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Effect of probiotic bacteria (*Lactobacillus rhamnosus*) on the survival, growth and immune responses of the fresh water prawn *Macrobrachium idae*

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ABSTRACT

Experiments were performed to study the influence of probiontic Lactobacillus species on growth enhancement of freshwater prawn Macrobrachiam idae. Feed intake, specific growth rate, and feed conversion efficiency of M.idae was significantly different in various treatments at the level of p≤0.05. However the specific growth rates differed among the treatments at p≤0.05 level of significance. Highest specific growth rate in M.idae was observed in the diets containing 3% level of probiotic of Lactobacillus. There were significant differences p≤0.05 in body weight and length gain between basal and Lactobacillus supplemented diets. The total haemocytes counts of M.idae fed with 3% Lactobacillus ranged from 2563-3956cells/mm3.

Disease challenge test on the vibro species infected in the fresh water prawn M.idae fed on the basal diets showed an increase in body weight and length. The oral feeding of the basal diets incorporated with probiotic had positive effects on the survival rate of M.idae. The total viable counts of V.alginolyticus found in midgut on M.idae diets showed increasing total population of V.alginolyticus as a function of experimental duration. The present study confirmed the hypothesis that probiotics could be used to prevent disease infections. Probiotics may prevent disease infection because they compete with pathogenic viruses or Bacteria for binding sites on epithelial cells. Probiotics might also inhibit the growth of pathogenic bacteria by producing bacteriocins such as niasin.

Key words: Probiotic –Lactobacillus species – Macrobrachiam idae –Immune response.

INTRODUCTION

The annual increase in farmed freshwater prawn production in the world during the decade ending 2001 has been estimated as 29%, and 48% during 1999-2001,with the production of all *Macrobrachium* species in 2001 being about 3,00,000 mt [1]. An alternative method to antibiotic treatment is the application of probiotics. Probiotics are defined as ''live microorganisms, which are consumed in adequate amounts, confer a health benefit for the host [2].

Lactic acid bacteria which have probiotic properties stimulate the growth of preferred micro-organisms, compete harmful bacteria, and reinforce the organism's natural defense mechanisms [3]. The application of probiotics and may result in elevated health status, improved disease resistance, growth performance, body composition, production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, inhibition of virulence gene expression or disruption of quorum sensing, improvement of water quality, enhancement of the immune response, source of macro or micronutrients and enzymatic contribution to digestion and improved gut morphology and microbial balance[4-9]. Application of probiotics for intensive shrimp cultivation is the most promising preventive methods developed to fight against diseases caused by *V. anguillarum, V. vulnificus, V. alginolyticus and V. harveyi.*

Many probiotics including *Lactobacillus* sp.[10-11], *Bacillus* sp.[12], yeast[13-14] and many Gram-negative bacteria l [15] have been reported that they effectively inhibited *Vibrios* in shrimp cultivation.

The objectives

*To study the growth of *Machrobrachium idae* using *Lactobacillus rhamnosus*.

*To estimate the survival rate of *Machrobrachium idae*.

*To assess the immune response of *Machrobrachium idae* to a pathogen.

MATERIALS AND METHODS

Lactobacillus rhamnosus was a live bacterium (Yoghurt). Nutrient agar was employed as a medium in determining the standard plate count. After isolation and initial screening for antibiotic activity, the lactobacillus probionts were maintained at -80 ^{0} C as well as agar slants.

Lactobacillus rhamnosus were grown for 3 days at 12^0 C in shaken bottles with full medium composed of Bacto trypton (0.5%) $KHPO_4$ (0.2%), glucose (0.5%) and 1% each of a standard mineral, vitamin and nucleotide solution. The culture were centrifuged for 15min at 4000rpm, and the pellets resuspended in sterile saline water to OD600mm = 2.0-2.5 before being sprayed on the pellets[16].

Experimental Diets: According to Gismondo *et al.*[17] basal diet (Control) containing 31.10% protein was prepared and basal diet was mixed with dietary level of 1%, 2% and 3% *Lactobacillus rhamnosus* (Experimental).

Feeding Trials: Four groups of prawns were collected and graded by size and each group of 10 prawns with the total weight of 0.334±0.23g/group was stocked into 25 litres trough. The basal diet was fed to all during 1 week conditioning period. Each diet was fed to prawns in triplicate groups at 3% of the body weight daily. The feeding trial was conducted for 4 weeks. Survival rate and growth in terms of percentage weight gain were calculated. The haemolymph of experimental prawn was analyzed using the standard methods [18-19].

Pathogen challenge test: Prawn in the control and treated groups 10 prawn / treatment) were immersed in a suspension of *Vibro alginolyticus* at ~ 10^7 CFU/ml $^{-1}$ according to Austin *et al.* [20] and survival was determined after 28 days of challenge. Antibacterial activity was determined as the diameter (mm) of the clear inhibitory zone formed around the well.

Data analysis

Data from both feeding trials and the bacterial challenge were suspected to analysis of standard deviation. Difference in treatment means considered significant at $p \leq 0.05$.

$$\text{Percentage of weight gain} = \frac{Wf - Wi}{Wi} \times 100$$

Wi-Initial average weight of the experimental animals at day 1

Wf-Final Average weight of experimental animals

$$\text{Survival rate (\%)} = \frac{(Ni - Nf)}{Ni} \times 100$$

Ni - Initial number of animals

Nf - Final number of animals

RESULT AND DISCUSSION

Feed intake and growth rate of *Machrobrachium idae* were significantly different in various treatments at the levels of $p \leq 0.05$ (Table 1). However growth rate of *M. idae* differed among the diets treatments at $p \leq 0.05$ level of significance, with highest growth rate in the diets containing highest level of probiotic of *Lactobacillus*. No health problems were detected in any group of prawn in the feeding trials.

There were significant differences of the body weight and length gain between basal diet and experimental diets containing different doses of *Lactobacillus* sps. (Table 2). The raising of fresh water prawn *M.idae* on the basal diets incorporate with *Lactobacillus rhamnosus* showed an increase in body weight and length. No obvious pollution effect on water quality was found. The survival of *M.idae* after first and second weeks was significantly different between the control and the treated group (Figure 1). Growth and survival rate of the ponds which was applied with probiotics was higher than that of control ponds [21].

Our result was supported by Amal S. Saad [22]. Experimental diets were identical in all aspects except for variation in the probiotics ratio. Generally, growth performance and survival of the probiotic fed groups were significantly higher (P<0.05) than the control group.

Table 1. Relative growth rate of *Macrobrachium idae* fed with different doses of *Lactobacillus rhamnosus* in 35 days of feeding trials

Diets	Growth rate mg/g wet wt/day					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Control	0.325±0.013	0.328±0.045	0.332±0.056	0.345±0.067	0.357±0.078	0.362±0.043
Basal diet + 1% Lactobacillus	0.340±0.023	0.351±0.034*	0.359±0.056	0.361±0.067*	0.379±0.078*	0389±0.024*
Basal diet + 2 % Lactobacillus	0.334±0.045	0.341±0.036	0.358±0.034**	0.367±0.023	0.374±0.029*	0.376±0.003*
Basal diet + 3 % Lactobacillus	0.330±0.023	0.335±0.034	0.356±0.045**	0.386±0.021	0.401±0.091	0.403±0.091**

Values are expressed as Mean ±S.E *=Significant at P≤0.05% ,**=P≤0.01%

Table 2. Growth performance of weight and length of *Macrobrachium idae*

Treatment	Weight in gm		Length in cm	
	Initial	Final	Initial	Final
Control	0.356±0.21	0.474±0.23	0.88±0.07	1.25±0.23
1% Lactobacillus	0.412±0.03	0.523±0.52	0.83±0.05	1.25±0.02*
2% Lactobacillus	0.325±0.45	0.457±0.45	0.96±0.01	1.46±0.29*
3% Lactobacillus	0.395±0.23	0.542±0.01	0.92±0.01	1.83±0.01*

Values are expressed as Mean± S.E *=Significant at P≤0.05%

Table 3. Total Haemocyte Counts of *Macrobrachium idae* fed with different doses of *Lactobacillus rhamnosus* in 35 days of feeding trials

Diets	Total haemocyte counts /Cells/mm^3					
	Day 1	Day7	Day 14	Day 21	Day 28	Day 35
Control	2235±36	2231±41	2413±21	2600±24	3130±35	3512±32*
Basal diet + 1%Lactobacillus	2365±45	2400±35	2623±45	2693±21	3251±36	3654±14*
Basal diet + 2%Lactobacillus	2436±14	2563±23	2700±31	2904±21	3015±25	3604±31
Basal diet + 3%Lactobacillus	2563±12	2896±35	3512±36	3584±35	3910±56	3956±24*

Values are expressed as Mean± S.E *=Significant at P≤0.05%

The total haemocytes of fresh water prawn M.*idae* fed with basal diets and experimental diets incorporated with probiotic are illustrated in Table 3. The Total haemocytes counts of M.*idae* fed with *Lactobacillus* ranged from 2563-3956 cells/mm^3. Ringø and Gatesoupe [23] stated that bacteria, capable of producing lactic acid fermentation may inhibit the proliferation of putrefactive microbes in the digestive tract of aquatic organisms and thus contribute to improved health status of the host. Further, the release of lactic acid resulting in modifications of the gut's pH may be another factor accounting for the effect observed in its microflora. Vázquez *et al.*[24] showed that lactic and acetic acids are responsible for the effects of nine potential lactic acid bacteria probiotics tested for their inhibitory effects on four common pathogens of turbot.

Fig. 1: Survival responses of *Macrobrachium idae fed* with basal and experimental diet incorporated with *Lactobacillus*

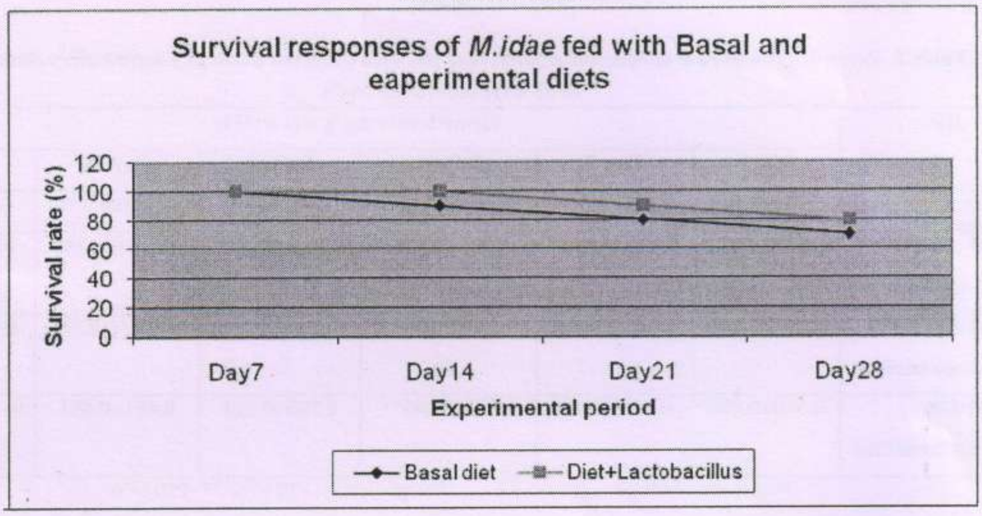


Fig. 2: Total Viable Counts of *Lactobacillus* Bacteria

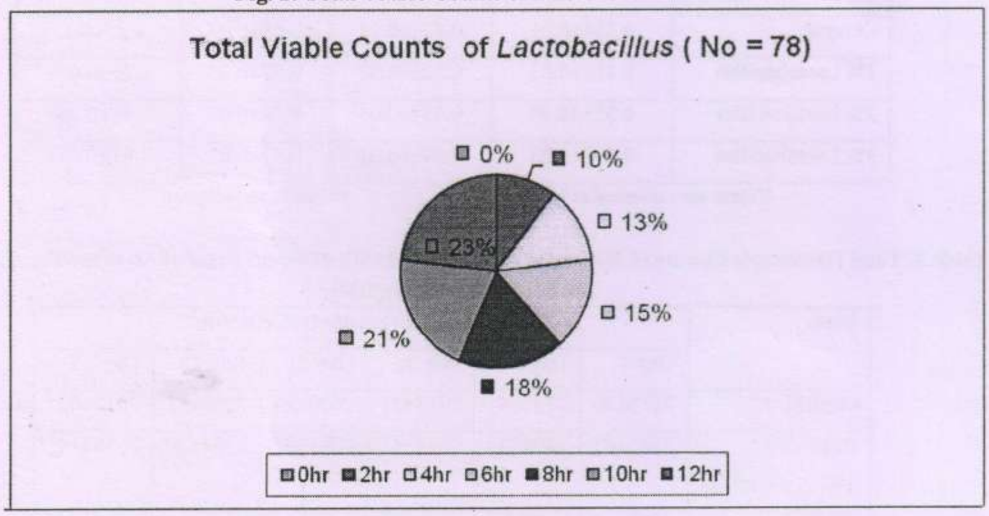
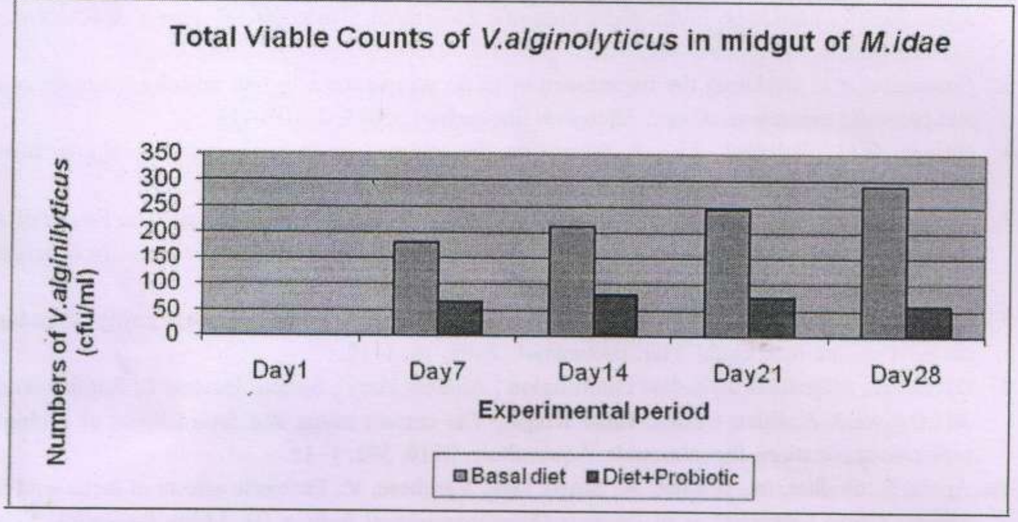


Fig. 3: Total viable counts of *V.alginolyticus* in midgut content of *Macrobrachium idae*



In the present investigation, the midgut of the prawn *M.idae* fed with basal diets showed increased total viable populations of *V.alginolyticus* than the basal diets incorporated with probiotics (Figure 2). This is in accordance to the investigation of Mathieu Castex et al.[25] who stated that the highest concentration of probiotic in the shrimp gut was obtained 2 hrs after feeding. During the first 2 weeks, prevalence and load of *V.nigripulchritudo* strains in haemolymph was lower in animals fed with the probiotic diet (Figure 2 & 3).

The present study showed that the growth of pathogenic *V. alginilyticus* was controlled by non-pathogenic *Lactobacillus rhamnosus* under invivo and invitro conditions (Figure 2). The studies of Ponpisit Utiswannakul1 *et al.*[26] also agree with these observations. When the regular feed of *P. monodon* was supplemented with BP11 at ~109 CFU g-1 feed, a higher shrimp growth, feed conversion ratio, survival and general health was obtained for postlarvae (PL) shrimp both in concrete tanks and in an earthen pond. Adapted probiotic used as an effective biocontrol agent for shrimp with high level of inhibition against *V.alginolyticus*.

CONCLUSION

During 35 day feeding trial, feed intake, growth rate of *M.idae* was significantly different in various treatments at the level of $p \leq 0.05$ significance. Highest growth rate was observed in 3% of *Lactobacillus* diets. There is significant body weight and length gain between basal and *Lactobacillus* diets.

The survival response of *M.idae* fed with basal and *Lactobacillus* incorporated diets on day 28. The oral feeding of the basal diets incorporated probiotics had positive effects on the survival rate of *M.idae*.

Disease challenge test against *Vibrio* species infected in the fresh water prawn *M.idae* fed on basal diets and diet incorporated with *Lactobacillus* showed an increase in body weight and length.

Consequently it proves to be suitable candidates for the oral administration to prawn, in commercial ventures to improve health and to protect them from *Vibrio* infections. *Lactobacillus* species seems to have a good potential for probiotics application.

REFERENCES

1. New M.P. (2003b).The role of fresh water prawn in sustainable aquaculture. In Fresh water prawn 2003.Souvenir of the international symposium on fresh water prawn, 2003. Cochin,20-23 August 2003 (by K.S.Purushan).
2. Reid, G., Sanders, M.E., Gaskins, H.R., Gibson, G.R., Mercenier, A.,Rastall, R.A., Roberfroid, M.B., Rowland, I., Cherbut, C., Klaenhammer, T.R., New scientific paradigms for probiotics and prebiotics. J. Clin. Gastroenterol., **2003**, 37: 105–118.

3. Villamil, L., Tafalla, C., Figueras, A., Novoa, B., Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus)*. Clin. Diagn. Lab. Immunol., **2002**, 9: 1318–1323.
4. Vine, N.G., Leukes, W.D., Kaiser, H., Daya, S., Baxter, J., Hecht, T., Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. *J. Fish Dis.,* **2004**, 27: 319–325.
5. Gatesoupe, F.J., Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. *J. Mol. Microbiol. Biotechnol*., **2008**, 4: 107–114.
6. Gómez, G.D., Balcázar, J.L., A review on the interactions between gut microbiota and innate immunity of fish FEMS. Immunol. *Med. Microbiol.,* **2008**, **52**: 145–154.
7. Ringø, E., Olsen, R.E., Gut health in aquatic species. World Nutrition Forum. The Future of Animal Production. Binder, E.M. and Schatzmayr, G. (Eds.). Nottingham University Press, Nottingham, UK, 2008, pp. 79–105.
8. Tinh, N.T.N., Dierckens, K., Sorgeloos, P., Bossier, P., A review of the functionality of probiotics in the larvi culture food chain. Mar. Biotechnol., **2008**, 10: 1–12.
9. Daniel, L., Merrifield, Arkadios Dimitroglou , Andrew Foey , Simon. Davies, J., Remi Baker, T.M., Jarl Bøgwald, Mathieu Castex, Einar Ringø., The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, **2010**, 302: 1–18.
10. Ajitha, S., Sridhar, M., Sridhar, N., Singh, ISB., Varghese, V., Probiotic effects of lactic acid bacteria against *Vibrio alginolyticus* in Penaeus (*Fenneropenaeus) Indicus* (H. Milne Edwards). Asian Fish Sci., **2004**, **17**: 71-80.
11. Chiu, CH., Guu, Y.K., Lui, CH., Pan, TM., Cheng, W., Immune response and gene expression in white shrimp, *Litopenaeus vannamei*, induced by *Lactobacillus plantarum*. Fish Shellfish Immun., **2007**, 23: 364-77.
12. Vaseeharan, B., Ramasamy, P,. Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *J Appl Microbiol*., **2003**, 36: 83-7.
13. Scholz, UG., Garcia, DD., Ricque, LE., Cruz, SF., Albores, V., Lalchford, J., Enhancement of vibriosis resistance in juvenile *Penaeus vannamei* by supplementation of diets with different yeast products. Aquaculture., **1999**, 176: 271-83.
14. Suphantharika, M., Khunrae, P., Thanardkit, P., Verduyn, C., Preparation of spent brewer's yeast as an immuno stimulatnt for black tiger shrimp, *Penaeus monodon*. Biores Technol., 2003, 88:55-60.
15. Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I., Nielsen, TF., Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. *Appl Environ Microbiol.,* **1999**, 65: 969-73.
16. Devriese., L.A., Kerckhove, A., Klipper – Balz, R., and Schleifer,K.H. Characretisation and identification of *Entero coccus* species isolated from the intestine of animals. *Int. J. Syst. Bacteriol.* **1987**, 37: 257-259.
17. Gismondo, M.R., Drago,L., and Lomborti, A., Review of probiotics available to modify gastrointestinal flora. *Int. J Antimicrob*., **1999**, 12: 287-292.
18. Yeager., I.F., and Touber, O.E., On the Haemolymph cell counts of some marine invertrbrete.S.Biol.Bull., 1995, 69-70.
19. Jones,J.C.S., Current concept concerning insect haemocytes. Zoologist. **1962**, 2: 209-246.
20. Austin., B., Stuckey, L.E., Robertson, P.A.W., Effendi, I. and Griffith, D.R.W. A probiotics strain of *Vibrio alginolyticus* effective in reducing disease caused by *Aeromonas salmonicida, Vibro anguillarum* and *Vibrio ordalli*. *Jounal of Fish Diseases*. **1995**, 18: 93-96.
21. Soundarapandian, P., Ramanan, V., and Dinakaran, G.K ., Effect of probiotics on the growth and survival of *Penaeus monodon* (Fabricius). *Journal of Social Sciences*. **2010**, 2(2): 51-57.
22. Amal Saad, S., Madlen, M., Habashy and Khadiga, M., Sharshar, Growth Response of the Freshwater Prawn, *Macrobrachium rosenbergii* (De Man),to Diets Having Different Levels of Biogen®. *World Applied Sciences Journal.*, **2009**, 6 (4): 550-556.

23. Ringo, E., and Gatesoupe,F.J. Lactic acid bacteria in fish: A review.Aguaculture., 1998,160,177-203.

24. Vázquez, J.A., González, M.P., Murado, M.A., Effects of lactic acid bacteria cultures on pathogenic microbiota from fish. Aquaculture., **2005**, 245: 149–161.

25.Mathieu Castex, Liet Chim, Dominique Pham, Pierrette Lemaire, Nelly Wabete ,Jean-Louis Nicolas, Philippe Schmidely, Catherine Mariojouls., Probiotic *P. acidilactici* application in shrimp *Litopenaeus stylirostris* culture subject to *vibriosis* in New Caledonia. Aquaculture., **2008**, 275: 182–193.

26. Ponpisit Utiswannakul1, Siripen Sangchai1 and Sirirat Rengpipa., Enhanced growth of black tiger shrimp *Penaeus monodon* by dietary supplementation with Bacillus (BP11) as a probiotic. *J Aquac. Res. Development*., **2011**.

