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# Effects of a Probiotic Bacterium, *Lactobacillus acidophilus*, on the Growth and Survival of Pearl Oyster (*Pinctada margaritifera*) Spat

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**Abstract**

The present study investigated the effect of a probiotic bacterium, *Lactobacillus acidophilus*, on the growth and survival of pearl oyster, *Pinctada margaritifera*, spat. The probiotic bacteria was fed together with a microalgal feed at 1:1 or 2:1 while control groups received no probiotic supplementation. The probiotic groups had significantly higher survival (78.7±8.1 and 85.7±2.9%, respectively) than the control groups (60.7±1.2%). Weight and length also increased significantly. The weight gains in the probiotic groups were 349.8±0.44 mg (1:1 level) and 396.8±0.49 mg (2:1 level) mg, compared to 300.9±0.51 mg in the control. The increases in dorso-ventral measurement were 20.08 mm (1:1 level) and 21.04 mm (2:1 level) in the probiotic groups, compared to 14.22 mm in the control.

## Introduction

The majority of cultured black pearls produced in tropical regions come from the blacklip pearl oyster, *Pinctada margaritifera*. Although techniques for hatchery production and rearing of *P. margaritifera* to nucleus implanting size have been achieved, high mortality of larvae and spat pose a persistent problem (Alagarswami et al., 1989; Southgate and Beer, 1997; Doroudi et al., 1999). In tropical rearing conditions, massive larvae mortality due to bacterial infection have been reported (Garland et al., 1983; Subhash et al., 2007).

Probiotics can increase the survival of fish and shellfish (Gomez-Gil et al., 2000). Lactic acid bacteria (LAB) and their metabolic products are potential aquaculture probiotics (Gatesoupe, 1999). Antimicrobial compounds produced by LAB provide these organisms with a competitive advantage over other microorganisms. The efficacy and spectrum of antimicrobial LAB products, including lactic

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and acetic acids, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins or bacteriocin-like substances, are broad (Mishra, 1996).

The optimum dose and combination 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. margaritifera* spat to combinations of probiotic and microalgae are evaluated.

## Materials and Methods

*Spat rearing*. The experiment was conducted at the Vizhinjam Research Center of the Central Marine Fisheries Research Institute (CMFRI). Laboratory-reared 100-day-old spat (mean dorso-ventral measurement 3.9±0.44 mm, avg wt 24.6±0.76 mg) from a single spawning were stocked at 100/trough in 10 liters of sea water. Three triplicate groups of each of the following treatments were established: (a) control (no probiotic), (b) 1:1 feed group, and (c) 2:1 feed group. Constant aeration was provided. The water exchange regime included a daily exchange of 50% and a complete exchange every five days.

*Feeding*. The control group was fed only the algae, *Chaetoceros calcitrans*. The concentration from the start of the experiment to day 60 was 0.6-1.1 million cells/spat/day. From day 61 to 70, 1.8 million cells/spat/day were provided. The ration was increased to 1.9 million/cells/spat/day on days 71-80 and 2.0 million cells/spat/day on days 81-90. In the experimental groups, *Lactobacillus acidophilus*, isolated from a commercial sporlac sachet and maintained in 3.0% NaCl incorporated nutrient agar (w/v), was provided together with the algae at a ratio of 1:1 or 2:1, i.e., beginning at 0.6-1.1 million or 1.2-2.2 million cells/spat/day and increasing together with the algae ration. The spat were fed once a day at 17:00.

*Hydrological parameters.* Water quality parameters were recorded as per APHA (1992). Temperature and pH were recorded daily, dissolved oxygen content once in three days, and salinity once a week.

*Estimation of bacterial load.* Water samples were collected aseptically prior to total water exchange (12 h after last feeding) and plated on nutrient agar prepared in aged sea water using the pour plate method.

*Growth, weight gain, and survival*. Spat growth was determined by measuring the mean dorso-ventral measurement (DVM) of 50 specimens in each triplicate with a 0.05-mm division scale at the start of the experiment and on days 30, 60, and 90. Average weight was determined on an electronic balance (Shimadzu AW 120, Japan). Percent survival was determined by counting dead spat during water exchanges.

*Statistical analysis*. All data were analyzed using one-way ANOVA in Microsoft Statistica Software, Version 2.01, and differences were considered significant when $p<0.05$.

## Results

*Hydrological parameters*. No significant differences in hydrological conditions were noted between the control and treated groups. The temperature (26.54±0.4 to 26.90±0.2°C), pH (7.64±0.06 to 7.76±0.19), dissolved oxygen (4.75±0.29 to 4.98±0.04 mg/l), and salinity (34.25±0.05 to 34.76±0.15 ppt) were within the optimal ranges for spat growth

*Bacterial load*. In the control group, the bacterial load ranged 0.17±0.01 to 0.70±0.1 x $10^3$ cfu/ml. In the 1:1 group, it ranged 0.15±0.01 to 21.0±0.08 and in the 2:1 group 0.19±0.03 to 29.5±0.04 x $10^3$ cfu/ml (Fig. 1).

*Growth and survival of spat*. Growth and survival are given in Table 1. The length, weight, and survival in the treated groups were significantly higher than in the control after 90 days of rearing (Table 2).

## Discussion

Probiotics are used in aquaculture to manipulate the microbial population of the environment and to reduce or eliminate pathogenic microorganisms, thereby leading to better growth and survival of the cultured species (Irianto and Austin, 2002). The present study suggests that improved survival and growth of pearl oyster spat results from the addition of probiotic *L. acidophilus.*

Under normal feeding regimes, *P. margaritifera* grows 0.15 mm/day (Alagarswami et

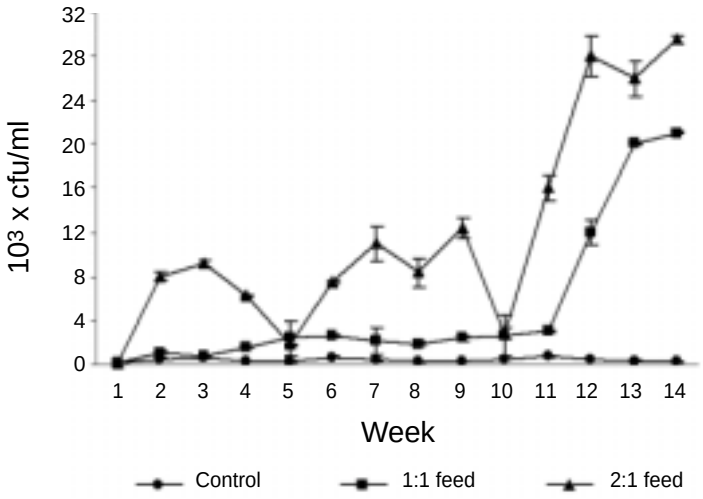


Fig. 1. Bacterial load in the rearing water.

Table 1. Survival and growth of *Pinctada margaritifera* spat fed *Lactobacillus acidophilus* in addition to micoalgae at a ratio of 1:1 or 2:1.

| | *0 day* | *30 days* | *60 days* | *90 days* |
|---|---|---|---|---|
| *Control* | | | | |
| Survival (%±SD) | 100.00 | 69.7±4.5 | 60.7±1.2 | 60.7±1.2 |
| DVM (mm±SD)* | 3.9±0.44 | 8.2±0.52 | 13.14±0.66 | 18.12±0.34 |
| Wt (mg±SD) | 24.6±0.76 | 123.18±0.56 | 223.83±1.4 | 325.52±0.25 |
| *1:1 feed* | | | | |
| Survival (%±SD) | 100.00 | 82.0±3.5 | 78.7±8.1 | 78.7±8.1 |
| DVM (mm±SD)* | 3.9±0.44 | 8.96±0.72 | 15.96±0.74 | 23.98±0.91 |
| Wt (mg±SD) | 24.6±0.76 | 139.83±1.5 | 257.51±0.98 | 374.41±1.2 |
| *2:1 feed* | | | | |
| Survival (%±SD) | 100.00 | 85.7±2.9 | 85.7±2.9 | 85.7±2.9 |
| DVM (mm±SD)* | 3.9±0.44 | 9.94±0.67 | 17.01±0.43 | 24.94±0.90 |
| Wt (mg±SD) | 24.6±0.76 | 154.3±1.63 | 287.28±1.48 | 421.4±1.2 |

* Dorso-ventral measurement

al., 1989). In our study, a similar growth rate of 0.16 mm/day was obtained in the control group while higher growth rates of 0.22 and 0.23 mm/day were obtained in the treated groups. A similarly enhanced growth rate was reported for the Pacific oyster, *Crassostrea gigas*, when the probiotic bacteria *Alteromonas* sp. was provided at a rate of 0.1 million cells/ml (Douillet and Langdon, 1994). Thus, the otherwise slow growth of bivalve spats can evidently be enhanced when treated with a LAB probiotic.

Table 2. Growth and survival of *Pinctada margaritifera* fed *Lactobacillus acidophilus* plus microalgae for 90 days.

| *Group* | *Survival (%±SD)* | *Growth rate (mm/d)* | *Wt gain (mg±SD)* |
|---|---|---|---|
| Control | 60.7±1.2[a] | 0.16[a] | 300.92±0.51[a] |
| 1:1 feed | 78.7±8.1[b] | 0.22[b] | 349.81±0.44[b] |
| 2:1 feed | 85.7±2.9[c] | 0.23[b] | 396.8±0.49[c] |

Values in a column with different superscripts significantly differ ($p$<0.05).

Survival was also significantly enhanced in the treated groups. When administrated through the water, the probiotic strain CA2 (*Alteromonas* sp.) increased survival in the Pacific oyster (Douillet and Langdon, 1994). Survival is increased by modification of the microbial composition of the water in such a way that pathogens are reduced by competitive exclusion.

The treated spat attained a significantly higher weight gain than the control. The overall increase in weight gain results from increased digestibility of nutrients as well as protection from infectious agents (Goldin, 1998). In general, LAB have the ability to attach to the gut epithelium and establish there (Vine et al., 2004). When in large presence, they saturate the adhesion receptors and prevent the attachment of pathogenic bacteria, thereby preventing the incidence of disease. Gatesoupe (1999) reported that the addition of a probiotic in feed resulted in improved digestive activity by synthesis of vitamins, cofactors, or enzyme activity. Though further studies are required, it is probable that these factors contributed to the weight gain in the treated groups.

The bacterial loads in the treated groups were higher than in the control. In a shrimp farm, the microbial load rapidly increased after application of a probiotic (Lipton et al., 2006). Their rapid growth helps beneficial bacteria colonize on epithelial surfaces of spat. Their ability to colonize epithelial surfaces, which in turn excludes pathogenic species, is an important advantage of using a probiotic (Fuller, 1992).

Probiotic protection can be result from mechanisms such as nutritional competition or production of antibacterial substances. Probiotics may account for growth factors or inhibit proliferation of pathogens by stimulating the non-specific immune response (Irianto and Austin, 2002). Feeding gram-positive and gram-negative probiotics at $10^7$ cells per g feed to rainbow trout led to stimulation of cellular immunity with increased erythrocytes, macrophages, lymphocytes, and lyzozyme activity within two weeks of feeding with probiotics (Irianto and Austin, 2002).

In the present study, temperature, pH, dissolved oxygen content, and salinity were within the standard limitations for spat rearing. Thus, probiotic treatment could be regarded as an effective alternative for enhancing spat health. Feeding the spats *L. acidophilus* together with algae at 1:1 or 2:1 produced better growth, survival, and weight gain compared to the control. The 2:1 ratio is more appropriate to the pearl oyster hatchery, as it can reduce the period necessary for growing the spats to nucleus-implanting size.

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