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Beneficial uses of probiotics in mass scale production of marine ornamental fish

Shanmugam Velmurugan* and Santhanam Rajagopal

CAS in Marine Biology, Annamalai University, Tamil Nadu 608502, India.

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Mass scale culture of marine ornamental fish is facing many problems caused by pathogenic microorganisms. The accumulation of unconsumed feed and bad water quality influence the growth of pathogenic forms of the *Vibrio* sp. To avoid these problems probiotics are used as an alternative agent *in vitro* for efficacy based on the inhibition of growth of pathogenic *Vibrio* sp and also it decreased the concentrations of ammonium, nitrite, nitrate and phosphate ions. The present study revealed that after the use of probiotics in experimental tank the beneficial microbial load is drastically increased but decreased the level of pathogenic Vibrios. The bacterial population changed during every sampling. THB in the experimental tank before the application of probiotics ranged from $2.39X10^3$ to $4.82X10^4$. After the application of probiotics, THB population varied between $4.21X10^6$ and $7.43X10^7$. In the control tank, THB count was found to be between $5.28X10^3$ and $2.1X10^5$, but *Vibrio* load was high. *Vibrio* sp counts ranged from $6.82X10^2$ to $9.21\ X10^3$ CFU/ml in the experimental tank prior to application of probiotics. After the application of probiotics, *Vibrio* population varied between $1.62\ X10^2$ and $3.96\ X10^2$ CFU/ml. In the control tank, *Vibrio* count was found to be between $8.56\ X10^2$ and $2.1\ X10^4$ CFU/ml.

Key words: Probiotics, ornamental fish, disease.

INTRODUCTION

Today ornamental fishes are the most popular pets of the world and ornamental fish keeping is the second popular hobby next to photography. To cope up with the emerging demand, in captive breeding and rearing programs are being carried out in various parts of the world. However, maintenance in laboratory conditions is plagued by a variety of diseases which require regular use of antibiotics and other chemicals to avoid the pathogens. In marine ornamental fish cultures the major threat is the disease out break due to the pathogenic form of the microbes. Pathogenic microorganisms implicated in these outbreaks were viruses, bacteria, rickettsia, mycoplasma, algae, fungi and protozoan parasites. One of the commonest bacterial diseases, affecting farmed ornamental fishes in marine conditions is caused by *Vibrio* species, which consists of luminescent Vibriosis such as *Vibriosis harveyi, Vibriosis splendidus, Vibriosis parahaemolyticus*. The use of probiotics in aquaculture is becoming increasingly popular, having recently been defined as 'microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health (Gatesoupe, 1999). The probiotics application came first as it is environmentally safe and cost effective (Moriarty, 1997). The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. This benefit of probiotics will be long lasting, and the application of probiotics will become a major field in the development of aquaculture (Ravi et al., 1998). Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Thampsonet et al., 1999; Verchuere et al., 2000).

In the present study, water probiotic was used. It is a unique combination of aerobic, microaerophilic and anaerobic microorganisms to maintain fish tank in an environmentally balanced condition. It is a blend of pure strains

*Corresponding author. E-mail: vel_cas@yahoo.com. Tel: 04144–243223*212. Fax: +914144243555.

fortified with nutrients for better growth of the microorganisms at the time of brewing. Aerobic bacterium in this formula helps in the continuous degradation of the organic matter by mineralization process. The bacterium cellulomonas helps in the degradation of unutilized feed. The microorganisms in water probiotics compete in growth with *Vibrio* sp bringing down *Vibrio* population due to competitive exclusion. These beneficial bacteria have the bactericidal effect, which targets specially *V. harveyi.* The organisms also produce exudates which keep the culture animals free from other secondary infections. The use of probiotics in fin and shell fish culture system is well documented. Currently more emphasis is being given to application of probiotics in larviculture and live food organisms. Studies on the applicability of probiotics to ornamental fishes are very limited. Apart from few works available on freshwater ornamental fishes, there is no work on probiotics in marine ornamental fishes. The present study aims at understanding the efficiency of a commercially available probiotics on the maintenance of a commonly traded and highly priced *Neopomacentrus nemurus* under captive conditions.

MATERIALS AND METHODS

Probiotics used in the experiment

PROACT is a probiotics commercially available. It was formulated and manufactured by Matrix Biosciences Ltd. It is a combination of aerobic, microaerophilic and anaerobic microorganisms. The bacterial strains used in this product were *Nitrosomonas* sp, *Nitrobacter* sp and *Bacillus* sp.

Activation of probiotics

The 2.5 gm of probiotics (PROACT) was dissolved in 500 ml of water sample in one liter conical flask, and left as such for 4 h with sufficient aeration provided. The foams were continuously emerging and the temperature increased to 32°C. Then the probiotics were applied in the experimental tanks.

Monitoring of water quality parameters

The water quality parameters of the probiotics treated and control tank were regularly monitored. The water salinity of the tank was measured by using a hand Refracto meter (Atago- Japan). The pH of the tank water was recorded by using electronic pH pen (Hanna Instrumental Company, Japan) . Water temperature was measured by using a standard centigrade thermometer. The dissolved oxygen was estimated by dissolved oxygen meter (YSI 55 model).

Nutrient analysis

Water samples were collected from both experimental and control tanks in well cleaned bottles for analysis of nutrients. The nutrients like, nitrate and nitrite were estimated following the standard methods described by Strickland and Parsons (1972).

Ammonia

Ammonia level was monitored regularly once in a week by adopting the method of Koroleff (1969).

Bacterial population

Collection of water samples

The water samples were collected from the tank (both control and experimental) in well cleaned, dried and sterile bottle for bacteriological examination. The water samples were collected before and after application of probiotics. After collection, 1 ml of the sample was transferred to sterile conical flask (150 ml) containing 99 ml of sterile diluents and serial dilution was performed to get 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} samples. THB was enumerated by adopting the spread plate method using Zobell marine agar medium (Hi-media, Mumbai).

In this method, sterile media were poured into Petri dishes aseptically and allowed to solidify. From the diluents 0.1 ml of the sample was pipetted out into sterile Petri-dish containing medium. It was made spread in the plate by spreader with the help of 'L'-rod. The plates were incubated in an inverted position at 28±2°C for 24 to 48 h. All the determinations were carried out in triplicates. After the incubation period the colonies were counted. The plates con-taining viable bacterial colonies were counted and expressed as CFU/ ml for water samples.

Vibrio isolation

Vibrio sp was enumerated by using TCBS agar medium (Thiosulphate Citrate Bile Salt Sucrose) (M189, Hi- media, Mumbai). TCBS medium weighing 89 g was suspended in 1000 ml distilled water and heated up to the boiling point to dissolve the medium completely. Then, it was cooled to 52°C and poured into the pre sterilized Petri plate and allowed to solidify. The 0.1 ml of the sample was inoculated in to the medium and plates were incubated at 37°C for 24–48 h. After the incubation the colonies were characterized based on their morphology and biochemical tests for confirmation.

Survival of fishes

At the end of the experimental period, survival rate of the fishes in the experimental and control tanks were estimated using the following formula:

$$\text{Survival rate\%: } \frac{\text{No. of fishes at the end of the experiment X 100}}{\text{No. of fishes stocked}}$$

RESULTS

Water quality parameters

The water quality parameters were measured in both experimental and control tanks. The results are given in the Table 1.

Nutrients

Concentration of nutrients were recorded from both experimental and control tanks and given in the Table 2.

Total heterotrophic population

THB in the experimental tank before the application of

Table 1. Water quality parameters of both experimental and control tanks.

Parameters	Experimental tank	Control tank
Salinity (ppt)	32- 35(33.82±1.17)	32- 35 (33.91± 1.04)
Temperature(°C)	24- 28 (25.83± 1.27)	24- 28 (25.92± 1.44)
pH	8.1- 8.4 (8.26±0.103)	7.9- 8.3(8.17±0.179)
Dissolved Oxygen(mg/l)	4.66- 5.38 (5.03±0.233)	4.55- 5.16 (4.93±0.176)

Table 2. Nutrient concentration of both experimental and control tanks.

Nutrients	Experimental tank		Control tank
	Before application	After application	
NO_2 (mg/l)	0.2- 0.8 (0.48±0.023)	0.5- 1.0 (0.72±0.192)	0.4-0.8 (0.62±0.148)
NO_3(mg/l)	0.4-0.7 (0.56±0.114)	0.7-1.0 (0.88±0.130)	0.3-0.7 (0.54±0.134)
NH_4(mg/l)	0-0.1(0.06±0.055)	-	0-0.1(0.08±0.045)

Table 3. THB load (CFU/ml) in the experimental and control tank.

S. No	Experimental tank		Control tank
	Before application	After application	
1	$2.39X10^3$	$4.21X10^6$	$5.28X10^3$
2	$5.11X10^3$	$6.73X10^6$	$8.26X10^3$
3	$1.83X10^4$	$3.25X10^7$	$6.24X10^4$
4	$4.82X10^4$	$7.43X10^7$	$2.1X10^5$

Table 4. Vibrio load (CFU/ml) in the experimental and control tank.

S.No	Experimental tank		Control tank
	Before application	After application	
1	$6.82X10^2$	$1.62\ X10^2$	$8.56\ X10^2$
2	$9.21\ X10^2$	$3.21\ X10^2$	$3.64\ X10^3$
3	$6.21\ X10^3$	$3.96\ X10^2$	$9.4\ X10^3$
4	$9.21\ X10^3$	$4.56\ X10^2$	$2.1\ X10^4$

probiotics ranged from 2.39X103 to 4.82X104. After the application of probiotics, THB population varied between 4.21X106 and 7.43X107. In the control tank, THB count was found to be between 5.28X103 and 2.1X105. THB load during the experimental study is given in Table 3.

Vibrio population

Vibrio sp isolated using specific media showed that counts ranged from $6.82X10^2$ to $9.21\ X10^3$ CFU/ml in the experimental tank prior to application of probiotics. After the application of probiotics, *Vibrio* population varied between $1.62\ X10^2$ and $3.96\ X10^2$ CFU/ml. In the control tank, *Vibrio* count was found to be between $8.56\ X10^2$ and $2.1\ X10^4$ CFU/ml. The two dominant pathogenic species were isolated and identified based on the biochemical characters (Table 5) and (Figure 1). *Vibrio* load in the experimental and control tanks during the experimental study is given in Table 4.

DISCUSSION

Ornamental fish trade considerably increased due to its beauty, market demand in both national and international markets. Bacterial diseases are the major constraints in ornamental fish culture. Marine ornamental fish culture production in much of the world is depressed by disease, particularly caused by *Vibrio* and viruses. Antibiotics, which have been used in large quantities, are in many cases ineffective or result in increases in virulence of

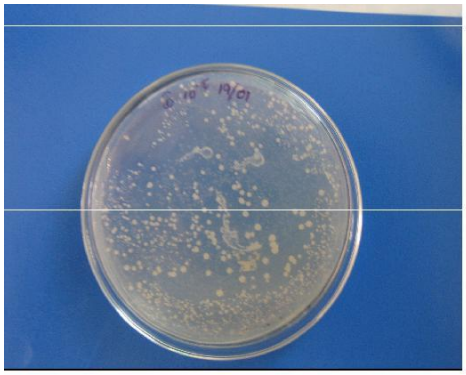
Table 5. Biochemical characteristics of Vibrio sp.

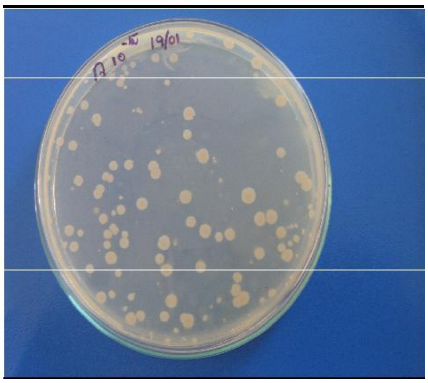
S. No.	Tests	*Vibrio parahaemolyticus*	*Vibrio harveyi*
1	Hydrogen sulphide (TSI agar)	-	-
2	Urea hydrolysed	+	-
3	Indole	+	+
4	Methyl red (37°C)	-	-
5	Voges-proskauer(37°C)	-	-
6	Citrate(Simmosons)	±	±
7	Growth in KCN	+	+
8	Catalase	+	-
9	Cytochrome oxidase	+	+
10	Growth in Peptone water	+	+
11	Growth at 42°C	+	+
12	Motility	+	+
13	Gelatin hydrolysis	±	-
14	Lysine decarboxylase	±	±
15	Nitrate reduction	+	+
16	Glucose acid, gas acid form	±	±
17	Arabinose	-	-
18	Lactose	+	+
19	Maltose	±	±
20	Mannitol	±	±
21	Sucrose	-	_
22	Trehalose	+	+
23	Xylose	+	+
24	Grams reaction	-	_

pathogens and furthermore are causes for concern in promoting transfer of antibiotic resistance to human pathogens. Probiotics technology provides a solution to these problems. The microbial species composition in hatchery tanks or large aquaculture ponds can be changed by adding selected bacterial species to displace deleterious normal bacteria. Virulence of *Vibrio* species can be controlled in this manner. Abundance of luminous *Vibrio* strains decreased in tanks where specially selected probiotic strains of *Bacillus* sp. were added.

The present study was undertaken to ascertain the efficiency of probiotics (PROACT) on the growth and survival of the most economic valued ornamental fish, *N. nemurus* and its influence on water quality parameters and changes in the bacterial load in the marine ornamental fish holding tanks. Physiochemical parameters such as temperature and salinity did not show any variations between the experimental and control tanks. Since temperature and salinity are the conservative parameters of water quality, they are not altered by biological processes (Prabhu et al., 1999) . pH in the experimental tank was slightly more (8.26 ± 0.103) than the control (8.17 ± 0.0179) tank. DO was also slightly greater in the experimental tank (4.93 ± 0.176). Increase in pH and DO in the experimental tank may be due to the beneficial effect of probiotics which favored mineralization of organic matter. Change in this equilibrium through way of impairment in water quality parameters can influence survival of organisms as they become vulnerable to disease due to stress, so also growth (Rengpipat et al., 1998). The presence of heterotrophic gram positive bacterial population in the experimental tank is more efficient in consuming organic matter as their sources of carbon and converting into CO_2 (Stain et al., 1965).

In the experimental tank, THB population was found to be $2.39X10^3$ to $4.82X10^4$ CFU/ml before the application of probiotics. After the application of probiotics, there was an increase in the THB, which ranged between $4.21X10^6$ and $7.43X10^7$. In the control tank, THB count was found to be $5.28X10^3$ to $2.1X10^5$. THB population was found to increase with the progress of the experiment. In the experimental pond, there was a decrease in the *Vibrio* population after the application of probiotics $1.62X10^2$ to$4.56X10^2$. *Vibrio* load was found to be varying between $6.52\ X10^2$ and $9.21\ X10^3$ CFU/ml and from $8.56\ X10^2$ to $2.1\ X10^4$ CFU/ml in the experiment and control tanks, respectively. The reduction in the *Vibrio* load in the experimental tank is due to competitive exclusion. The survival of the yellow tail damselfishes was higher (100%) in the experimental tank than in the control tank showing that addition of probiotics improved the water quality conditions and reduced stressful conditions leading to better





THB population in experimental tank THB population in control tank

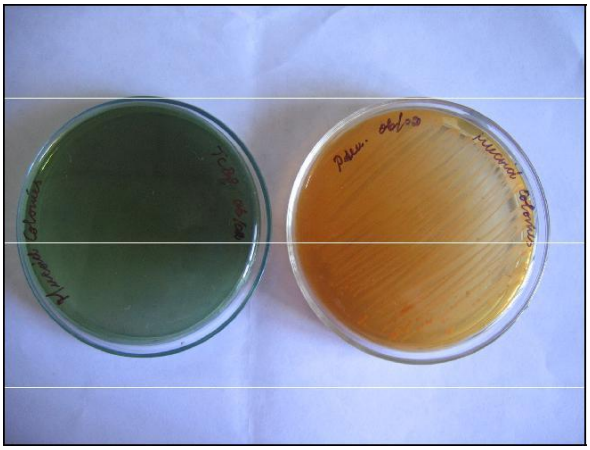


Figure 1. Biochemical characteristics of Vibrio sp. Vibrio sp (green colour indicate (-); yellow colour indicate (+).

maintenance of the fishes. The rationale is that gram-positive bacteria are better converters of organic matter back to Co_2 than gram-negative bacteria (Verschuere, 2000). During the production cycle, high levels of gram-positive bacteria can minimize the building up of dissolved and particulate organic carbon. A similar observation was found in the present study. The culture tank treated with the PROACT was abundant with *Bacillus* sp., showing a low level of ammonia, which was converted into nitrate through nitrite.

The results of this study demonstrated that incorporation of probiotics in the ornamental fish tanks favorably maintained the water quality parameters and microbial load in optimum levels which together improved the survival of the fishes. Globally the demand for marine ornamental fishes is increasing and with microbial intervention like probiotics, it would be possible sooner than later to meet this demand by improving the holding facilities and reducing holding mortality.

The general conclusion obtained from the present study is that the probiotics plays a vital role in growth, survival and disease resistance of the animal by maintaining good water quality parameters throughout the culture period. It is clear from the microbial load data that *vibrio* sp. is dominant in the control tank when compared to the probiotic applied tank.

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