth and survival of postlarvae of *M. rosenbergii* and their bacteriological study indicated that the gut microflora of postlarvae were devoid of LAB. The probiotic strains were found to have inhibitory effects against the Gram-ve bacterial flora present in the gut. Growth of the probiotic fed groups was significantly higher than the control group. Significantly higher growth, percentage weight gain (132.5%) and specific growth rate (1.41%) were recorded in the group fed with *Artemia* bioencapsulated *L. sporogenes*, over the control group. Although insignificant, growth-promoting effects of *L. sporogenes* were found to be higher than *L. acidophilus*. Survival of the postlarvae was not affected by probiotics in the diet.

2.7.3. *Pseudomonas* spp.

Pseudomonads are common inhabitants of the aquatic environment including shrimp culture ponds (Otta *et al.*, 1999). *Pseudomonas* I-2 is non-pathogenic to shrimp larvae even at very high levels of 10^6 cells / ml. This

strain has the properties of a biocontrol agent for use in shrimp hatcheries and farms. *Pseudomonas* I-2 antagonized shrimp pathogenic *V. harveyi, V. fluvialis, V. parahaemolyticus, V. vulnificus* and *Photobacterium damsela* by means of low molecular weight inhibitors (Chythanya *et al.*, 2002).

2.7.4. *Vibrio* spp.

Vibrio harveyi, a luminous species known to cause losses in hatcheries has been found as a major cause of disease in growout ponds (Nithimathachoke *et al.*, 1995). The application of probiotics lessened pathogenic vibrios and enhanced beneficial *Bacilli* in the culture, improved water quality, promoted growth and survival rates and increased the health status of the shrimp without stress and disease outbreaks (Dalmin *et al.*, 2001).

Griffith (1995) reported that a disease characterized by a change in the bacterial population affected shrimp larvae reared in Ecuadorian hatcheries. The proportion of *Vibrio alginolyticus* decreased, whereas *V. parahaemolyticus* increased. The strain of *V. alginolyticus* was isolated and used as probiotic in many hatcheries, where shrimp survival was restored to the level obtained before disease outbreak.

Garriques and Arevalo (1995) reported that the use of *V. alginolyticus* as a probiotic agent might increase survival and growth in *Fenneropenaeus vannamei* postlarvae in Ecuador by competitively excluding potential pathogenic bacteria, and could effectively reduce or eliminate the need for antibiotic prophylaxis in intensive larvae culture systems. They believed that in nature a very small percentage of *Vibrio* sp. is truly pathogenic, and the addition of potentially pathogenic bacteria to aquaculture system through water, algae, and/or *Artemia* was recognized. In their study, the addition of the bacteria *V. alginolyticus* as a probiotic to mass larval culture tanks resulted in increased survival rates and growth over the controls and the antibiotic prophylaxes.

Various amounts of heat killed *Vibrio vulnificus* antigen were delivered by immersion to *P. monodon*. The antigens were detectable in haemolymph plasma and haemocytes one day following delivery. It was suggested that *Vibrio* antigen delivered by immersion could be absorbed by shrimp via either the circulatory or the digestive system, which indicates that the systemic rather than local defense system of tiger shrimp could be enhanced (Sung and Song, 1996). Significant levels of protection were conferred to *Penaeus indicus* larvae for at least 48 h when either fresh or freeze-dried *Vibrio harveyi* vaccines were administered by immersion, but not when such vaccines were administered orally (Alabi *et al.*, 1999).

2.7.5. Other Probiotics

Several commercial probiotics have been tested by different authors to determine their effect on the growth, survival and maintenance of water quality parameters in hatchery rearing and growout of many economically important species of fish and shellfish. The treatment of rotifer with antibiotics or feeding them with probionts, lactic acid bacteria and spores of *Bacilli toyoi* was proposed by Gatesoupe (1989). Maeda and Nogami (1989) reported the use of a ciliate *Strombidium sulcatum* as a food source for the larvae of *Penaeus monodon*. The addition of this ciliate resulted in increased survival and moult rates of the larvae. Abraham *et al.* (2001) suggested the use of a marine bacterium *Alteromonas* sp. to combat epizootics in intensive larviculture systems of shrimp. Maeda and Liao (1992) isolated a strain PM-4 of *Thalassobacter utilis* from the rearing waters of *Penaeus monodon* larvae. It improved the survival rate of larvae of *P. monodon* and the swimming crab, *Portunus trituberculatus*. These bacteria also depressed the growth of *Vibrio anguillarum* (Maeda *et al.*, 1997).

Mohamed (1995) used live heterotrophic bacteria as 50% replacement to microalgae as diet of *P. monodon* larvae. Sunilkumar (1995)

found that *P. monodon* larvae could be reared to PL-1 stage using live heterotrophic bacteria, *Pseudomonas* sp. and *Micrococcus* sp., as 50% replacement diet instead of diatoms. Such larvae were reported to show quick metamorphosis and had better survival. 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. Sridhar and Chandrasekar (1996) evaluated the effects of feeding 5 strains of bacterial biomass (comprised by 2 strains of *Bacillus*, one strain of *Pseudomonas* and 2 strains of *Micrococcus* respectively) to larvae of *P. indicus*. Both *Bacilli* BTM-01 and BTM-05 and the *Micrococcus* (BTM -12) promoted survival rates from 64-70%. Groups of *P. indicus* larvae fed with 100% *Micrococcus* BTM-12 strain showed good growth, initially which, however declined with advancement. Though complete feeding with *Bacillus* spp. and the *Pseudomonas* spp. yielded poor development with total mortality, the study revealed a possibility that bacteria could be used for feeding larval shrimp.

The use of yeast (YB) and fungi (CAL1) as probiotics in *Penaeus vannamei* larviculture improved its growth rate and performance (Intriago *et al.*, 1998). Li Jian *et al.* (2001) reared *P. vannamei* in indoor tanks and treated with 3 kinds of the probiotics, GMA sub (1), GMA sub (2) and Dy-1 individually, and the survival rates were 95.79, 91.78 and 88.00% respectively, while that of the control was 81.67%. Enrichment of *Artemia* nauplii with a known probiotic yeast *Saccharomyces boulardii* (SB) and its role in enhancing resistance against the pathogen *Vibrio harveyi* was investigated by Patra and Mohamed (2003) who found a profound beneficial effect on the nauplii by increasing their resistance to a pathogenic *Vibrio* infection.

Gullian *et al.* (2004) studied the selection of probiotic bacteria and their immunostimulatory effect in *Penaeus vannamei*. The probiotic effect *in vitro* was evaluated using the agar diffusion technique. Three strains

identified as *Vibrio* P62, *Vibrio* P63 and *Bacillus* P64 showed inhibitory effects against *V. harveyi* (S2). The strains P62, P63 and P64 achieved colonization percentages of 83%, 60% and 58% respectively. The competitive interaction with *V. harveyi* (S2) was evaluated in shrimp using RAPDS and monoclonal antibodies. The inhibition percentage against S2 reached by strains P62, P63 and P64 was 54%, 19%, and 34% respectively. The isolated strain *Bacillus* P64 showed both probiotic and immunostimulatory features, while *Vibrio* P62 showed good probiotic properties.

Direkbusarakom *et al.* (1997) reported *Vibrio* spp., which dominate in shrimp hatchery against some fish pathogens. Two isolates of *Vibrio* spp., which are the dominant composition of the flora in shrimp hatchery, were studied for antiviral activity against infectious haematopoietic necrosis virus (IHNV) and *Oncorhynchus masou* virus (OMV). Both strains of bacteria showed the antiviral activities against IHNV and OMV by reducing the number of plaque. Their results demonstrated the possibility of using the *Vibrio* flora against the pathogenic viruses in shrimp culture.

Probiotic treatment with Wunapuo-15 and DMS probiotic had profound effect in reducing the total *Vibrio* counts in the shrimp culture pond water and pond sediment. Higher counts of *V. harveyi* were found in probiotic untreated pond sediments (2.5 ± 0.22 x10^3 cfu/g). Wunapuo-15 treated pond sediments showed lower total *Vibrio* counts (1.97 ± 0.19x10^3 cfu/g) than untreated ponds. DMS treated pond sediment exhibited a lower *Vibrio* counts (1.7 ± 0.15x10^3 cfu/g) than in Wunapuo-15 treated ponds (Vaseeharan and Ramasamy, 1999).

Marine shrimp and wheat bran fermented with *B. licheniformis* and *Beauveria* spp. fungi compounded and fed to postlarvae of *P. indicus* at different concentrations for a period of 45 days showed that probiotics enhanced growth and survival in shrimp (Sridhar and Chandrasekar, 1996). Jiravanichpaisal *et al.* (1997) reported the use of *Lactobacillus sp.* as the probiotic bacteria in the giant tiger shrimp, *P. monodon.* They designed to

investigate an effective treatment of *Lactobacillus sp.* against vibriosis and white spot disease in *Penaeus monodon.* They investigated the growth of some probiotic bacteria, and their survival in the 20 ppt seawater for at least 7 days. Inhibiting activity of two *Lactobacillus* sp. against *Vibrio* sp., *E. coli*, *Staphylococcus* sp. and *Bacillus subtilis* was determined.

The formulated diet incorporated with probiotic (*Gp-5*) @7.5 g /kg of diet, consisting of combinations of *Lactobacillus sporogenes* (45,000 million cfu), *Lactobacillus acidophilus* (45,000 million cfu), *Bacillus subtilis* (30,000 million cfu), *B. licheniformis* (30,000 million cfu), *Saccharomyces cervisiae* (125,000 million cfu) and seaweed extract (100 g /kg) resulted in better growth and better feed utilization of postlarvae of *M. rosenbergii* during 15 days of indoor nursery rearing (Indulkar and Belsare, 2003).

The prophenoloxidase activating system of shrimp haemocytes was enhanced by *in vitro*-ß-glucan treatment. The phenoloxidase activity of haemocyte lysate supernatent (HLS) from both *P. monodon* and *M. rosenbergii* was enhanced significantly by treating with ß-1, 3-1,6-glucan at the rate of 1 mg/ml (Sung *et al.*, 1998).

The efficacy of probiotics product Epicin (Epicore Networks Inc., Canada) on growth and survival of *Macrobrachium rosenbergii* larvae was tested by Nayak *et al.* (2003). Epicin applied @ 0.2 ppm to the larval rearing tanks resulted in a reduction in the ammonia, nitrite and nitrate levels, and these parameters were maintained within the optimum range throughout the larval rearing period of 50 days. The average survival achieved in probiotics treated tanks was 39% as against the 12% in the control tanks (Nayak *et al.*, 2003).

Shirgur and Indulkar (1999) reported that the use of growth promoter, *Bioboost* in the diet improved the growth rate in *Macrobrachium rosenbergii* juveniles. Uma *et al.* (1999) prepared a commercial livestock probiotic feed supplement, Lacto-sacc given at a concentration of 2.5g/kg basal feed. The probiotic contained microencapsulated bacteria

(*Enterococcus faecium* and *Lactobacillus acidophilus*) and yeast. They found that the growth and survival of shrimp juveniles were significantly improved by the addition of Lacto-sacc in shrimp feed at different levels ranging from 2.5 to 7.5 g/kg basal feed. Yazid *et al.* (1999) revealed that the combination of probiotic microorganisms exerts better inhibitory effect against enteropathogenic *E. coli* than a single probiotic dose. AquazynTM-1000 (Quinn India Ltd., Hyderabad), a probiotic which is claimed by the manufacturer to remove H_2S and NH_3 from polluted waters, stimulate growth of prawn and fish, breakdown organic and faecal wastes, reduce bottom sludge and to maintain good water quality, contains bacteria viz., *Bacillus subtilis, B. megaterium, B. licheniformis, B. polymyxa, Remeno-coccusalbus* sp., *Aspergillus oryzae*, enzymes, stabilizers, nutrients and minerals. It can work in aerobic and anaerobic conditions (Sharma and Bhukhar, 2000).

Ravichandran and Jalaluddin (2001) used the probiotics, ENVIRON-AC (Wockhardt, Mumbai, India) with an initial dose of 25 kg /ha and subsequently 5 to 10 kg /ha at weekly intervals and found that the pathogen levels could be reduced and suitable environment maintained in the ponds. Nayak *et al.* (2003) reported that the production increased by 60% in ponds where the probiotics Aqualact (Wockhardt, Mumbai, India,) was used, compared to control.

Maeda and Nagami (1989) reported some aspects of the biocontrolling method in aquaculture. In their study, bacterial strains possessing vibriostatic activity, which improved the growth of prawn and crab larvae, were observed. By applying these bacteria in aquaculture, a biological equilibrium between competing beneficial and deleterious microorganisms was produced, and results showed that the population of *Vibrio* spp., which frequently causes large-scale damage to the larval production, was decreased. Survival rate of the crustacean larvae in these experiments was much higher than those without the addition of bacterial strains. They also found that addition of these strains of bacteria repressed

the growth of *Vibrio* spp., fungi and other pathogenic microorganisms. Their data suggested that controlling the aquaculture ecosystem using bacteria and protozoa is quite possible and if this system is adopted, it will maintain the aquaculture environment in better condition, which will increase the production of fish and crustaceans.

Nogami and Maeda (1992) isolated a bacterial strain from a crustacean culture pond. The bacterial strain was found to improve the growth of crab (*Portunus trituberculatus*) larvae and repressed the growth of other pathogenic bacteria, especially *Vibrio* spp., but would not kill or inhibit useful micro algae in seawater when it was added into the culture water. Among the bacterial population present in the culture water of the crab larvae, the numbers of *Vibrio* spp. and pigmented bacteria decreased or even became undetectable when the bacterial strain was added into culture water. The production and survival rate of crab larvae were greatly increased by the addition of the probiotic bacteria into the culture water. They also suggested that the bacterium might improve the physiological state of the crab larvae by serving as a nutrient source during its growth. This bacterium may have a good effect in the crab larval culture as a biocontrolling agent in the future.

Maeda and Liao (1992) reported the effect of bacterial strains obtained from soil extracts on the growth of *P. monodon* larvae. Higher survival and moult rates of shrimp larvae were observed in the experiment treated with soil extract, and the bacterial strain that promoted the growth of prawn larvae was isolated. They assumed that if a specific bacterium was cultured and added to the prawn ecosystem to the level of 10 million cell/ml, other bacteria might hardly inhabit the same biotype because of protozoan activity, which could be one of the ways to biologically control the aquaculture water biotype and ecosystem.

Maeda *et al.* (1992) reported the utility of microbial food assemblages in culturing the crab, *Portunus trituberculatus*. Assemblages of microorganisms were produced by adding several nutrients, urea, glucose

and potassium phosphate, to natural seawater with gentle aeration in which bacteria and yeast were prevailing. When these cultured microbes were added to seawater where crab larvae of *P. trituberculatus* were reared, bacteria numbers decreased very rapidly, followed by the decrease in flagellated protozoa and diatoms. Their results suggested that the crab larvae were fed on these microorganisms successively. They found some strains of bacteria promoted larval growth, although yeasts did not support its growth. By adopting these assemblages of microorganisms a high yield was obtained for a prawn larva *Penaeus japonicus*, although the success was not always consistent.

Douillet and Langdon (1994) reported the use of probiotics for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunbeerg). They added probiotic bacteria as a food supplement to xenic larval cultures of the oyster *C. gigas* that consistently enhanced growth of the larvae during different seasons of the year. Probiotic bacteria were added, at 0.1 million cells/ml, to cultures of algal-fed larvae, the proportion of larvae that were set to produce spat, and subsequently the number of spat increased. Manipulation of bacterial population present in bivalve larval cultures is a potentially useful strategy for the enhancement of oyster production. They suggested that the mechanisms of action of probiotic bacteria are providing essential nutrients that are not present in the algal diets or improving the oyster's digestion by supplying digestive enzymes to the larvae or removing metabolic substances released by bivalves or algae.

Maeda and Liao (1994) reported microbial processes in aquaculture environment and their importance in increasing crustacean production. They suggested that based on the photosynthesis of micro algae mainly, it was clarified that bacteria, protozoa and other microorganisms from microbial food assemblages use the organic matter produced by the algae and that these assemblages play a significant role in the aquatic food chain. The growth of the larvae and their production were markedly promoted by the probiotic bacteria. They also described the presence of a bacterial

clump, stained with a fluorescent dye, inside the digestive organ of the crab *P. trituberculatus*.

Zhenguo *et al.* (1994) evaluated three strains of photosynthetic bacteria used in shrimp (*Penaeus chinensis*) diet preparation. Addition of the photosynthetic bacteria in the food or culture water was found to improve the growth of the prawn and the quality of the water. Jinggin *et al.* (1997) reported the application of photosynthetic bacteria in the hatchery rearing of *P. chinensis*. They used a mixture of several kinds of photosynthetic bacteria (*Rhodomonas* sp.) as water cleaner and auxiliary food. Their results showed that the water quality of the pond treated with the bacteria was remarkably improved, the fouling on the shell of the larvae was reduced, the metamorphosis time of the larvae was 1 day or even earlier, and the production of postlarvae was more than that of the control.

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL SET UP

The experiments were conducted in fifteen circular fibreglass tanks of 70 l capacity each (53 cm diameter, 33 cm height), filled with soil collected from a fishpond to a depth of 5 cm, and then with freshwater to a depth of 25 cm. These tanks were kept under shade inside the hatchery shed of College of Fisheries, Kochi. Out of 15 tanks, 5 were kept as control (C), 5 for first treatment (T_1) and 5 for second treatment (T_2).

Macrobrachium rosenbergii postlarvae (PL) measuring 16.5 mm length were stocked @ 500 no./m^2 in all tanks. They were fed with a commercial pelletised feed (Higashimaru-S I; Higashimaru Feeds India Ltd., Alappuzha) @100% of body weight. The feed was introduced in a petridish and the leftover, unconsumed feed remaining in the petridish was collected, dried and weighed to determine the quantity of feed consumed by the animal. Mild aeration was provided in the tanks with diffusion stones. The individual weight of the PL was also noted before introducing into the tanks.

Two commercial probiotics namely, The Waves (Udang, Taipei, Taiwan) and Epicin (Epicore Networks Inc., Canada) were applied at doses of 0.5 mg/l (T_1) and 0.25 mg/l (T_2) respectively at an interval of 10 days to the experimental tanks. The control (C) was maintained without the application of any probiotics. The PL were reared for a period of 30 days as per standard practices, except that the water exchange was limited to periodic replenishment of evaporation loss using fresh distilled water.

Plate 1. Experimental Tanks

Plate 2. Experimental Tanks

3.2. ESTIMATION OF GROWTH PARAMETERS

The following formulae were used to determine the respective growth parameters.

3.2.1. Food Conversion Ratio

$$FCR = \frac{\text{Feed consumed (dry weight)}}{\text{Increase in mass of animal (wet weight)}}$$

3.2.2. Average Gain in Weight

Average Gain in Weight = Final weight –Initial weight

3.2.3. Average Gain in Length

Average Gain in Length = Final Length –Initial Length

3.2.4. Specific Growth Rate

$$\text{Specific growth Rate} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 100$$

where,

W_2 =Weight at time t_2

W_1 = Weight at time t_1

3.2.5. Percentage Survival

$$\text{Percentage survival} = \frac{\text{No. of animals survived}}{\text{No. of animals stocked}} \times 100$$

3.3. STATISTICAL DESIGN AND ANALYSIS

The experiment was conducted using completely randomised design with three treatments and five replications for each treatment. The data obtained in each case was subjected to analysis of variance (ANOVA) at a significance level of $P<0.05$ (Snedecor and Cochran, 1967) after making angular transformations for percentage values.

3.4. ESTIMATION OF MICRO ORGANISMS IN WATER AND SOIL

The microorganisms were estimated (a) before introducing probiotics in the tank and (b) once in 15 days after applying probiotics.

3.4.1. Pour Plate Technique

From each tank, 1 ml of water was taken from different places using sterile pipettes in a test tube and mixed well. Then 10 ml water was added to 90 ml alkaline peptone water. This formed 10^{-1} dilution. For further dilutions, 1 ml from this dilution was mixed with 9 ml of the diluent (10^{-2} dilution) and so on to get 10^{-3} dilution. From each dilution tube, 1 ml was taken and transferred to sterile petriplates in duplicate, and above that 15-20 ml of molten plate count agar was poured at about 45°C and rotated 6 times clockwise and 6 times anticlockwise. After it got solidified, the plates were incubated at room temperature in inverted position for 48 h. Then the colonies were counted.

3.4.1.1. Composition of Alkaline Peptone Water

Peptone	10g

Sodium chloride	5 g
Distilled Water	1000 ml
pH	9.1 ± 0.1

These were sterilized at 121°C for 15 min in autoclave.

3.4.1.2. Composition of Plate Count Agar

Yeast Extract	2.5 g
Pancreatic digest of casein	5 g
Glucose	1 g
Agar	20g
Distilled Water	1 litre

These were sterilized at 121°C for 15 min in autoclave.

Dose: 28 g in 1000 ml distilled water.

3.4.2. Streak Plate Technique

About 15-20 ml of plate count agar (45°C) was poured on the sterile petridishes and allowed to solidify. A loopful of water was streaked over the agar and it was inverted and kept for 24-48 h in room temperature to allow them to grow. Streaking was done to get isolated colonies. After 48 h, the morphology was noted.

3.4.3. Enrichment of Isolated Colonies

In test tubes, about 5 ml nutrient broth were taken and sterilized at 121°C for 15 min. The colonies from the petridish were picked up with a

inoculation loop and introduced in nutrient broth. They were incubated at room temperature for 24 hours.

3.4.3.1. Composition of Nutrient Broth

Peptone	10.0 g
Beef extract	3.0g
NaCl	5.0 g
Distilled Water	1 litre
pH	7.0 ± 0.1

These were sterilized at 121°C for 15 min in autoclave.

Dose: 13 g in1000ml distilled water.

3.4.4. Staining

3.4.4.1. Preparation of Smear

Young cultures (16-24 h old) from nutrient broth were subjected to staining. Dust, dirt and oil free microscopic glass slides were taken. In the middle of the slide, a speck of young culture was emulsified with a drop of sterile platinum wire or nichrome wire loop and spreaded uniformly. It was then dried in air and fixed by passing the slide 3-4 times through the blue flame of Bunsen burner. Care was taken not to allow the smear to get charred.

3.4.4.2. Staining Method

The slides were placed on a staining bridge, made with 2 glass rods. Then smear was flooded with Gram's crystal violet and allowed to act for 2-

3 minutes and washed with water. Then it was flooded with Gram's iodine; allowed to act for 1 minute, and washed with water. Destaining was done with dropwise addition of alcohol until washings were free from violet colour and washed with water. Then counterstaining was done with carbol fuchsin for 1 minute and washed. After drying in air they were viewed under oil immersion microscope (100X). The colour and morphology of the organisms were noted. Red colour indicated Gram negative and violet/ purple colour indicated Gram-positive organisms.

3.4.4.3. Composition of Gram's Crystal Violet

Solution A	
Crystal violet	2 g
Ethyl alcohol	20 ml
Solution B	
Ammonium oxalate	0.8 g
Distilled water	80 ml

Solutions A and B were mixed, filtered and kept overnight before use.

3.4.4.4. Composition of Gram's Iodine

Potassium iodide	2 g
Iodine crystals	1 g
Distilled water	300 ml

All these were dissolved and filtered.

3.4.4.5. Composition of Carbol Fuchsin

Solution A	
Basic fuchsin	1 g

Ethanol (95%)	10 ml
Solution B	
Phenol	5 g
Distilled water	100 ml

Solutions A and B were mixed, and allowed to be aged for 1-2 weeks.

3.4.5. IMViC Tests

Isolated cultures from nutrient broth were subjected to IMViC tests.

3.4.5.1. Indole Test

Indole medium was prepared in test tubes (4-5 ml) with sufficient amount of Tryptophan and sterilized by autoclaving at 121°C for 15 min. A loopful of culture was inoculated into the medium. It was allowed to grow for 48 h, at room temperature. Then 0.5 ml of Kovax reagent was added to each tube. Red ring on the surface indicated positive indole test.

3.4.5.1.1. Composition of peptone water

Peptic Digest of Animal Tissue	10 g/l
Sodium Chloride	5g/l
Final pH (at 25 °C)	7.2 ± 0.2
Dose: 15g/1000 ml distilled water	

3.4.5.1.2. Composition of Kovac's reagent

P-dimethylaminobenzaldehyde	5 g
N-butyl alcohol (or amyl alcohol)	75 ml
Conc. HCl	25 ml

3.4.5.2. MRVP Test

MRVP medium was prepared and 4 ml was poured into two sets of test tubes and then autoclaved at 121°C for 15 min. Then a loopful of culture from nutrient broth was added to each test tube and allowed to grow for 48 h. Then 0.25 ml (or 5 drops) of Methyl red indicator was added in one set of test tubes. A red colour indicated positive test. In second set of test tubes, VP test reagents A (0.6 ml) and B (0.2 ml) were added to 1 ml of culture in test tubes. They were mixed well and a small crystal of creatinine was added to enhance the reaction. It was then allowed to stand up to 4 h. Eosine pink colour indicated positive VP test.

3.4.5.2.1. Composition of MRVP medium

Peptone	0.5 g
D-glucose	0.5 g
K_2 H PO_4	0.5 g
Distilled water	100 ml
pH	6.9 ±0.1

3.4.5.2.2. MR test reagents

Methyl red	50 mg
Alcohol	150 ml
Distilled water	100 ml

3.4.5.2.3. VP test reagents

Solution A	
a-Naphthol	0.25 g
Alcohol	5 ml
Solution B	
KOH	2g
Distilled water	5 ml

3.4.5.3. Citrate Utilization Test

The liquid citrate broth was prepared and 4-5 ml was poured in test tubes and autoclaved at 121°C for 15 min. Then a loopful of culture was inoculated in each test tube and allowed to grow for 24-48 h. Presence of turbidity indicated positive test.

3.4.5.3.1. Composition of Citrate

Sodium ammonium phosphate	1.50g/l
Monopotassium phosphate	1.00g/l
Magnesium sulphate	0.20 g/l
Sodium citrate	3.00g/l
Final pH (at 25°C)	6.7 ± 0.2

Dose: 5.7g/1000 ml distilled water

3.4.6. Identification of Micro organisms at Generic Level

Based on the results of IMViC tests and staining, the organisms were identified.

3.5. ESTIMATION OF MICROORGANISMS IN THE ANIMAL

About 10 g of animal was weighed aseptically and ground well using sterile pestle and mortar. Then 90 ml of sterile alkaline peptone water was added to it and mixed well. This formed 10^{-1} dilution. Then about 1 ml was taken from this and added to 9 ml of sterile alkaline peptone water in another test tube. This formed 10^{-2} dilution. Thus up to 10^{-6} dilutions were made. Then pour plating, streak plating etc. were done as above.

3.6. ESTIMATION OF WATER QUALITY PARAMETERS

The water quality parameters such as total hardness and ammonia nitrogen were estimated using auto water analyser (Hach, USA). The parameters such as temperature, pH and dissolved oxygen were estimated using thermometer, pH meter and DO meter respectively. All the water quality parameters were estimated once in 15 days.

Results

4. RESULTS

4.1. BIOLOGICAL OBSERVATIONS

4.1.1. Food Conversion Ratio

The food conversion ratios (F.C.R) of the *M. rosenbergii* PL treated with different probiotics are given in Table 1 and Fig 1. Analysis of variance of the data (Table 2) showed significant difference in F.C.R. values ($P<0.01$) between the Epicin and the control. The lowest F.C.R. of 0.76 ± 0.17 was obtained for T_2 followed by T_1 (1.10 ± 0.25), while the control had the highest F.C.R. of 2.03 ± 0.80.

4.1.2. Average Gain in Weight

The average gain in weight of the *M. rosenbergii* PL treated with different probiotics are given in Table 3 and Fig 2. The highest gain in weight was obtained in T_2 (895.80 mg). In T_1 the average gain in weight was 869.96 mg followed by the control (650.17 mg). Analysis of variance of the data on average gain in weight (Table 4) indicated that there was significant difference ($P<0.01$) in the gain in weights of the two treatments compared to the control. However, pair wise comparison using *t* test has shown that the two treatments were identical.

4.1.3. Average Gain in Length

The data on the average gain in length of the *M. rosenbergii* PL treated with different probiotics are given in Table 5 and Fig 3. The maximum gain in length was obtained for T_2 (31.25 mm), followed by T_1 (28.14 mm) and control (26.4 mm). Analysis of variance of the data (Table 6) showed that the treatment T_2 was significantly different ($P<0.05$) from the control. In the case of T_1 the difference was not statistically significant.

4.1.4. Specific Growth Rate

The data on the effect of the two probiotics on specific growth rate (S.G.R.) of the *M. rosenbergii* PL treated with different probiotics are given in Table 7 and Fig 4. The S.G.R. obtained for the two treatments were 11.33 ± 0.04 for T_2 and 11.23 ± 0.17 for T_1. In the case of control, the SGR was only 10.29 ± 0.24. The S.G.R. for the two treatments showed significant variation (Table 8) from the control at 1% level.

4.1.5. Percentage Survival

The percentage survival of the *M. rosenbergii* PL treated with different probiotics are given in Table 9 and Fig 5. Analysis of variance of the data is given in Table 10. The highest survival of 64.76% was obtained for T_2. In T_1, the survival was 63.81% that, however, showed no significant difference with T_2, on pair wise comparison. The lowest survival was obtained in the control (58.67%). There was no significant difference between the treatments and the control.

Table 1. Food conversion ratio of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Treatment	Replication	Total Initial Weight (mg)	Total Final Weight (mg)	Total Live Weight Gain (mg)	Weight of Feed Consumed (mg)	Food Conversion Ratio	Average F. C. R. (Mean ± SD)
T_1	1	3255	68590.60	65335.60	45384.21	0.69	1.10 ± 0.25
	2	3255	51219.44	47964.44	53430.00	1.11	
	3	3255	66919.93	63664.93	63410.27	0.996	
	4	3255	55519.18	52264.18	69098.29	1.32	
	5	3255	59325.34	56070.34	78542.39	1.4	
T_2	1	3255	41988.80	38733.80	41836.38	0.60	0.76 ± 0.17
	2	3255	65231.22	61976.22	6142.00	0.58	
	3	3255	75850.00	72595.00	52303.49	0.72	
	4	3255	67843.20	64588.20	56733.50	0.88	
	5	3255	64446.00	61191.00	63087.92	1.03	
C	1	3255	43470.00	40215.00	52077.73	1.29	2.03 ± 0.80
	2	3255	41427.54	38172.54	101827.82	2.67	
	3	3255	47699.22	44444.22	75555.17	1.70	
	4	3255	45052.77	41797.77	51290.00	1.23	
	5	3255	30247.79	26992.79	87454.93	3.24	

Table 2. ANOVA for F.C.R. of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Sources of Variation	df	SS	MS	F	Table values of F (2,12)
Probiotics	2	4.29	2.14	7.13**	3.88 (5%) 6.93 (1%)
Error	12	3.63	0.3		
Total	14	7.92			

*Statistically significiant (P<0.01)

Critical difference = 1.06

Comparison of Treatment Means

Treatments	Means
T_1	1.10
T_2	0.76
C	2.03

Treatments connected with lines are not significantly different.

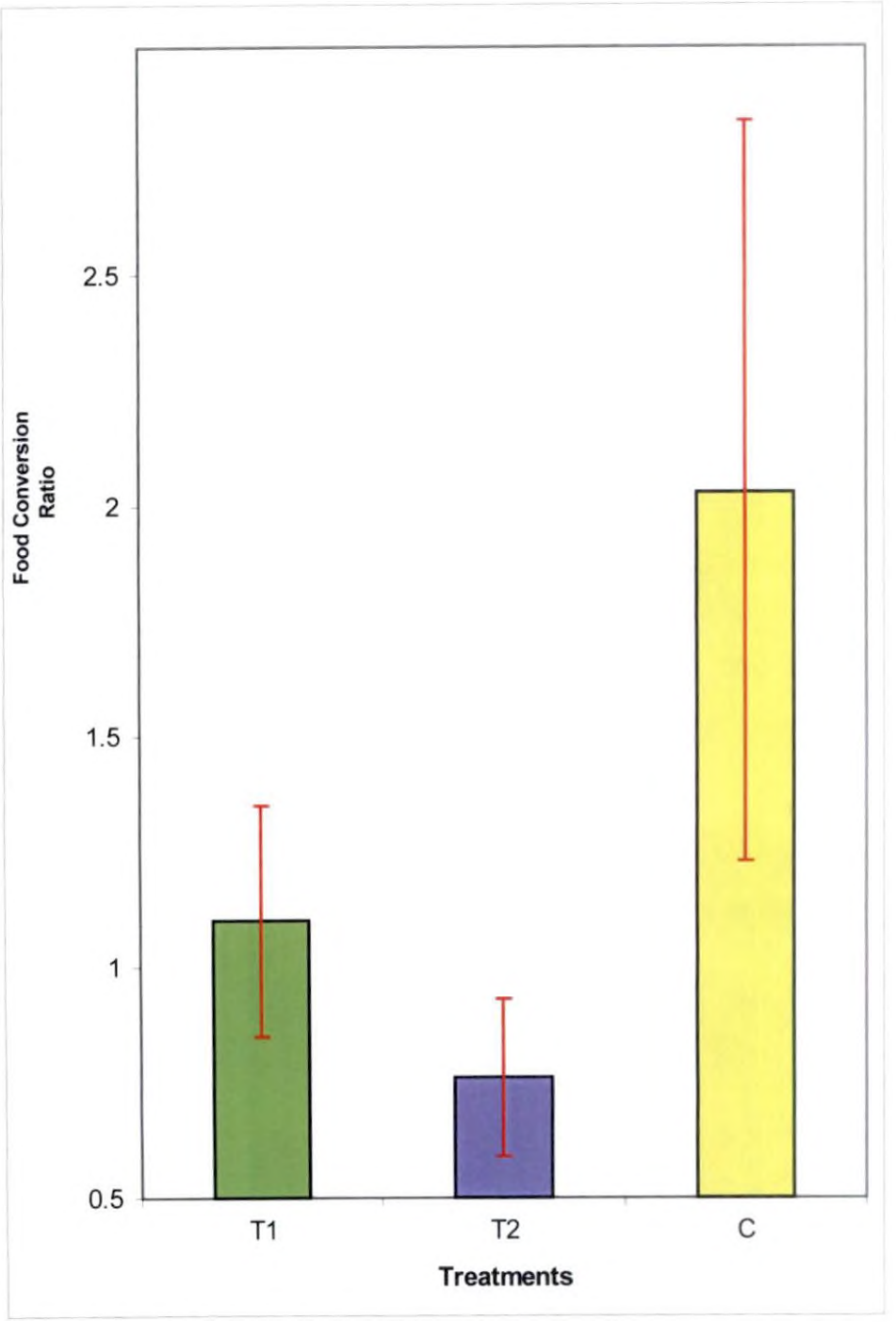

Figure1. Food Conversion Ratio of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Table 3. Average gain in weight of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Treatment	Replication	Initial Weight (mg)	Final weight (mg)	Gain in Weight (mg)	Average Gain in Weight (mg) (Mean ± S. D)
T_1	1	31	926.90	895.90	869.96 ± 45.93
	2	31	826.12	795.12	
	3	31	869.09	838.09	
	4	31	941.00	910.00	
	5	31	941.67	910.67	
T_2	1	31	912.80	881.80	895.80 ± 11.80
	2	31	945.38	914.38	
	3	31	925.00	894.00	
	4	31	916.80	885.80	
	5	31	934.00	903.00	
C	1	31	630.00	599.00	650.17 ± 49.12
	2	31	627.69	596.69	
	3	31	671.82	640.82	
	4	31	738.57	707.57	
	5	31	737.75	706.75	

Table 4. ANOVA for average gain in weight of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Sources of Variation	df	SS	MS	F	Table values of F (2,12)
Probiotics	2	182182.41	91091.20	46.897**	3.88 (5%) 6.93 (1%)
Error	12	23308.37	1942.36		
Total	14	205490.78			

**Statistically significant (P<0.01).

Critical difference = 85.15

Comparison of Treatment Means

Treatments	Means
T_1	869.96
T_2	895.80
C	650.17

Treatments connected with line are not significantly different.

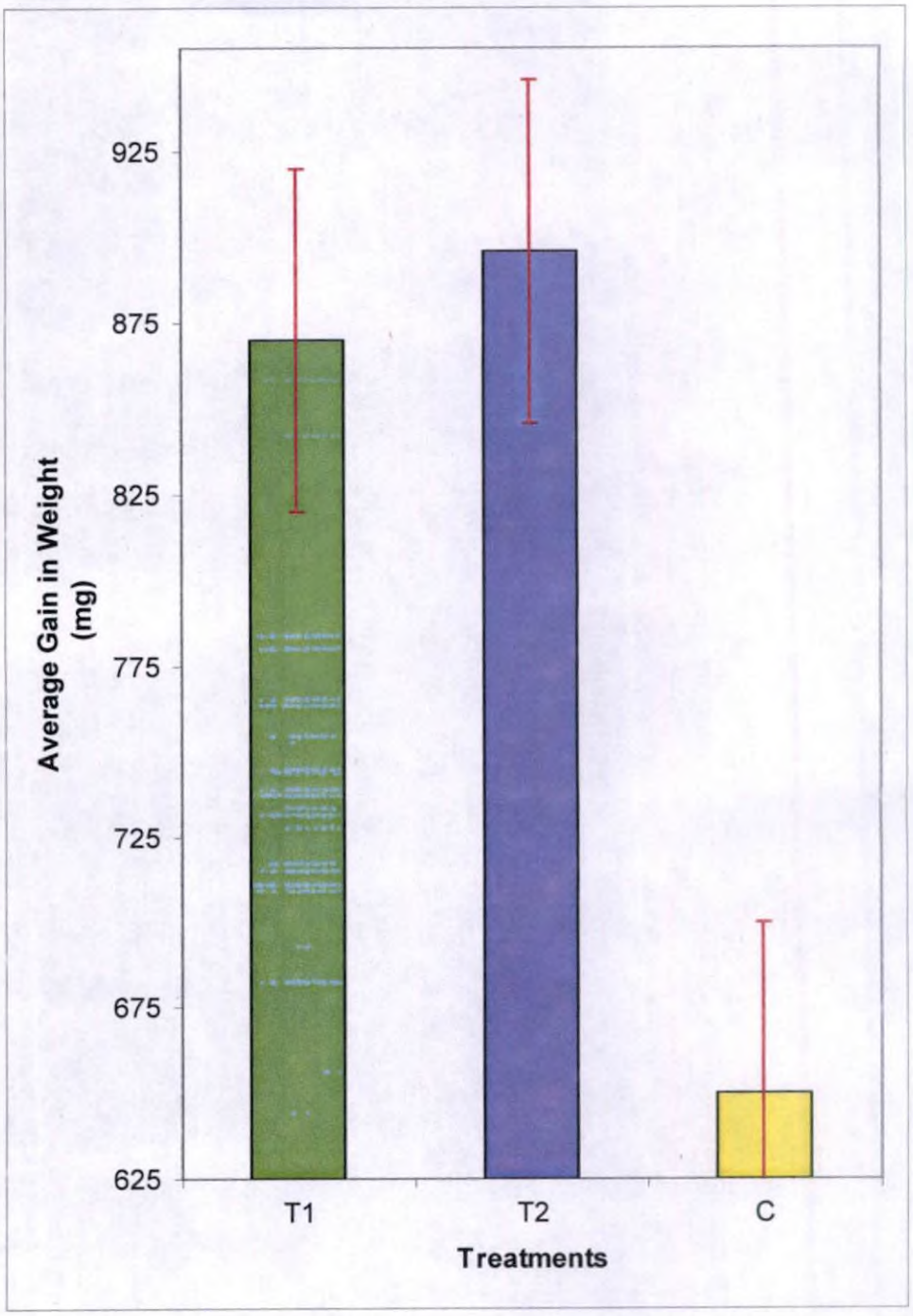

Figure 2. Average Gain in Weight of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Table 5. Average gain in length of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Treatment	Replication	Initial Length (mm)	Final Length (mm)	Gain in Length (mm)	Average Gain in Length (mm) (Mean ± SD)
T_1	1	16.56	38.89	22.33	28.14 ± 3.11
	2	16.56	45.18	28.62	
	3	16.56	47.64	31.08	
	4	16.56	44.72	28.16	
	5	16.56	47.08	30.52	
T_2	1	16.56	44.89	28.33	31.25 ± 1.86
	2	16.56	50.61	34.06	
	3	16.56	47.21	30.65	
	4	16.56	48.59	32.03	
	5	16.56	47.75	31.19	
C	1	16.56	40.73	24.17	26.4 ± 1.93
	2	16.56	41.69	25.13	
	3	16.56	42.27	25.71	
	4	16.56	43.86	27.3	
	5	16.56	46.25	29.69	

Table 6. ANOVA for average gain in length of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Sources of Variation	df	SS	MS	F	Table values of F (2,12)
Probiotics	2	60.42	30.21	4.296*	3.88 (5%) 6.93 (1%)
Error	12	84.38	7.032		
Total	14	144.804			

* Statistically significiant (P<0.05)

Critical difference = 3.654

Comparison of Treatment Means

Treatments	Mean Gain in Length (mm)
T_1	28.14
T_2	31.25
C	26.4

Treatments connected with lines are not significantly different.

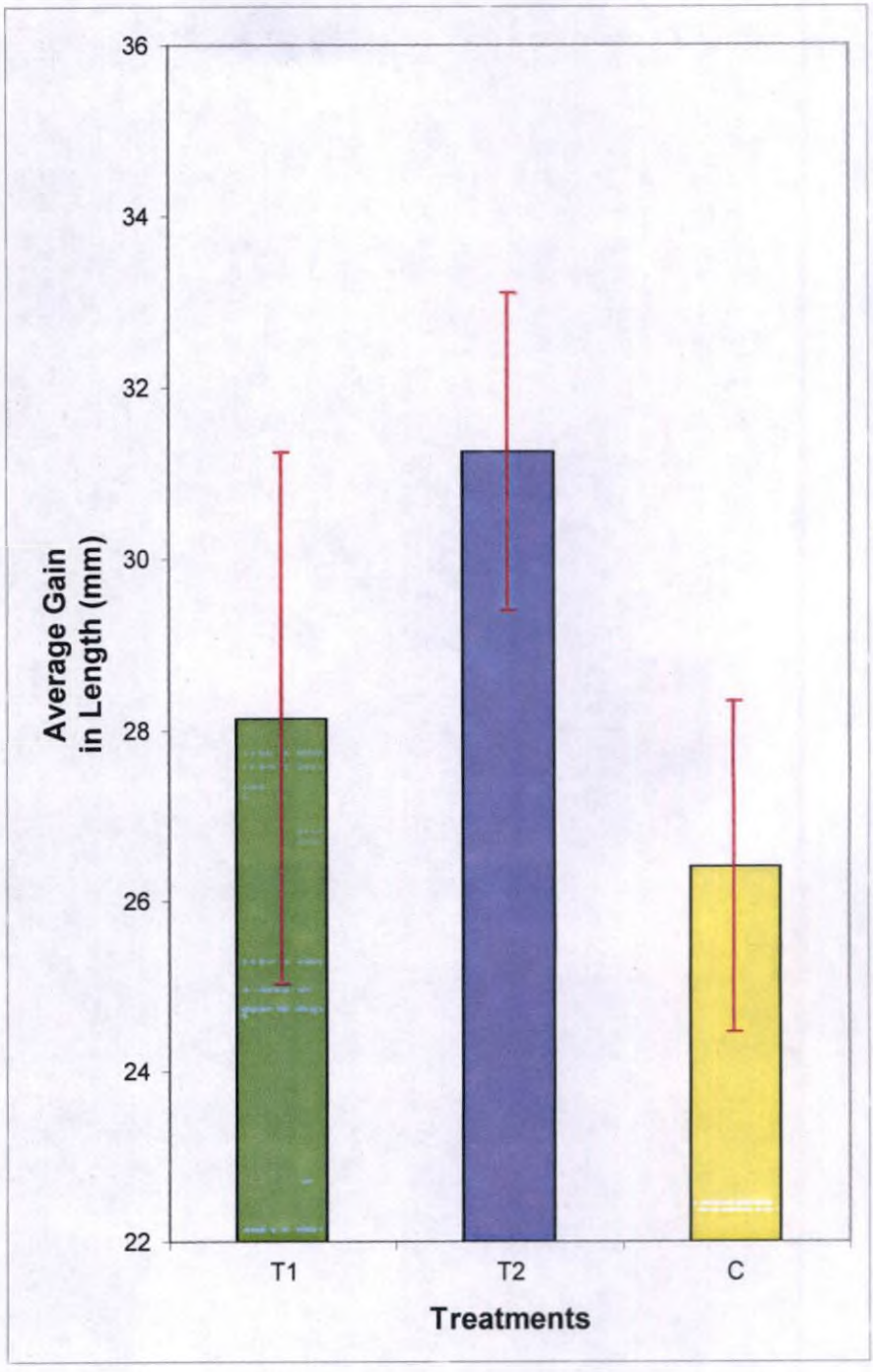

Figure 3. Average Gain in Length of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Table 7. Specific growth rate of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Treatment	Replication	Initial Weight (mg)	Final Weight (mg)	Specific Growth Rate (%)	Specific Growth Rate (Mean± SD)
T_1	1	31	926.90	11.33	11.23 ± 0.17
	2	31	826.12	10.94	
	3	31	869.09	11.11	
	4	31	941.00	11.38	
	5	31	941.67	11.38	
T_2	1	31	912.80	11.28	11.33 ± 0.04
	2	31	945.38	11.39	
	3	31	925.00	11.32	
	4	31	916.80	11.29	
	5	31	934.00	11.35	
C	1	31	630.00	10.04	10.29 ± 0.24
	2	31	627.69	10.03	
	3	31	671.82	10.25	
	4	31	738.57	10.57	
	5	31	737.75	10.57	

Table 8. ANOVA for specific growth rate (arc sine transformed) of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Sources of Variation	df	SS	MS	F	Table values of F (2,12)
Probiotics	2	2.79	1.40	46.67**	3.88 (5%) 6.93 (1%)
Error	12	0.39	0.03		
Total	14	3.18			

**Statistically significant (P<0.01)

Critical difference = 0.334

Comparison of Treatment Means

Treatments	Mean S. G. R.
T_1	11.23
T_2	11.33
C	10.29

Treatments connected with line are not significantly different.

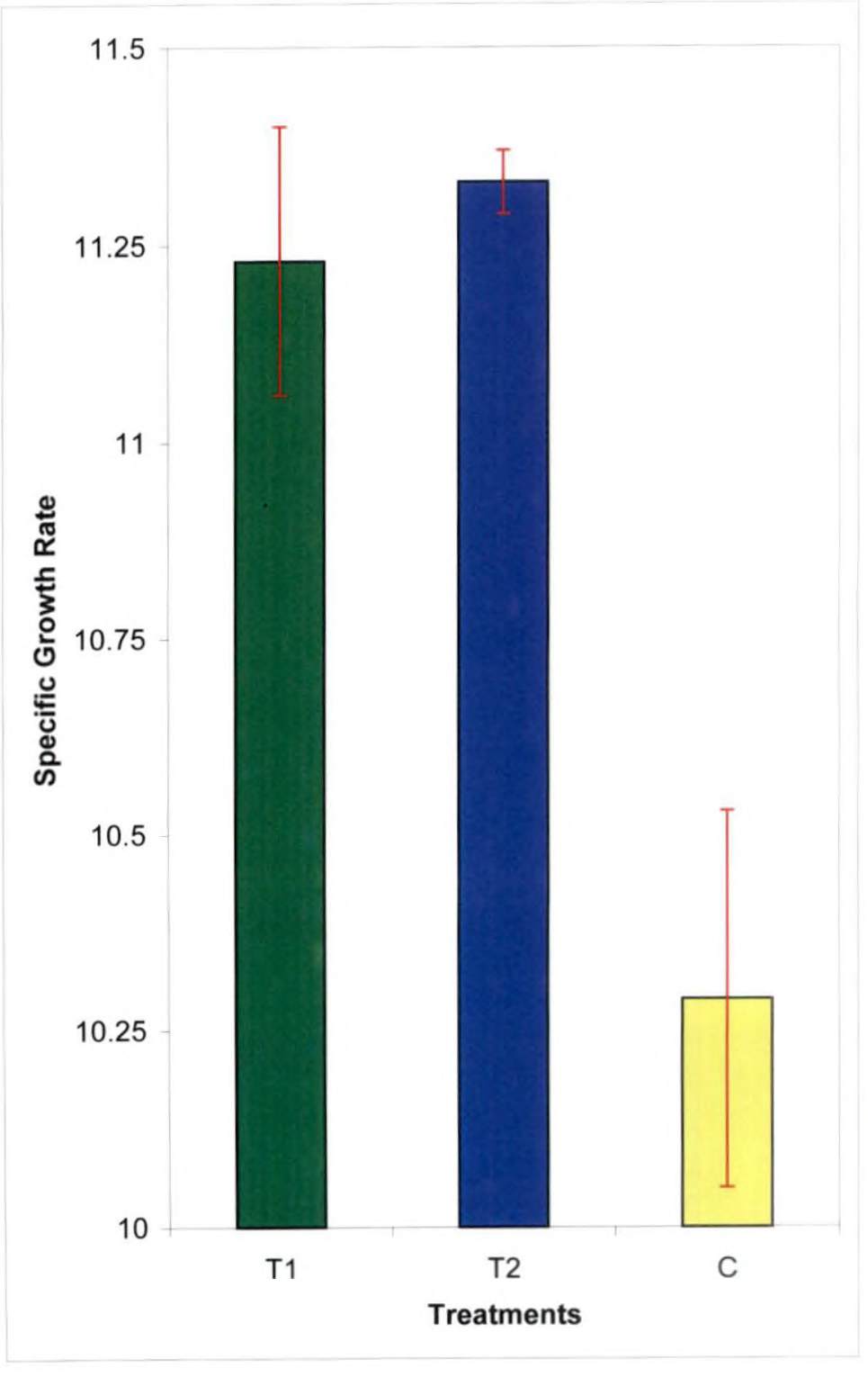

Figure 4. Specific Growth Rate of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Table 9. Percentage survival of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Treatment	Replication	Initial Stocking Number	Final Number Survived	Percentage Survival	Average Percentage Survival (Mean± SD)
T_1	1	105	74	70.48	63.81 ± 6.79
	2	105	62	59.05	
	3	105	77	73.33	
	4	105	59	56.19	
	5	105	63	60.00	
T_2	1	105	46	43.81	64.76 ± 11.41
	2	105	69	65.71	
	3	105	82	78.10	
	4	105	74	70.48	
	5	105	69	65.71	
C	1	105	69	65.71	58.67 ± 10.32
	2	105	66	62.86	
	3	105	71	67.62	
	4	105	61	58.10	
	5	105	41	39.05	

Table 10. ANOVA for percentage survival (arc sine transformed) of *Macrobrachium rosenbergii* reared in different probiotic treatments.

Sources of Variation	df	SS	MS	F	Table values of F (2,12)
Probiotics	2	39.63	19.81	0.48 (NS)	3.88 (5%) 6.93 (1%)
Error	12	496.89	41.41		
Total	14	536.52			

NS-Statistically not significant (P>0.05)

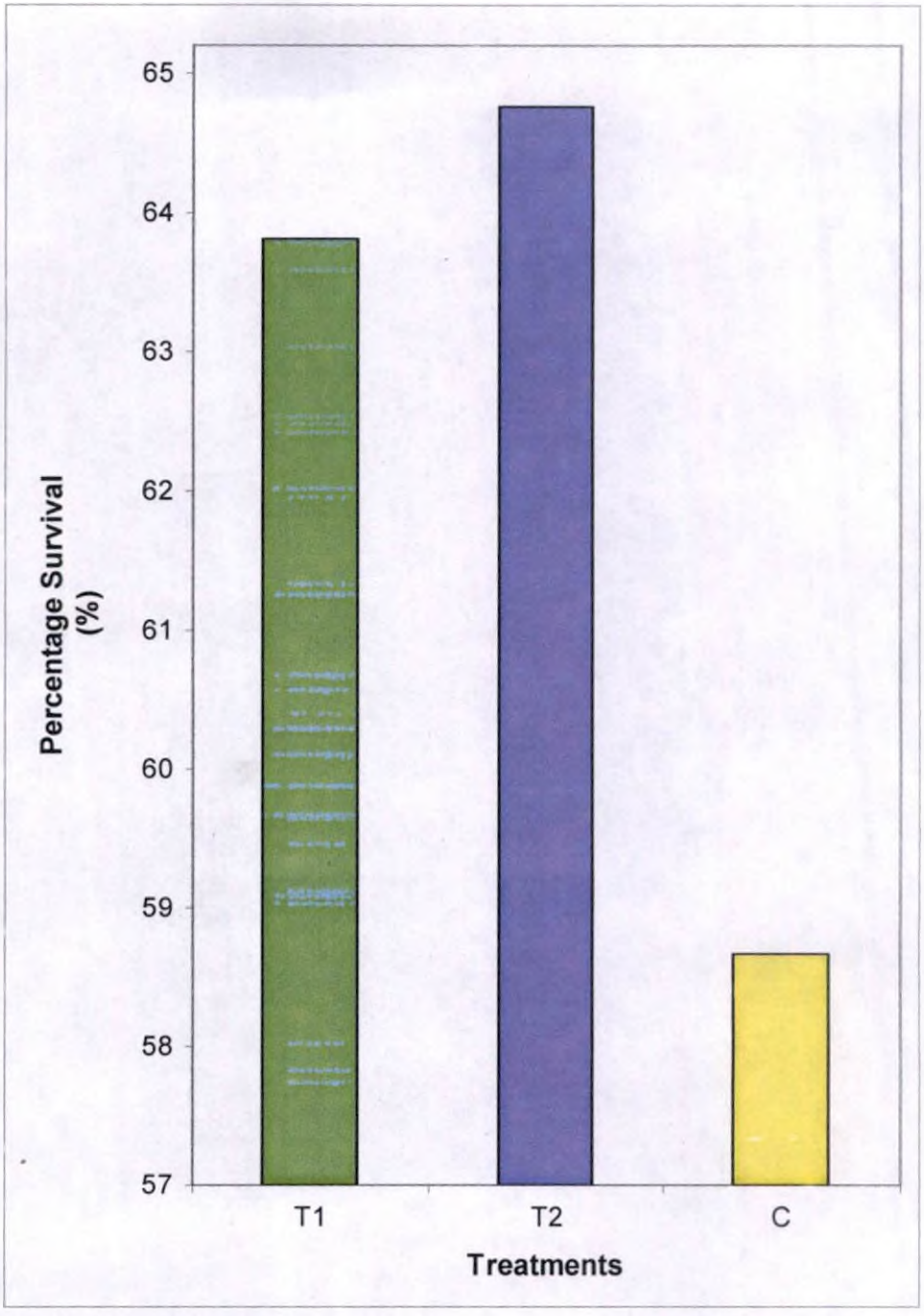

Figure 5. Percentage Survival of *Macro brachium rosenbergii* reared in different probiotic treatments.

4.2. MICROBIOLOGICAL OBSERVATIONS

4.2.1. Microorganisms in Water and Soil

The different microorganisms present in soil and water during various days of the experiment are shown in Table 11. The Total Plate Count in soil and water are given in Table 12 and Fig 6. Though soil from the same pond and water from the same source were used, there were variations in the initial TPC and microbial flora. The initial TPC was 1.72×10^2 cfu/ml for T_1, which increased to 3.2×10^3 cfu/ml on the 15th day and to 1.3×10^4 cfu/ml on the 30th day, the bacteria found being *Aeromonas caviae*, *A. sobria*, *A. hydrophila*, *Escherichia coli*, *Enterobacter aerogenus*, *Salmonella, Hafnia alvei* and *Streptococci*. The initial TPC for T_2 was $>3\times10^3$ cfu/ml which increased to 3.3×10^4 cfu/ml on 15th day but slightly reduced to 1.866×10^4 cfu/ml on the 30th day. The predominant bacteria found were *A. hydrophila*, *A. sobria*, *A. caviae*, *E. coli*, *Salmonella, E. aerogenus*, *Hafnia alvei*, *Streptococci* etc. For the control, the initial TPC of 2.49×10^2 cfu/ml was increased to 1.03×10^5 cfu/ml on day 15 and then registered a marginal decline to 1.008×10^5 cfu/ml on the 30th day. The microbial composition of the control was almost similar to the two treatments.

The probiotics seem to have not greatly affected the species composition of bacteria in the final samples. However, the *E. coli* found initially were not seen beyond 15th day of rearing in T_1 and T_2, although it continued to be present in the control.

Table 11. Microorganisms in water and soil during various days

Treatments	Day 0	Day 15	Day 30
T_1	*Aeromonas caviae* *Aeromonas hydrophila* *Aeromonas sobria* *Enterobacter aerogenus* *E. coli* *Hafnia alvei* *Salmonella* *Streptococci*	*Aeromonas sobria* *A. caviae* *Hafnia alvei* *Salmonella* *Streptococci*	*A. hydrophila* *A. caviae* *Aeromonas sobria* *Enterobacter aerogenus* *Hafnia alvei* *Salmonella*
T_2	*A. caviae* *A. sobria* *A. hydrophila* *E. coli* *Hafnia alvei* *Salmonella*	*A. caviae* *A. sobria* *Hafnia alvei* *Salmonella* *Streptococci*	*A. caviae* *E. aerogenus* *Hafnia alvei* *Salmonella* *Streptococci*
C	*A. caviae* *A. sobria* *E. coli* *Hafnia alvei* *Salmonella* *Streptococci*	*A. caviae* *A. sobria* *E. coli* *Hafnia alvei* *Salmonella* *Streptococci*	*A. caviae* *E. aerogenus* *E. coli* *Hafnia alvei* *Salmonella* *Streptococci*

Table 12. Total plate count of water and soil during various days

Treatment	Mean Total Plate Count (CFU/ml)		
	Day 0	Day 15	Day 30
T_1	1.72×10^2	3.2×10^3	1.3×10^4
T_2	$>3\times10^3$	3.3×10^4	1.87×10^4
C	2.49×10^2	1.03×10^5	1.01×10^5

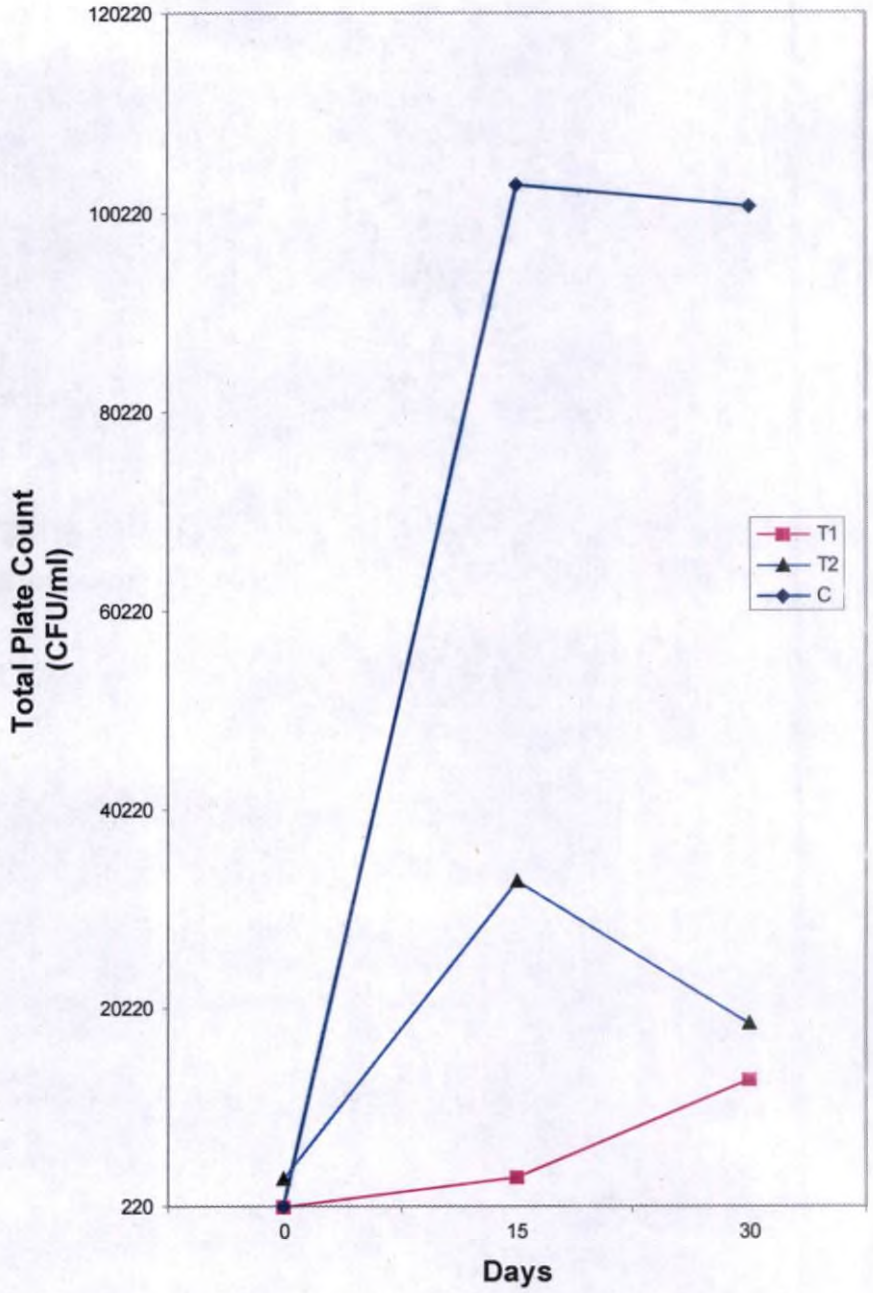

Figure 6. Total Plate Count of water and soil during various days of the experiment.

4.2.2. Microorganisms in PL

The different microorganisms present in the *M. rosenbergii* PL initially and at the end of the experiment are given in the Table 13. The total plate counts are given in Table 14.

Table 13. Microorganisms present in PL

Treatment	Microorganisms	
	Initial	Final
T_1	*A. sobria* *A. hydrophila* *E. aerogenus* *Hafnia alvei*	*E. aerogenus*, *A. caviae*, *Hafnia alvei*
T_2		*A. caviae*, *Citrobacter* *Hafnia alvei*
C		*Aeromonas caviae* *A. sobria*, *E. coli*

Table 14. Total plate counts of microorganisms present in PL

Treatment	Mean Total Plate Count (CFU/g) animal	
	Initial	Final
T_1	2.88×10^4	9.15×10^4
T_2		1.69×10^5
C		1.78×10^5

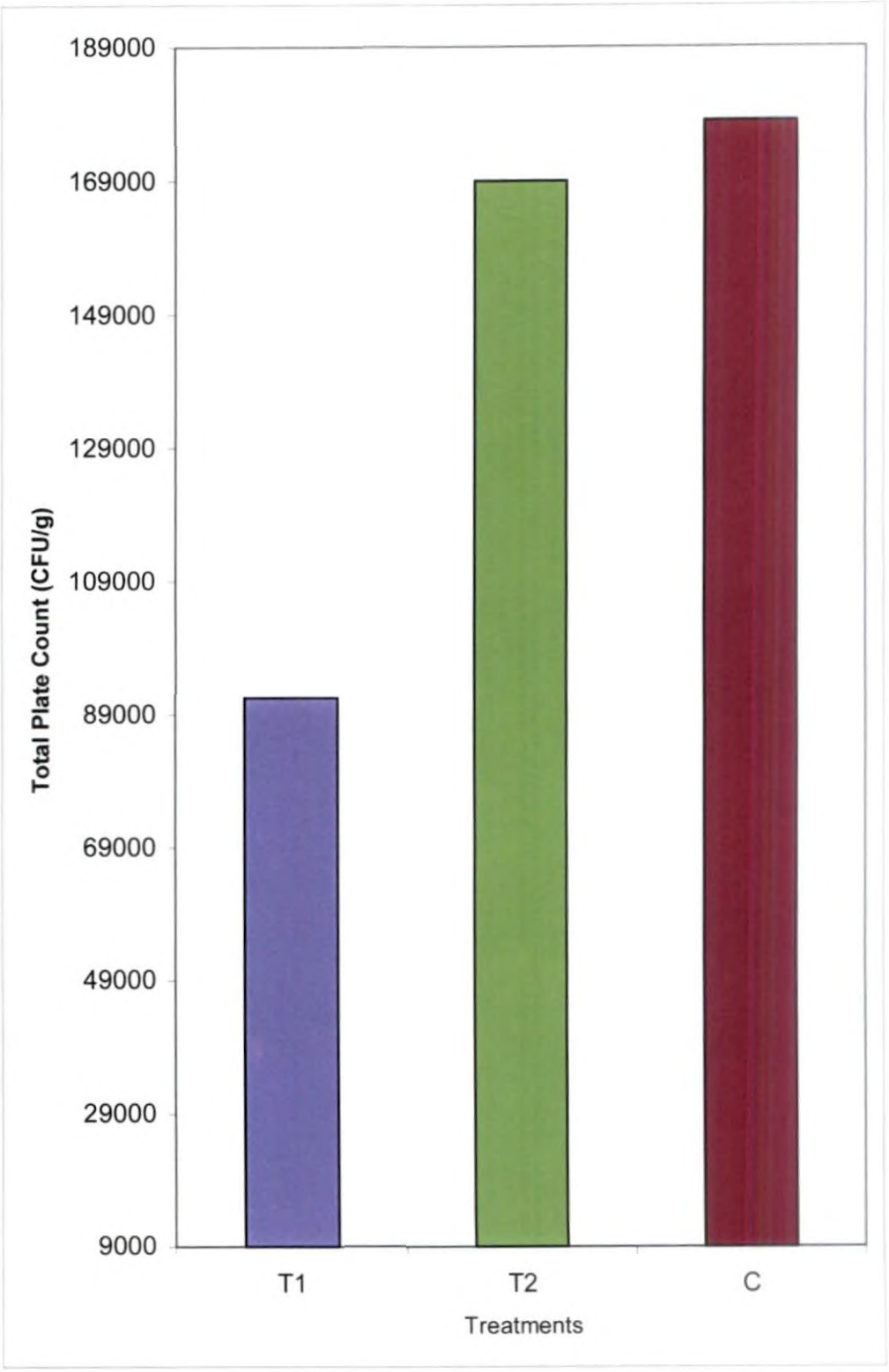

Figure 7. Total Plate Count in the body of PL after the experiment.

4.3. WATER QUALITY PARAMETERS

The water quality parameters in the rearing tanks remained within the optimum range during the course of the experiment. The water temperature ranged from 23.5 to 29.7 ^{0}C (Table 15) while the pH from 7.17 to 7.8 (Table 16 and Fig 8). The initial pH of the water ranged from 7.08 to 7.25 in T_1; 7.73 to 7.86 in T_2 and 7.25 to 7.3 in control. During day 15, it varied from 7.54 to 7.95 in T_1; 7.25 to 7.77 in T_2 and 7.24 to 8.2 in control. During day 30, it varied from 7.41 to 7.79 in T_1; 7.32 to 7.72 in T_2 and 7.42 to 7.8 in control. The pH and temperature in the experimental tanks remained stable throughout the experiment.

The total hardness of water varied from 103.6 to 105.8 mg/l as $CaCO_3$. There was not much difference in total hardness among the experimental tanks (Table 17 and Fig 9). However, there was marked reduction in the hardness of water as the days progressed during the experiment. The hardness was increased from the initial value of 104.5 to 105.8 and 105.6 mg/l in T_1, 104.5 to 104.9 and 103.7 mg/l in T_2 and 104.5 to 103.9 and 103.6 mg/l in control on the 15th and 30th day, respectively, during the experiment.

The fluctuations in the concentration of ammonia-nitrogen of water were found to be markedly different among the various experimental tanks (Table 18 and Fig 10). There was marked reduction in the ammonia concentration in the rearing water in T_1 and T_2 compared to the control. The mean ammonia-nitrogen declined from an initial value of 0.04 to 0.03 and 0.01 mg/l as NH_3-N in T_1, 0.05 to 0.01 and 0.00 mg/l as NH_3-N in T_2, but increased from 0.04 to 0.16 and 0.38 mg/l as NH_3-N in the control, on the 15th and 30th day, respectively.

The initial dissolved oxygen content of water varied from 4.17 to 4.20 mg/l in T_1; 4.18 to 4.24 mg/l in T_2 and 4.22 to 4.27 mg/l in control. During day 15, it ranged from 4.13 to 6.07 mg/l in T_1; 3.69 to 5.70 mg/l in T_2 and

2.01 to 4.69 mg/l in control. During day 30, it varied from 4.47 to 5.47 mg/l in T_1; 3.81 to 4.80 mg/l in T_2 and 2.74 to 4.47 mg/l in control. There was not much difference in the dissolved oxygen content among the treatment tanks (Table 19 and Fig 11).

Table 15. Temperature of water during various days of the experiment.

Treatment	Mean Temperature (°C)		
	Day 0	Day 15	Day 30
T_1	29.4	23.5	24.5
T_2	29.7	23.5	24.5
C	29.4	23.5	24.5

Table 16. pH of water during various days of the experiment.

Treatment	Mean pH		
	Day 0	Day 15	Day 30
T_1	7.17	7.79	7.61
T_2	7.8	7.55	7.53
C	7.28	7.79	7.55

Table 17. Total Hardness of water during various days of the experiment.

Treatment	Mean Hardness (mg/l as $CaCO_3$)		
	Day 0	Day 15	Day 30
T_1	104.5	105.8	105.6
T_2	104.5	104.9	103.7
C	104.5	103.9	103.6

Table 18. Ammonia-Nitrogen of water during various days of the experiment

Treatment	Mean Ammonia-Nitrogen (mg/l as NH_3-N)		
	Day 0	Day 15	Day 30
T_1	0.04	0.03	0.01
T_2	0.05	0.01	0.00
C	0.04	0.16	0.38

Table 19. Dissolved Oxygen of water during various days of the experiment

Treatment	Mean Dissolved Oxygen (mg/l)		
	Day 0	Day 15	Day 30
T_1	4.18	4.99	4.87
T_2	4.21	4.71	4.45
C	4.24	3.95	3.94

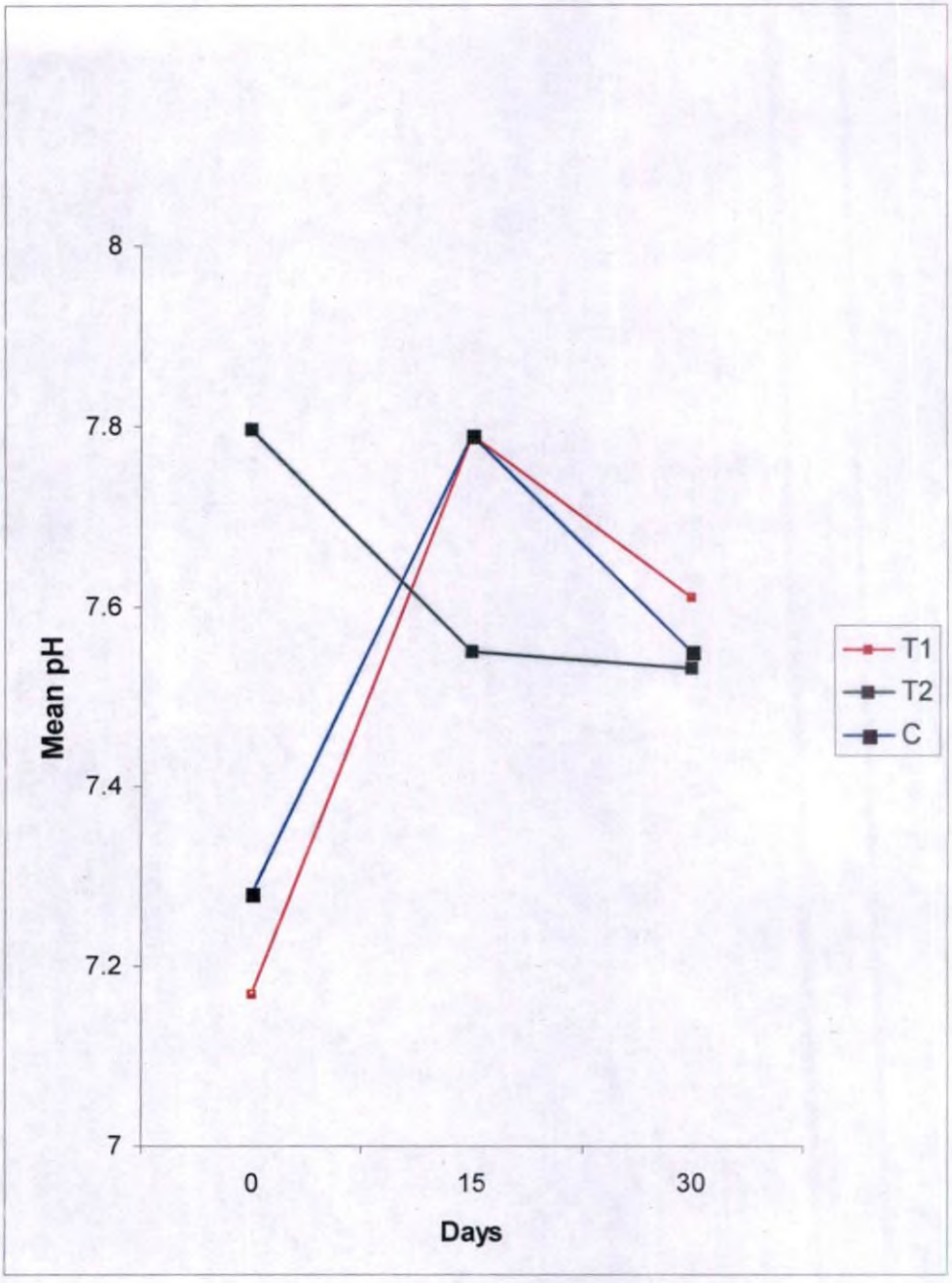

Figure 8. pH of water during various days of the experiment.

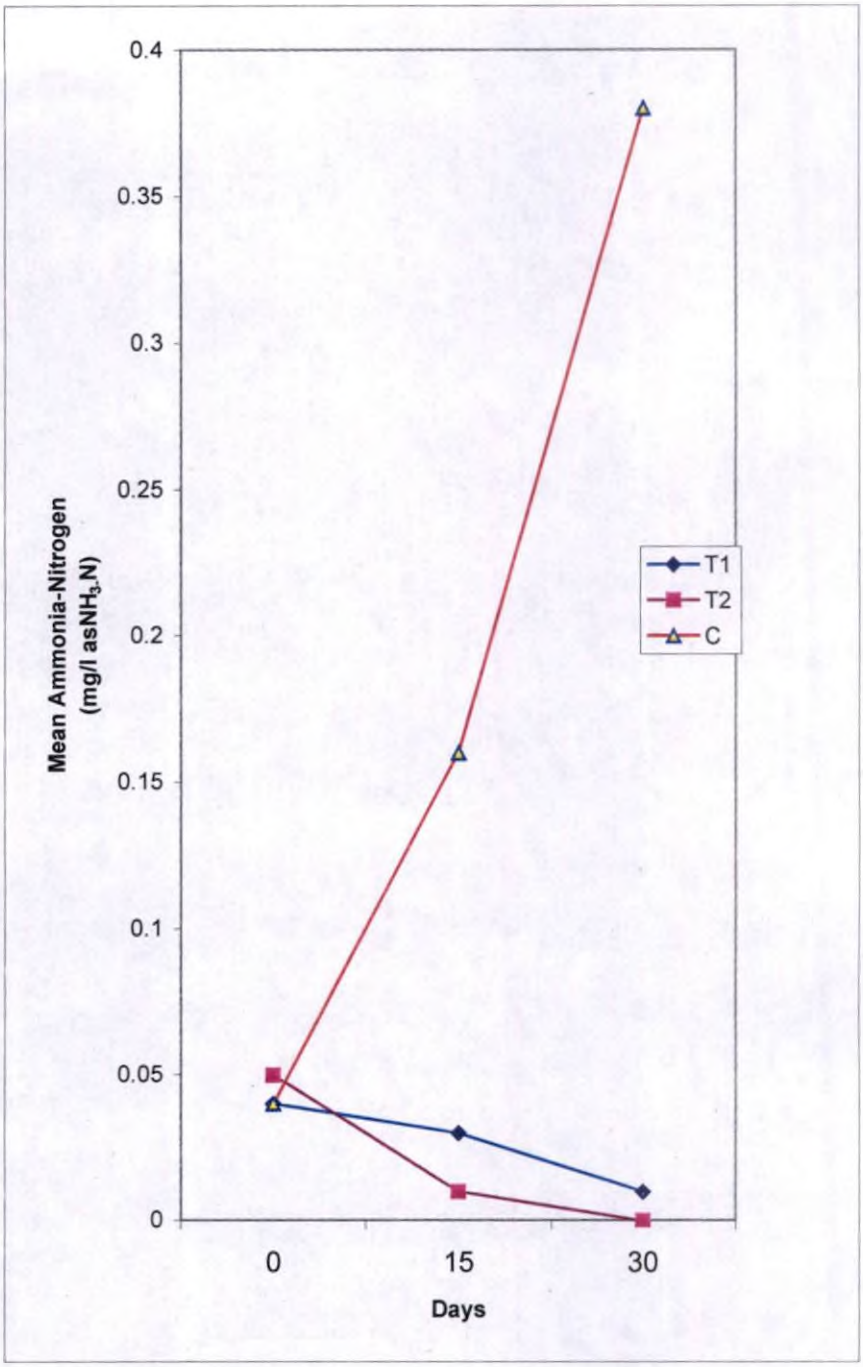

Figure 9. Ammonia-Nitrogen of water during various days of the experiment.

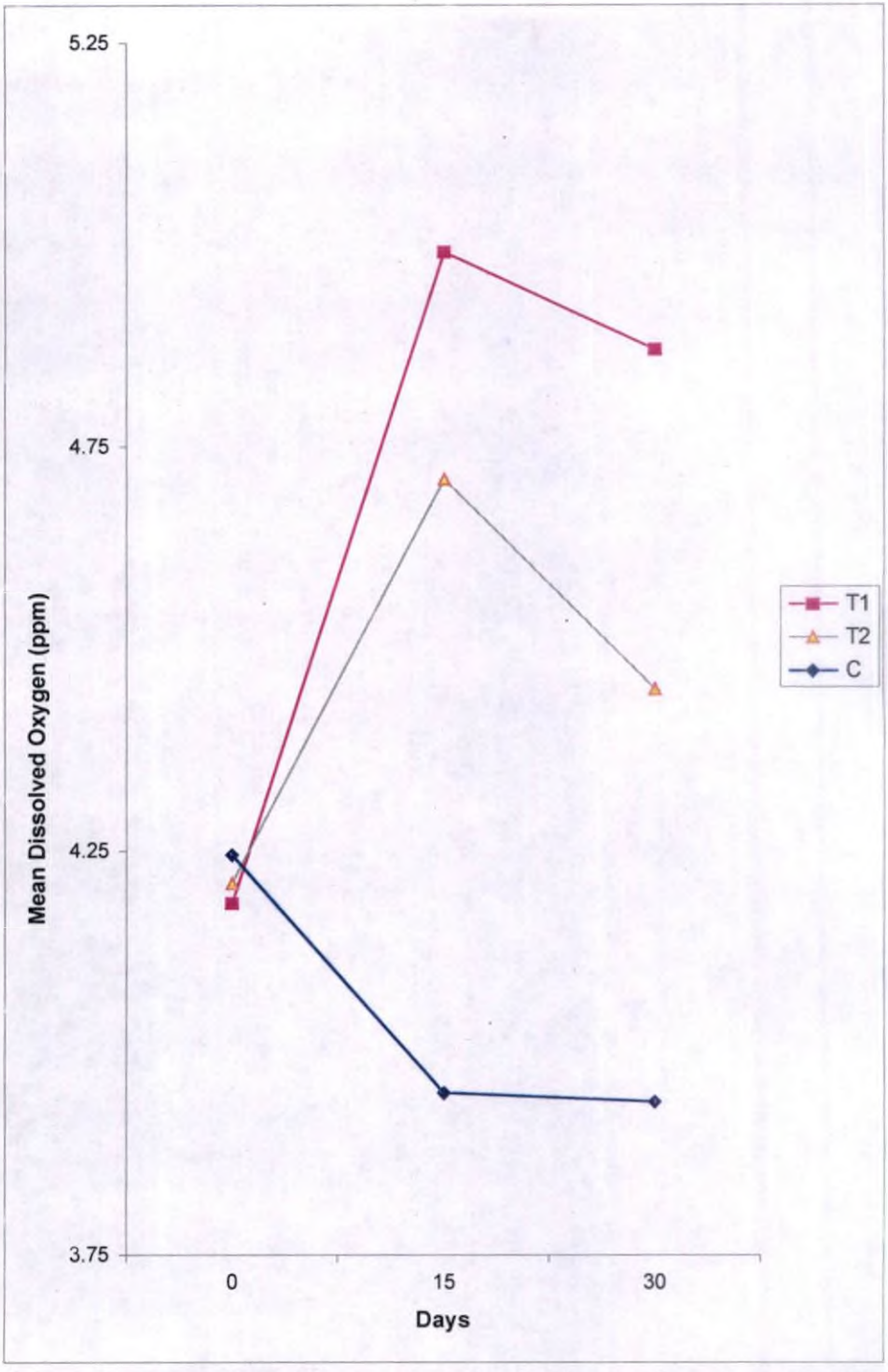

Figure 10. Dissolved Oxygen of water during various days of the experiment.

Discussion

5. DISCUSSION

5.1. BIOLOGICAL OBSERVATIONS

5.1.1. Food Conversion Ratio

The food conversion ratio of post larvae of *M. rosenbergii* varied in the range of 0.33 to 0.53 when fed the control diet and diets containing different probiotics (Indulkar and Belsare, 2003). In another experiment, they reported that the FCR of post-larvae of *Macrobrachium rosenbergii* fed the control diet and diets containing various doses of probiotic varied in the range of 0.36 to 0.54.

In the present study, the food conversion ratio of post larvae of *Macrobrachium rosenbergii* was 0.76 in Epicin, 1.10 in Waves and 2.03 in control, which is in agreement with the results obtained by Indulkar and Belsare (2003).

In the case of *Fenneropenaeus indicus* fed with Lacto-sacc, the food conversion ratio in the treatment groups ranged from 1.698 to 2.95 and in the control group it was 3.28 (Uma *et al.*, 1999).

5.1.2. Average Gain in Weight

The average gain in weight was 895.8 mg in Epicin treated tanks followed by Waves with 869.96 mg and the control sets with 650.17 mg, which showed significant difference between the treatments and control.

The observations made by Indulkar and Belsare (2003) showed that the average gain in weight of *M. rosenbergii* PL was found to vary from 26.97 to 35.79 mg in treatment groups incorporated with different probiotics, in the control it was only 22.37 mg as in agreement with the results obtained in the present study. In another experiment, they got 24.63 mg in control group and 29.00 to 36.28 mg in treatment groups.

Rengpipat *et al.* (1998a) recorded mean shrimp weights of all probiont treatment groups, as 7.06±0.48 g while it was 3.99 ± 0.38 g in control after 100 days treatment. Uma *et al.* (1999) observed that the mean total wet weight gain was almost doubled in shrimp fed with Lactosacc at 2.5 g/kg feed level compared to the control group. In the treatment it ranged from 1.14 to 1.87g and in the control it was 0.96g.

5.1.3. Average Gain in Length

In two separate experiments carried out by Indulkar and Belsare (2003), the average gain in length of *M. rosenbergii* PL was found to vary from 8.70 to 11.48 mm in treatment groups incorporated with different probiotics, in the control it was only 7.21 mm and it was 8.42 to 11.18 in treatment groups and 7.05 in the control group.

In the present study also the average gain in length was 31.25 mm in Epicin treated tanks followed by Waves with 28.14 mm and the control sets with 26.4 mm. The results also showed significant variation between the control and the treatment.

5.1.4. Specific Growth Rate

In an experiment by Indulkar and Belsare (2003), the specific growth rate (% day^{-1}) of post larvae of *Macrobrachium rosenbergii* varied in the range of 12.09 to 13.71 in the groups fed with probionts, Gp-1, Gp-2, Gp-3, Gp-4 and Gp-5. In the control group, it was found to be 11.07. In another experiment, they got 11.87 to 13.15 in treatment groups and 10.99 in the control.

In the present study also the specific growth rate was higher in the probiotic treated tanks than in the control. It was 11.33 in Epicin treated tanks and 11.23 in Waves. In the control sets it was only 10.29.

Uma *et al.* (1999) got 2.23 to 3.02 in treatment groups fed with Lactosacc and 2.015 in control groups of *Fenneropenaeus indicus,* while Sridhar and Raj (2003) got 0.204 to 0.846 in control diet and diets incorporated with probiotics in *F. indicus* PL.

5.1.5. Percentage Survival

Rengpipat *et al.* (1998a) got 33.3±4.4% survival after 100 days in the probiont treatment groups of shrimps, *P. monodon* while it was 15.8 ± 5.2% in control. After 10 days challenge with *V. harveyi* D 331, survival was 100% in probiotic treatment groups and 26% in control.

Uma *et al.* (1999) got 100% survival in the case of *Fenneropenaeus indicus* fed with Lacto-sacc, against 77.66% in control group. Alston and Sampaio (2000) observed a survival rate of 80% in well-managed research hatcheries and 40-60% in commercial hatcheries of *M. rosenbergii.* Sridhar and Raj (2003) got 82.5 to 96.5% in control diet and diet incorporated with probiotics in *F. indicus* PL. Nayak *et al.* (2003) got 39% survival in probiotics treated tanks of *Macrobrachium rosenbergii,* whereas, it was 12% in the control tanks.

Indulkar and Belsare (2003) got 90 to 95% survival in the post-larvae of *Macrobrachium rosenbergii* fed for 15 days on control diet as well as on diets incorporated with different probiotics. In another experiment, they got 90 to 96.67% survival in the post-larvae of *Macrobrachium rosenbergii* fed on both the control diet and the diets containing probiotic in different doses.

In the present study, the survival rate was 64.76% in Epicin treated tanks, 63.81 % in Waves and 58.67% in the control. The results were identical on statistical analysis with no difference between the control and treatments showing that the results of the present study confirm with the observations made by Sridhar and Raj (2003).

5.2. MICROBIOLOGICAL OBSERVATIONS

5.2.1. Microbiological Observations in Soil and Water

5.2.1.1. Microorganisms Present in Soil and Water

In the present study, the soil and water before introducing the probiotics contained microorganisms such as *Aeromonas caviae, A. sobria, A. hydrophila, Hafnia alvei,* faecal *Streptococci, E. coli, Salmonella,* and *Enterobacter aerogenus*. Vanderzant *et al.* (1971) found Coryneformes and species of *Flavobacterium, Moraxella* and *Bacillus* to be dominant in pond water of brown shrimp, *Penaeus aztecus*. Fujioka and Greco (1984) enumerated *Vibrio* spp. in the larval culture medium of *Macrobrachium*. Maeda and Liao (1992) isolated a strain PM-4 of *Thalassobacter utilis* from the rearing waters of *P. monodon* larvae. Hameed (1993) also found *Alcaligenes* (19-24%) *Vibrio* (16-20%), *Pseudomonas* (10-13%), *Aeromonas* (7-11%) and *Flavobacterium* (6-12%) in the larval rearing tank waters of *Penaeus indicus*. Direkbusarakom *et al.* (1997) reported *Vibrio* spp. to be dominant in shrimp hatchery.

Vaseeharan and Ramasamy (1999) found higher counts of *Vibrio harveyi* in untreated pond sediments. Phatarpekar *et al.* (2002) found *Aeromonas, Alcaligenes* and *Pseudomonas* to be dominant in the larval rearing waters of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Alteromonas* and *Xanthomonas* were also seen in water. Gullian and Rodriguez (2002) identified *Vibrio* P62 and *Bacillus* P64 from the *F. vannamei* rearing ponds.

After the regular introduction of probiotics for 30 days, in the present case, microorganisms such as *Aeromonas caviae, A. sobria, A. hydrophila, Hafnia alvei, E. aerogenus, Salmonella* and *Streptococci* were seen in the water and soil of both T_1 and T_2 and the control. The *E. coli*, which was

found originally, before the experiment, was not seen in the treatment tanks on the 15^{th} and 30^{th} days of analysis. No new species of bacteria was found to have developed in the treatment tanks as a result of the introduction of probiotics.

5.2.1.2. Total Plate Count in Soil and Water

In the present study, before introducing the probiotics, the total plate count ranged from 1.72×10^2 to $>3 \times 10^3$. During the course of the experiment, the TPC in the control tanks shot up to 1.03×10^5 in 15 days time, while for the rest 15 days, the growth was stagnant, and remained at 1.01×10^5 on the 30^{th} day. In T_1, the TPC registered a regular pattern of growth. It increased from the initial 1.72×10^2 to 3.2×10^3 on the 15^{th} day and then to 1.3×10^4 on the 30^{th} day. The treatment T_2 showed sudden rise from $>3 \times 10^3$ to 3.3×10^4 on the 15^{th} day, and then slightly declined to 1.87×10^4 on the 30^{th} day.

Aquacop (1977) recorded 2.6×10^5 to 4.9×10^5 cfu/ml; 6×10^0 to 1×10^6 cfu/ml (Miyamoto *et al.*, 1983) and 3.7×10^3 to 5.6×10^6cfu/ml (Anderson *et al.*, 1989) in tank water. Hameed (1993) found the bacterial flora in the larval rearing waters of *F. indicus* to have ranged from 9.0×10^2 to 1.0×10^5 cfu/ml.

Vaseeharan and Ramasamy (1999) reported that the Total Plate Count of *Vibrio harveyi* in untreated pond sediments was $2.5 \pm 0.22 \times 10^3$ cfu/g; while that in Wunapuo-15 treated pond sediments was $1.97 \pm 0.19 \times 10^3$ cfu/g, and DMS-treated pond sediment was $1.7 \pm 0.15 \times 10^3$ cfu/g. Dharma (2000) observed that the total heterotrophs ranged from 41.66×10^3 to 603.33×10^3 cfu/ml in the water treated with probiotics and in control. Phatarpekar *et al.* (2002) found that the Total Count for water ranged from 0.1×10^4 to $28 \pm 4.0 \times 10^4$cfu/ml. Total Viable Count of water of ranged from $1.1 \pm 0.6 \times 10^4$ to $9.8 \pm 1.5 \times 10^6$ cfu/ml.

The results obtained for the present study also showed similar patterns as reported by various authors (Miyamoto *et al.*, 1983; Anderson *et al.*, 1989; Hameed, 1993; Vaseeharan and Ramasamy, 1999; Dharma, 2000 and Phatarpekar *et al.*, 2002).

5.2.2. Microbiological Observations in PL

5.2.2.1. Microorganisms Present in PL

Yasuda and Kitao (1980) found *Vibrio* to be the dominant genus in larvae of *F. japonicus*. Miyomoto *et al.* (1983) and Colorni (1985) recorded *Vibrio* in *M. rosenbergii* larvae. Singh (1986) and Hameed (1993) also reported a similar observation in *F. indicus*. Anderson *et al.* (1989) observed *Vibrio* in the washed larval tissue-slurries of *M. rosenbergii*.

Sakata (1989) reported that aquatic invertebrates, including crustaceans, annelids and molluscs harbour gut bacteria. Phatarpekar *et al.* (2002) identified 16 genera from rearing water, eggs, larvae and different organs of berried prawns and the most frequently isolated ones were *Pseudomonas*, *Aeromonas*, *Alcaligenes* and of the family Enterobacteriaceae. Bacteria isolated from the samples were predominantly gram-negative, although Gram-positive ones were represented by *Micrococcus*, *Staphylococcus* and *Streptococcus*. Nine genera were isolated from larvae and the most frequently isolated genera were *Alcaligenes, Pseudomonas*, *Streptococcus* and of family, Enterobacteriaceae. He also found *Cytophaga, Photobacterium* and *Xanthomonas* in *M. rosenbergii* larvae.

In the present study, microorganisms such as *A. sobria, A. hydrophila, Hafnia alvei* and *E. aerogenus* found in larvae, continued in the treatment and control tanks after the experiment period of 30 days. In T_2, a new group of bacteria, *Citrobacter* was seen to have developed in the gut of the animal, while in the control, *E. coli* was observed. Citrobacter also

belongs to enterobacteriaceae group, which was seen in the control. The results showed similar pattern to Phatarpekar *et al.* (2002).

5.2.2.2. Total Plate Count in PL

Yasuda and Kitao (1980) observed an increase in the bacterial population from egg to mysis of *Penaeus japonicus* and thereafter the population declined as the larva transformed to the post-larval stage. Miyamoto *et al.* (1983) noted higher bacterial counts in larvae ($1.9x10^3$ to $4.3x10^7$ cfu /g) than rearing water in two *Macrobrachium rosenbergii* hatcheries in Hawaii.

A general trend of increased bacterial cell count per gram of larval tissue with increased age of larvae was noted in freshwater prawns by Miyomoto *et al.* (1983) and Anderson *et al.* (1989).

Singh (1986) noted that the bacterial population increased from egg to protozoa, then decreased in the mysis stage, and again increased in the post-larval stage. Anderson *et al.* (1989) noted $4.4x10^8$ cfu/g in *M. rosenbergii* larvae.

Hameed (1993) found that the total number of colony forming units per gram (cfu/g) for post-larvae of *Fenneropenaeus indicus* ranged from 9.5×10^5 to 1.2×10^8. Seema *et al.* (1997) detected $1.37x\ 10^5$ TPC/g in *P. monodon.* Sridhar and Raj (2001) observed probiotic organism 10^6 cfu/shrimp in the gut of shrimp PL.

Phatarpekar *et al.* (2002) found Total Viable Count (TVC) of larvae of *Macrobrachium rosenbergii* ranged from $2.5\pm1.4\times10^4$ to $1.6\pm1.0\times10^8$ cfu/g. A high TC count of $76.0\pm7.0\times10^4$ cfu/g was observed in the first larval stage and similar counts were not detected until the tenth larval stage.

In the present study, the total plate count in the PL was found to be $2.88\ x\ 10^4$ cfu/g, which agreed with the results of Phatarpekar *et al.* (2002) and Miyomoto *et al.* (1983). After the end of the experiment, the TPC was

found to be $1.78x10^5$ cfu/g in the control prawns while in the case of treatment groups it was 9.15×10^4 cfu/g and 1.69×10^5 cfu/g, which agreed with the results of Miyomoto *et al.* (1983), Seema *et al.* (1997) and Phatarpekar *et al.* (2002).

5.3. WATER QUALITY PARAMETERS

During this study the temperature of water in the experimental tanks varied from 23.5°C to 29.7°C, which was found to be in the optimum range, recommended for the culture of *M. rosenbergii* postlarvae (Fujimura, 1974; Smith and Sandifer, 1979; Sandifer *et al.*, 1983; Pillay, 1990).

Ammonia-Nitrogen which was 0.04 mg/l during the start of the experiment, reduced to 0.01 mg/l on the 15^{th} day and 0.00 mg/l on the 30^{th} day in tanks treated with Epicin (T_2). In T_1 where the tanks treated with Waves, the NH_3-N reduced to 0.03 mg/l on the 15^{th} day and 0.01 mg/l on 30^{th} day. But in the control, it increased to 0.16 from 0.04 mg/l on the 15^{th} day and 0.38 mg/l on the 30^{th} day.

Prabhu *et al.* (1999) also reported that ammonia values increased from 0.22 mg/l on DOC 44 to 2.5 mg/l on 109^{th} day in the control pond. While in the experimental ponds, there was rhythmic lowering after the application of probiotics.

Dissolved oxygen levels should be maintained at approximately 5 mg/l in intensive culture (Sandifer *et al.*, 1983). Boyd (1989) reported that an oxygen content of 3.5ml/l is highly essential to support better growth of cultivable fin and shellfishes. The D.O. content of experimental pond treated with Buck-up remained above 3.6 ml/l while in the control pond it remained lower throughout and towards the end got reduced to 2.7 and 2.6 ml/l (Rani, 1996).

Prabhu *et al.* (1999) also got a similar result. In the control pond the oxygen level varied from 4 to 4.8 mg/l. The level was more in the

experimental ponds treated with probiotics and it varied from 4.6 to 5.8 mg/l.

Dissolved oxygen which was initially around 4.24 mg/l slightly decreased in the control to 3.94 mg/l on the 30th day of the experiment, but it increased to 4.87 mg/l and 4.45 mg/l in tanks treated with Waves and Epicin, respectively.

Hardness ranged from 103.6 to 105.8 mg/l as $CaCO_3$ in the experimental tanks, which was found to be in the optimum range (Cripps and Nakamura, 1979; New and Singholka, 1985; Vasquez *et al.* 1989).

Results of the experiment showed that the introduction of the probiotics (both Epicin and Waves) has raised the survival and growth of *M. rosenbergii* in the nursery tanks, compared to the control. But analysis of the bacteria during the course of experiment in soil, water and animal indicates that no new bacteria colony has developed in the tanks treated with the probiotics, nor the bacteria originally present has disappeared from the water and soil as a result of the introduction probiotics, other than the *E. coli* which disappeared from the treatment tanks when the probiotics were introduced. It seems that beneficial bacteria present in the probiotics could not have detected because they might have not developed in the plate count agar used in the present experiment. These two probiotics may be more effective in marine conditions than in freshwater. In the experiment, no quantification of the individual bacteria was made. It seems that some of the harmful bacteria like *Salmonella*, which was initially present might have reduced in its proportion at the end of the experiment. That might be the reason for the increase in growth and decrease in the FCR. The NH_3-N in the culture tanks treated with probiotics also reduced considerably compared to the control.

Summary

6. SUMMARY

The objective of the present study was to find out the effect of two commercial probiotics namely, Waves and Epicin on the chemical and biological characteristics of soil and water in the nurseries of *Macrobrachium rosenbergii* PL and its resultant effect on the growth and survival of the animal. The methodology, results and conclusions of the study are given below.

1. *Macrobrachium rosenbergii* PL of length 16.56 mm were selected for the study.
2. PL were reared in 70 litre capacity fiberglass tanks filled with 5 cm soil collected from a fish pond. Water was filled to a level of 25 cm. The stocking density was 500 no/m^2.
3. PL were fed with common freshwater prawn starter feed 1 (Higashi).
4. There were two treatments and a control each with five replications.
5. The probiotics Waves and Epicin were applied @ 0.5 ppm and 0.25 ppm respectively in the water at an interval of 10 days.
6. The microorganisms present in the water and soil and also the water quality parameters were recorded at an interval of 15 days.
7. The duration of the experiment was 30 days, after which, food conversion ratio, average gain in weight, average gain in length, specific growth rate and percentage survival were worked out.
8. The experiment was conducted using Completely Randomised Design and results were analysed by ANOVA technique.
9. Analysis before the introduction of the probiotics showed that microorganisms such as *Escherichia coli*, *Salmonella*, Faecal *Streptococci*, *Hafnia alvei*, *Aeromonas caviae*, *A. hydrophila*, *A. sobria* were present in the water and soil.
10. But probiotics did not affect the species composition of the bacteria in the water, soil and body of the prawn. But *E. coli*, which was

initially present and continued in the control, was not detected in the tanks treated with the probiotics.

11. After the experiment was over the body of the PL was taken and tested for microorganisms. *Enterobacter aerogenus, A. caviae, Citrobacter* sp. and *Hafnia alvei* were found in the body of PL.

12. The lowest mean food conversion ratio of 0.76 was recorded for the PL treated with Epicin, followed by 1.10 with Waves and 2.03 in the control group with significant difference ($P<0.01$) between the Epicin and control groups.

13. The animals treated with Epicin showed the maximum mean average gain in weight of 895.80 mg followed with 869.96 mg in Waves. In the control the average gain in weight was 650.17 mg. The differences were significant ($P<0.01$) between control and treatment groups.

14. A mean average gain in length of 31.25 mm was obtained for the PL treated with Epicin, 28.14 mm for Waves and 26.4 mm for the control group. There was significant difference ($P<0.05$) between control and Epicin group.

15. Maximum mean SGR of 11.33 was recorded in the PL treated with Epicin, followed by 11.23 with Waves and 10.29 in control group with significant difference ($P<0.01$) between control and treatment groups.

16. The highest mean percentage survival of 64.76% was obtained for the PL treated with Epicin, followed by 63.81% with Waves and 58.67% in the control. But differences were not found to be significant on statistical analysis.

17. Ammonia -nitrogen level was 0.04 mg/l as NH_3-N in day 0 in all tanks. The level continuously increased in control tanks as 0.16 mg/l as NH_3-N in day 15 and 0.38 mg/l as NH_3-N in day 30. In Waves it decreased to 0.03 mg/l as NH_3-N in day 15 and finally reached 0.01 mg/l as NH_3-N in day 30. In Epicin tanks, it decreased to 0.01 mg/l

as NH_3-N in day 15 and finally reached 0.00 mg/l as NH_3-N in day 30.

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COMMERCIAL PROBIOTICS IN TANK REARED NURSERY PHASE OF FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The efficacy of two commercial probiotics, Waves and Epicin on the growth and survival of *M. rosenbergii* PL and on the chemical and biological characteristics of water and soil were studied. The experimental set up consisted of fiberglass tanks of 70 l capacity with two treatments and a control, each with 5 replicates. Soil from a fishpond was filled to a height of 5 cm and water filled to a height of 25 cm. The post larvae were stocked @ 500 no/m^2 and mild aeration provided. Commercial freshwater prawn feed starter 1 (Higashi) was given @ 100% of body weight. Analysis before the introduction of probiotics showed the presence of microorganisms such as *E. coli, Salmonella, Hafnia alvei,* Faecal *Streptococci, Aeromonas caviae, A. sobria, A. hydrophila* and *Enterobacter aerogenus* in the water and soil. Enterobacteria such as *Enterobacter aerogenus, Hafnia alvei, Aeromonas sobria* and *A. hydrophila* were present in the PL. The probiotics Waves and Epicin were added @ 0.5 ppm and 0.25 ppm respectively to the experimental tanks once in ten days and the microbiological observations and water quality parameters were studied once in 15 days. The introduction of the probiotics increased the growth and survival and reduced the F.C.R. of *M. rosenbergii* in nursery system. The species composition of the bacteria in the soil, water and body of the animal were not affected by treatment with probiotics, other than that the *E. coli* present originally and continued in the control was not detected in the tanks treated with probiotics. The T.P.C. was also less in the treatments compared to the control. Probiotics considerably reduced the level of NH_3-N in culture tanks. Epicin was found to have reduced the NH_3-N level effectively than the Waves.