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Influence of probiotic bacterium *Lactobacillus acidophilus* on the survival and growth of pearl oyster *Pinctada fucata* spat

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ABSTRACT

Combination of micro-alga *Chaetoceros calcitrans* and the probiotic bacterium *Lactobacillus acidophilus* evaluated at 1:1 and 1:2 levels revealed that in the probiotic treated group, *Pinctada fucata* spat registered significantly high survival of 79.7 and 89.0 % ($P<0.05$) respectively compared to that of 65.0 % survival in control. The probiotic treated groups also showed significant improvement in growth in terms of length and weight as compared to the control group. The probiotic treated spat attained a weight gain of 346.0 ± 1.57 mg (1:1 level) and 382.0 ± 11.76 mg (1:2 level) compared to 296.4 ± 9.04 mg in control group. The length in terms of dorso-ventral measurement (DVM) increased to 18.68 mm (1:1 level) and 19.6 (1:2 level) mm compared to 13.56 mm in control group.

Introduction

Sustained mass production of seed and fully-grown pearl oysters are essential for successful marine pearl culture. Although techniques for hatchery production of seed and further rearing of the Indian pearl oyster, *Pinctada fucata* to nucleus implanting size was achieved, high mortality of larvae and spat posed a persistent problem (Alagarswami *et al.,* 1987). In tropical rearing conditions, massive larval mortalities due to bacterial infection were reported (Garland *et al*., 1983; Lipton *et al*., 2003).

The beneficial bacteria are useful to increase the survival of fish and shellfish and offer much scope in bivalve culture (Gomez-Gil *et al*., 2000). Recently, there has been great interest in the use of lactic acid bacteria (LAB) and their metabolic products as potential probiotics in aquaculture (Gatesoupe, 1999).

The exact dose and combinations of probiotic bacteria such as *Lactobacillus* sp. with microalgae in bivalve larvae and spat culture have not been evaluated. In the present study, the survival and growth response of *P. fucata* spat towards combinations of probiotic bacterium and microalgae as feed are evaluated and presented.

Materials and methods

Spat rearing

Experiments were conducted at Vizhinjam Research Centre of Central Marine Fisheries Research Institute. Laboratory reared 120 days old spat from single spawning with mean dorso ventral measurement (DVM) of 4.2 ± 0.44 mm and average weight of 22.5 ± 0.20 mg were used. The spat were stocked @ 100 no. / trough with 10 l of seawater. Three groups *viz.*, i. control, ii. experimental with 1:1 feed and iii. with 2:1 feed were maintained in triplicate. Constant aeration was provided. Fifty percentage water was exchanged daily and complete water exchange was done once in five days.

Experimental feeding

The control group was fed with *Chaetoceros calcitrans* alone. In the experimental groups, *Lactobacillus acidophilus* isolated from commercial sporlac (I.P) maintained in 3.0 % (w/v) NaCl incorporated nutrient agar was used along with micro algae at 1:1 and 2:1 proportions. The cells provided during different days of spawning are given in Table 1. The spat were provided with the above sets of experimental diet everyday at 17 h.

Estimation of water quality parameters

Water quality parameters were recorded as per the method of APHA (1992). Temperature and pH were recorded daily. Dissolved oxygen content and salinity were evaluated once in three days and once in a week respectively.

Estimation of bacterial load

Water samples were collected aseptically prior to water exchange once in a week and plated on nutrient agar prepared in aged seawater using the pour plate method.

Evaluation of growth, weight gain and percentage of survival

Spat growth was determined by measuring the mean DVM of 50 specimens in each triplicate set with a 0.05 mm division centimeter scale at the respective time intervals such as before the experiment, after 30, 60, and 90 days. Average weight of individual spat was determined at the first and last day of the experiment. The percentage of

TABLE 1: *Number of* Chaetoceros calcitrans *and* Lactobacillus acidophilus *added in the spat rearing tanks*

Days from spawning	Cells (in million) / spat / day		
	Control	Trough A	Trough B
121 – 130	0.6 Cc	0.6 Cc + 0.6 Lb	0.6 Cc + 1.2 Lb
131 – 140	0.7 Cc	0.7 Cc + 0.7 Lb	0.7 Cc + 1.4 Lb
141 – 150	0.8 Cc	0.8 Cc + 0.8 Lb	0.8 Cc + 1.6 Lb
151 – 160	0.9 Cc	0.9 Cc + 0.9 Lb	0.9 Cc + 1.8 Lb
161 – 170	1.0 Cc	1.0 Cc + 1.0 Lb	1.0 Cc + 2.0 Lb
171 – 180	1.1 Cc	1.1 Cc + 1.1 Lb	1.1 Cc + 2.2 Lb
181 – 190	1.8 Cc	1.8 Cc + 1.8 Lb	1.8 Cc + 3.6 Lb
191 – 200	1.9 Cc	1.9 Cc + 1.9 Lb	1.9 Cc + 3.8 Lb
201 – 210	2.0 Cc	2.0 Cc + 2.0 Lb	2.0 Cc + 4.0 Lb

Cc - *Chaetoceros calcitrans* Lb - *Lactobacillus acidophilus*

survival was determined by enumerating the dead spat during water exchange. All the statistical analysis was conducted using Microsoft Statistica Software Version 2.01.

Results

Hydrological parameters

There was no significant variation (ANOVA, $P>0.05$) in hydrological conditions between the control and treated groups (Table 2).

Bacterial load

Bacterial load in the control group ranged between 1.0 ± 0.01 to 5.8 ± 0.1 x 10^2 cfu/ml, whereas in the *Lactobacillus* treated groups, it ranged from 1.7 ± 0.01 to 19.4 ± 0.08 and 1.4 ± 0.03 to 32.4 ± 0.04 x 10^2 cfu/ml respectively (Fig. 1).

Growth and survival of spat

During the experimental period, survival in the control and in the probiotic-treated group was 65.0, 79.7 and 89.0 % respectively (Fig. 2). After 90 days of rearing, the increase in DVM of treated and control group was 18.68 (0.21mm/day), 19.6 mm (0.22 mm/day) and 13.56 mm (0.15 mm/day) as noted from Fig. 3. Average weight gain of individual spat was 296.4 ± 9.04, 346.0 ±

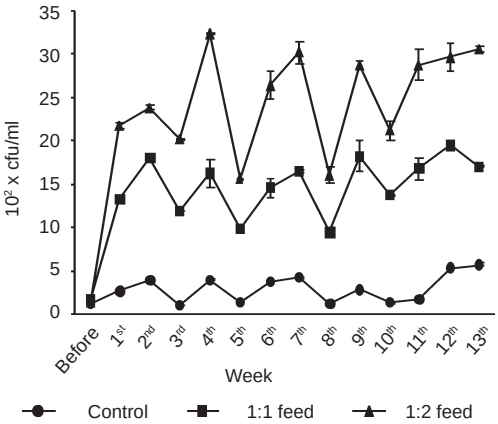

Fig. 1. Bacterial load in the rearing water

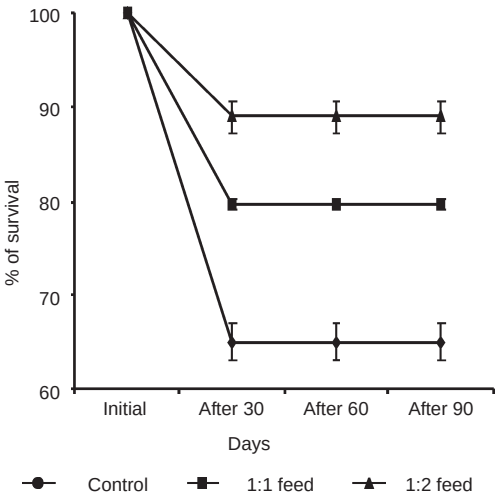

Fig. 2. Survival of spat of *P. fucata* during the rearing period

TABLE 2: *Mean temperature, pH, dissolved oxygen and salinity during spat rearing period*

Parameters	Control			1:1 feed			1: 2 feed		
	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Temperature (°C)	26.07 ± 0.4	26.08 ± 0.3	25.63 ± 0.4	26.00 ± 0.4	26.12 ±0.31	25.60 ±0.14	25.50 ± 0.2	26.20 ±0.3	25.67 ±0.1
pH	7.76 ±0.14	7.52 ±0.06	7.58 ±0.11	7.70 ±0.15	7.50 ±0.06	7.50 ±0.01	7.74 ±0.15	7.45 ±0.05	7.54 ±0.21
DO (mg/l)	4.60 ±0.24	4.64 ±0.19	4.84 ±0.09	4.60 ±0.2	4.74 ±0.2	4.80 ±0.01	4.70 ±0.02	4.78 ±0.2	4.82 ±0.05
Salinity (ppt)	34.37 ± 0.05	34.56 ± 0.05	34.56 ±0.05	34.30 ± 0.07	34.50 ± 0.15	34.46 ±0.05	34.38 ± 0.02	34.42 ± 0.19	34.46 ±0.07

1:1 & 1:2 feed = *Chaetoceros calcitrans* : *Lactobacillus acidophilus* (as given in Table 1)

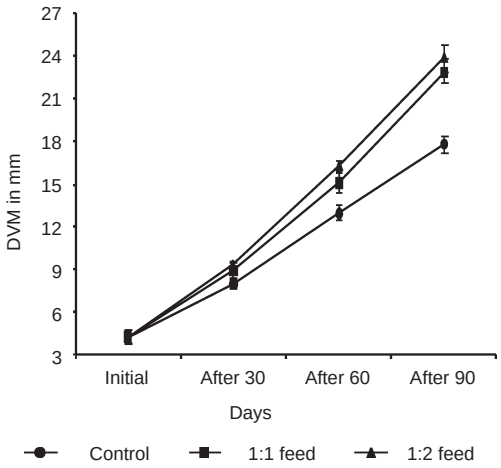

Fig. 3. Growth of spat of *P. fucata* as DVM during the rearing period

1.57 and 382.0 ± 11.76 mg in control, 1:1 and 1:2 feed groups respectively.

Discussion

Probiotics are presently used in aquaculture to modify and manipulate the microbial population of the environment and to reduce or eliminate selected pathogenic species of micro-organisms leading to better growth and survival of the candidate species (Irianto and Austin, 2002). From the results it is inferred that the improved survival and growth of pearl oyster spat is due to the addition of probiotic *Lactobacillus acidophilus*.

Under the normal feeding regimes, growth of *Pinctada fucata* spat has been reported to range from 0.03 and 0.04 mm/day in 1.5 and 5 t FRP tanks respectively (Victor *et al.*, 2001). Only spat of larger species such as *Pinctada margaritifera* has registered growth of about 0.15 mm/day (Alagarswami *et al.*, 1989). A similar trend of growth rate of 0.15 mm/day in terms of DVM in control group in *P. fucata* was obtained in the present study as that of the larger species of *P. margaritifera*. The treated groups showed a significant increase in growth rate (ANOVA, $P<0.05$) to the tune of 28.83 and 34.01 % compared to that of the control as well as that of the earlier published results for *Pinctada margaritifera*. A similar trend of enhanced growth rate of pacific oyster, *Crassostrea gigas* was reported by Douillet and Langdon (1994) when probiotic bacterium *Alteromonas* sp. at a rate of 0.1 million cells/ml were administered. Thus the otherwise slow growth of bivalve spat can evidently be enhanced to much higher rates when treated with LAB probiotic.

In addition to the growth, survival was also enhanced significantly in the probiotic treated groups (ANOVA, $P<0.05$). Better survival of 79.7 and 89.0 % was recorded in the treated groups while low survival of 65.0 % was observed in the control group. Victor *et al.* (2001) reported 30.1 and 19.0 % survival respectively under normal condition in 1.5 and 5 t. FRP tanks. Thus, it is possible to enhance the survival by three times by adding probiotic bacteria. Douillet and Langdon (1994) revealed that the probiotic strain CA2 (*Alteromonas* sp.) increased the survival of pacific oyster, *Crassostera gigas* when administered through water. Rengpipat *et al.* (2000) reported increased survival of shrimp, *Penaeus monodon* after feeding with the probiotic *Bacillus* S11 for 90 days. This could be achieved by modification of the microbial composition of water/sediments in such a way the pathogenic members are reduced through competitive exclusion.

The results indicated that the probiotic treated spat attained a significant ($P<0.05$) weight gain of 346.0 ± 1.57 and 382.0 ± 11.76 mg compared to 296.4 ± 9.04 mg in control group. The overall increase in weight gain results

from increased digestibility of nutrients as well as protection from infectious agents (Goldin, 1998). In general, the lactic acid bacteria (LAB) have the ability to attach with the gut epithelium and establish there. By their large presence, they saturate the adhesion receptors and prevent the pathogenic bacteria from attachment and thereby prevent the incidence of disease (Vine *et al.,* 2004). Improvements in the digestive activity by synthesis of vitamins, cofactors or enzyme activity were reported due to the addition of probiotic in feed (Gatesoupe, 1999). Though further studies are required, it is probable that these factors could have contributed to the weight gain in the treated group of spats.

The bacterial load in the treated groups (ranging from 1.7 ± 0.01 to 19.4 ± 0.08 and 1.4 ± 0.03 to 32.4 ± 0.04 x 10^2 cfu/ml respectively) was higher than that of the control (1.0 ± 0.01 to 5.8 ± 0.1 x 10^2 cfu/ml) due to the rapid multiplication of probiotic bacteria. Recent observation by Lipton *et al.* (2006) in shrimp farms revealed rapid increase in microbial load after the application of probiotics. Such rapid growth of beneficial bacteria helps them to colonize in the epithelial surfaces of spat. This ability to colonize epithelial surfaces, which in turn exclude pathogenic species is considered to be an important advantage of using probiotic (Fuller, 1992). Jiravanichpaisal *et al.* (1997) demonstrated the inhibitory activity of *Lactobacillus* spp. against *Vibrio* spp., *Escherischia coli*, *Staphylococcus* spp. and *Bacillus subtilis* in laboratory culture.

Probiotic protection can be due to different mechanisms such as nutritional competition or production of antibacterial substances. They may provide growth factors and inhibit the proliferation of pathogen by stimulating the non-specific immune response (Irianto and Austin, 2002).

Although there were no obvious effects of water quality parameters such as temperature, pH, dissolved oxygen content and salinity, the probiotic treatment could be regarded as an effective alternative for enhancing the spat health in the hatchery. Thus feeding the spats with *Lactobacillus acidophilus* along with algal diets at 1:1 and 2:1 proportions provided a better growth, survival and weight gain. The 2:1 ratio was more appropriate to the pearl oyster hatchery, which has the evident advantage of reducing the growth period for the nucleus-implanting size.

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