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IMMUNOMODULATORY AND GROWTH-PROMOTING POTENTIAL OF LOW-COST PROBIOTIC PRODUCT IN *Penaeus monodon* CULTURE SYSTEM

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ABSTRACT

The capability of a low-cost probiotic product was evaluated in *Penaeus monodon* reared under an outdoor culture system for a period of 90 days. The low-cost probiotic product was formulated by mixing the broth culture of *Bacillus cereus* along with commercial chalk powder in 1:1 ratio. The prepared probiotic product as a powdered supplement was incorporated along with the shrimp diet at various concentrations (1 to 5%). The viability of *B. cereus* in the experimental diet was tested once in 30 days up to 90 days during the experimentation. After 90 days of feeding experiments, the growth performance of shrimp was determined with a maximum production of 11.98 ± 0.367 g, SGR of 5.030 ± 0.171% and FCE of 86.63 ± 1.300% in *P. monodon* fed D4 diet (4% probiotic supplement). The immunological parameters determined at the end of the culture experiment showed variation among diets. The total haemocyte count (273.66 ± 3.09 x 10^5 cells/ml), phenoloxidase activity (0.132 ± 0.007 OD) and respiratory burst activity (0.291 ± 0.020 O.D) were high in *P. monodon* fed D4 diet. Similar effects were observed for plasma protein concentration (68.00 ± 1.41 mg/ml), lysozyme activity (0.483 ± 0.014 U/ml) and bactericidal activity (81.0 ± 1.33%) in shrimp fed D4 diet. The results of the present investigation conclude that the probiotic product at a concentration of 4% was effective in elevating the growth and immune performances in shrimp *P. monodon.*

How to Cite

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INTRODUCTION

Probiotic application plays an important role in aquaculture. Administration of antimicrobial drugs and pesticides has resulted in resistant strains of bacteria that cause havoc to aquaculture industry (Esiobu et al., 2002; Boyd and Massaaut, 1999). Therefore, a safe alternative is to use probiotic supplement for aquatic animals to create a sustainable and eco-friendly culture environment (Gatesoupe, 1999). Enormous benefits of probiotics include digestive enzymes that facilitate digestion, antagonistic activity against pathogens, growth and better immune response (Verschuere et al., 2000). Several reports are available for the beneficial effect of probiotics for fish and

shrimp (Mohanty et al., 1993; 1996; Sharma and Bhukhar, 2000; Wang et al., 2005; Wang and Xu, 2006). The cellular function of shrimp has been stimulated by probiotic bacteria (Vargas-Albores et al., 1998; Gullian et al., 2004). The administration of probiotics can be done through food supplement or as a water additive (Moriarty, 1998). Probiotics play a crucial role in improving the immunity in shrimp (Verschuere et al., 2000). *Bacillus* bacteria are widely used as potent probiotics and their health-promoting effects have been studied well in shrimp (Rengpipat et al., 1998, 2000; Sugita et al., 1998).

The beneficial effects of incorporating the probiotic strains in the diets of fish, crustaceans, molluscs and amphibians have been reported by many researchers (Aly et al., 2008; Ignatova et al., 2009; Soleimani et al., 2010; Veizaj-Delia et al., 2010; Gatesoupe, 1999). Such benefits include balancing the intestinal flora of animals, preventing diseases of digestive tract, improving the digestion capability and causing better utilization of nutrients (Fuller, 1992; Balcázar et al., 2006). Probiotics widely used in aquaculture contributed by the genus *Bacillus* species, *Bifidobacterium* sp. (lactic-acid secreting bacteria) and *Saccharomyces cerevisiae* (Lee et al., 1999; Sanders and Klaenhammer, 2001). The genus *Bacillus* are capable of secreting exoenzymes (proteases, lipases and carbohydrates) that facilitate digestion and nutrient absorption and further promote better growth in cultured organism (Ninawe and Selvin, 2009). Our previous study (Navin Chandran et al., 2014) inferred that the lyophilized form of *B. cereus* at a concentration of 0.4%/100 g feed was efficient in boosting up the immunity and growth performance of shrimp. But the production cost to develop it in a lyophilized form is high. Therefore, the present study was undertaken mainly to develop a low-cost probiotic product supplemented dietary source and to evaluate its performance on growth and immune response in shrimp *P. monodon* culture system.

MATERIALS AND METHODS

Isolation and identification of probiotic bacterium

The probiotic bacterium used in the present study was *Bacillus cereus*; the detailed procedure on isolation and identification of this strain was clearly mentioned in our earlier report (Navin Chandran et al., 2014).

Preparation of low-cost probiotic product

B. cereus 138.23 ± 1.05 x 10^{-4} CFU/ml enriched MRS broth culture (38-42h) was mixed with chalk powder at 1:1 ratio under aseptic condition. The contents were mixed well and dried in a hot air oven at 40°C for 48 h. The dried probiotic

mass was powdered using mortar and pestle under aseptic conditions and then the obtained probiotic product was stored at 32°C in an airtight plastic container until further use.

Diet preparation

The prepared probiotic product was incorporated along with the basal diet at 1-5% respectively in D1-D5 diets as shown in Table 1. A control diet (C) with lack of this probiotic product was also prepared. The respective feeds were prepared as per the method proposed by Navin Chandran et al. (2014).

Table 1. Feed ingredients used for the preparation of control (C) and low-cost probiotic supplemented experimental diets (D1 to D5)

Feed ingredients	Type of feed / Amount of feed ingredients					
	C	**D1**	**D2**	**D3**	**D4**	**D5**
Fish meal (g)	45	45	45	45	45	45
Ground nut oil cake (g)	25	25	25	25	25	25
Soya meal (g)	13	13	13	13	13	13
Wheat bran (g)	7	7	7	7	7	7
Seaweed powder (g)	2	2	2	2	2	2
Tapioca powder (g)	2	2	2	2	2	2
Vitamin and mineral (g)	2	2	2	2	2	2
Cod liver oil (g)	2	2	2	2	2	2
Gelatin (g)	2	2	2	2	2	2
Low-cost probiotic supplement (%)	-	1	2	3	4	5

Source: Navin Chandran et al. (2014)

Evaluation of the viability of supplemented probiotic in experimental diets

The viability of *B. cereus* in experimental diets was determined at various time intervals of 30th, 60th and 90th days. In brief, 10 mg of feed pellet was taken individually from different diets (D1-D5) and ground well using 1 ml of phosphate buffer saline (pH 7.2) in a sterile condition using mortar and pestle. The contents were serially diluted and then spread on MRS agar plates and finally kept in an incubator for 48 h. The *B. cereus* colonies obtained were counted to check the viability; simultaneously in each set of diets, triplicate plates were maintained.

Collection of experimental animal P. monodon postlarvae

P. monodon postlarvae (PL-15) were obtained from Aqua Marine Hatchery, Pondicherry and transported to the laboratory carefully in oxygenated polythene bags. The postlarvae were stocked in one tonne capacity FRP tank containing 25 ppt filtered seawater. The PL were fed on commercial starter feed (CP Nova) twice a day. Proper aeration was provided and the unfed remains were siphoned out daily prior to water exchange. Fresh filtered sea water (25 ppt) was added to maintain the initial water level.

Experimentation

The entire experiment was conducted in outdoor culture system for a duration of 90 days in 750 L capacity FRP tanks. Equal amount of clay soil (5 cm height) at the pH level of 7.86 ± 0.32 was applied uniformly at the bottom of all tanks and then they were finally filled with filtered seawater of 25 ppt salinity. The tanks were aerated well using Boyu (U-9900) air pump. *P. monodon* postlarvae with the weight range from 0.115 ± 0.002 g to 0.134 ± 0.006 g were collected from the stocking tank and then stocked into the individual experimental tanks at the rate of 50 PL/tank. For each diet of control (C) and experimental (D1-D5) group, triplicate tanks were maintained. The shrimp PL were fed on respective control and experimental diets twice daily (6 a.m. and 6 p.m.) for a duration of 90 days.

Evaluation of survival and growth performance of shrimp P. monodon

At the end of the culture period (90th day), the survival and growth performances of *P. monodon* were determined. The different growth parameters followed are given below.

Production (g) = Final weight − Initial weight

Food Conversion Ratio (FCR) = Total amount of feed given (g dry weight) / Total production of shrimp (g dry weight)

Specific Growth Rate (SGR - %) = (In final weight (g) − In initial weight (g) / Experimental period (days)) x 100
Where ln= natural log

Food Coversion Efficiency (FCE - %) = Wet weight of the shrimp produced (g) / Dry weight of the feed given (g) x 100

Evaluation of immunological parameters

To evaluate the immunological parameters, haemolymph was collected individually from control and experimental groups of shrimp according to the methodology of Navin Chandran et al., 2014. The following immunological parameters were performed in triplicates.

Total haemocyte count

The haemocyte count (THC) was determined by placing a drop of haemolymph /anticoagulant mixture stained by giemsa stain on a haemocytometer. Haemocytes were counted using a light microscope (Hund- Wetzlar -3550 (h500)) at a magnification of 400x and the result was expressed as cells/ml.

Phenoloxidase assay

Phenoloxidase activity was measured by the method of Hernandez − Lopez et al. (1996) with slight modification. The formation of dopochrome was recorded at 490 nm using UV-vis spectrophotometer (Tecomp 8500) and the result was expressed as increase in optical density (OD).

Superoxide anion assay

Nitroblue tetrazolium (NBT) reduction and the formation of formazan were taken as the measure of superoxide anion production and it was performed according to the method of Song and Hsieh (1994) with slight modification. Laminarin (L. 9634, Sigma) was used as an elictor and the result was expressed in optical density per 100 μl of haemolymph.

Plasma protein concentration

Plasma protein concentration was determined according to the standard method of Lowry et al. (1951). The result was expressed as mg/ml.

Lysozyme assay

Lysozyme activity was determined using *Micrococcus lysodeikticus* suspended in 0.05 M sodium phosphate buffer (pH 6.2). The initial absorbance of *M. lysodeikticus* was read at UV-vis spectrophotometer at 530 nm and immediately 200 μl of haemolymph was added to it. The reduction in absorbance was recorded at 0.5 min and 4.5 min of time intervals and the result was expressed as U/ml (Parry et al., 1965).

Bacterial clearance efficiency

The overnight culture of *Vibrio harveyi* cultured in Zobell marine broth was used as a pathogenic strain for testing the bacterial clearance efficiency. The broth was centrifuged, washed in sterile saline solution (2%) and further diluted in saline that served as a pathogenic sample. Haemolymph was subjected to centrifugation at 9700 rpm using 5% sodium citrate for 20 min. Further, 100 μl of cell-free haemolymph was incubated with 100 μl of bacterial suspension and the

samples were transferred to sterile microtubes and again incubated for 3 hrs at 25°C. Further, 100 µl of sample was collected from each microtube and then spread on TCBS agar plates, then the plates were incubated overnight at 25°C and the colony forming units (CFU) were counted (Adams, 1991).

Statistical analysis

The data obtained in the present study were expressed as Mean ± SD and were analyzed using one-way ANOVA at 5% significance level. Further a multiple comparison of SNK test was conducted to compare the significant differences among the parameters using STATISTICA 6.0 software package (Statosoft, Bedford, UK).

RESULTS

Experimental probiotic bacterium

The reference probiotic bacterium isolated previously from the gut of *P. monodon* and identified as *B. cereus* by 16Sr RNA sequence analysis was used in the present study.
The result on the viability of *B. cereus* in experimental diets (D1-D5) during different days of storage is given in Table 2. The viability of *B. cereus* was TNTC (too numerous to count) in D4 and D5 diets during the 30th, 60th and 90th days of analysis.
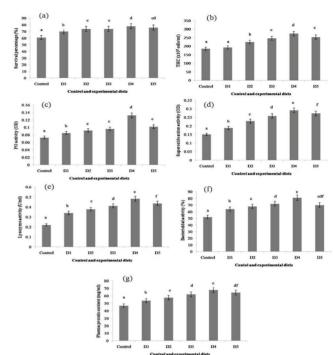

Fig 1. Evaluation of survival and immunological parameters in *P. monodon* during the 90th day of experimentation: (a) Survival, (b) Total haemocyte count, (c) Phenoloxidase, (d) Superoxide anion, (e) Lysozyme, (f) Bacterial clearance activity and (g) Plasma protein content

Each value is the Mean ± S.D of triplicate analysis, bars represented in different superscript letters are statistically significant (One way ANOVA test: P<0.005; further *post hoc* multiple comparison with SNK test)

Table 2. Viability of *B. cereus* in the prepared diets (D1-D5) at various duration of storage

Experimental duration	Diets / viability (CFU/g)				
	D1	**D2**	**D3**	**D4**	**D5**
30th day	40.00 ± 1.40	126.00 ± 1.54	220.00 ± 2.40	TNTC	TNTC
60th day	38.00 ± 1.34	124.00 ± 1.50	215.00 ± 2.13	TNTC	TNTC
90th day	35.00 ± 1.30	120.00 ± 1.48	210.00 ± 2.10	TNTC	TNTC

Each value is the mean ± SD of triplicate analysis
TNTC: Too numerous to count

But in D1, D2 and D3 diets, it showed many variations; accordingly, in D1 diet, the viability was 40.00 ± 1.40 CFU/g during the 30th day of analysis, but it declined to 35.00 ± 1.30 CFU/g on the 90th day. A similar trend was noticed in D2 and D3 diets with the viability of 126.00 ± 1.54 and 220.00 ± 2.40 CFU/g respectively on the 30th day of analysis, but it declined respectively to 120.00 ± 1.48 and 210.00 ± 2.10 CFU/g during the 90th day of analysis.

Survival and growth performance of P. monodon

At the end of the culture experiment, survival of *P. monodon* fed on different diets was recorded (Fig. 1a).
The result indicated that the survival was significantly higher

(P<0.05) in shrimp fed different concentrations of probiotic supplemented diets (70.0 ± 1.11 to 78.0 ± 2.05%) when compared to a lower survival of 61.0 ± 1.68% recorded in shrimp fed control diet. Overall growth performance indicated that the production was high (11.98 ± 0.367 g) in the group fed D4 diet, whereas it was low (7.694 ± 0.345 g) in the group fed control diet. The FCE was higher (86.63 ± 1.300%) in shrimp fed D4 diet, whereas it was only 60.07 ± 2.209% in shrimp fed control diet. A similar trend was also noticed for specific growth rate (SGR); here shrimp fed D4 diet displayed a higher SGR of 5.030 ± 0.171%, however, a low (4.640 ± 0.191%) SGR was attained by shrimp fed control diet. A better FCR of 1.15 ± 0.055 was registered in shrimp fed D4 diet when compared to shrimp fed control and other diets (Table 3).

Table 3. Overall growth response of *P. monodon* fed on control (C) and low-cost probiotic supplemented experimental diets (D1 to D5) at the end of culture experiment

Parameters	Growth responses					
	Control	**D1**	**D2**	**D3**	**D4**	**D5**
Initial weight (g)	0.120 ± 0.004	0.118 ± 0.017	0.115 ± 0.002	0.118 ± 0.001	0.131 ± 0.002	0.134 ± 0.006
Final weight (g)	7.814 ± 0.351	8.219 ± 0.246	8.900 ± 0.077	9.312 ± 0.088	12.12 ± 0.079	9.240 ± 0.108
Production (g)	a 7.694 ± 0.345	b 8.101 ± 0.242	c 8.785 ± 0.273	c 9.194 ± 0.086	d 11.98 ± 0.367	c 9.106 ± 0.155
Food consumed (g)	12.80 ± 0.355	13.01 ± 0.465	13.27 ± 0.457	13.20 ± 0.367	13.82 ± 0.146	13.31 ± 0.661
FCE (%)	a 60.07 ± 2.209	a 62.25 ± 2.661	b 66.19 ± 0.281	c 69.63 ± 1.920	d 86.63 ± 1.300	c 68.41± 1.290
AGR (g/body weight/day)	a 0.085 ± 0.006	b 0.090 ± 0.006	c 0.097 ± 0.005	d 0.102 ± 0.007	e 0.133 ± 0.014	d 0.101 ± 0.005
SGR (%)	a 4.640 ± 0.171	a 4.715 ± 0.187	a 4.832 ± 0.137	a 4.853 ± 0.312	b 5.030 ± 0.171	a 4.703 ± 0.160
FCR	a 1.66 ± 0.041	a 1.60 ± 0.042	b 1.51 ± 0.081	c 1.43 ± 0.022	d 1.15 ± 0.055	c 1.46 ± 0.069
Growth (%)	a 8.54 ± 0.146	b 9.00 ± 0.216	c 9.76 ± 0.114	c 10.21 ± 0.194	d 13.31 ± 0.220	c 10.11 ± 0.322

Each value is the Mean ± SD is the average of three individual estimates, values in a row superscripted with different alphabets are statistically significant (one way ANOVA test: P < 0.05; further post hoc multiple comparison with SNK test)

Immunological parameters

Total haemocyte count (THC) was higher ($273.66 \pm 3.09 \times 10^5$ cells/ml) in shrimp fed D4 diet, whereas in shrimp fed control diet, it was lower ($185.66 \pm 3.29 \times 10^5$ cells/ml). In shrimp fed other probiotic supplemented diets, the THC recorded ranged between 192.66 ± 2.49 and $253.66 \pm 2.62 \times 10^5$ cells/ml (Fig. 1b).

Phenoloxidase activity (PO) also showed the same trend as that of THC; here it was higher (0.132 ± 0.007 OD) in shrimp fed D4 diet, but lower (0.073 ± 0.0020 OD) in the group fed control diet. The PO activity of 0.085 ± 0.001, 0.092 ± 0.001, 0.096 ± 0.001 and 0.102 ± 0.002 OD was noticed in *P. monodon* fed D1, D2, D3 and D5 diets, respectively (Fig. 1c).

Superoxide anion activity was significantly (P <0.05) higher in *P. monodon* fed probiotic diets than the control shrimp (Fig. 1d). A higher superoxide anion activity of 0.291 ± 0.020 OD was noticed in *P. monodon* fed D4 diet, but this activity was lower (0.150 ± 0.016 OD) in shrimp fed control diet. In shrimp fed other probiotic diets, this activity was in the order of 0.188 ± 0.013, 0.228 ± 0.012, 0.258 ± 0.014 and 0.273 ± 0.006 OD respectively in shrimp fed D1, D2, D3 and D5 diets.

Plasma protein concentration (PPC) was higher (68.00 ± 1.41 mg/ml) in *P. monodon* fed D4 diet, whereas it was lower (47.00 ± 1.63 mg/ml) in *P. monodon* fed control diet (Fig. 1g), whereas shrimp fed other probiotic diets showed higher PPC when compared to control, and it was recorded as 53.66 ± 1.69, 57.66 ± 1.24, 62.10 ± 1.84 and 64.50 ± 1.47 mg/ml respectively in *P. monodon* fed D1, D2, D3 and D5 diets.

The result on lysozyme activity recorded in the haemolymph of shrimp fed control and experimental diets is shown in Fig.

1e. *P. monodon* fed D4 diet displayed a maximum lysozyme activity of 0.483 ± 0.014 U/ml when compared to a minimum value of 0.221 ± 0.002 U/ml registered in *P. monodon* fed control diet. The lysozyme activity recorded in *P. monodon* fed D1 and D2 diets showed marginal variation with 0.345 ± 0.010 and 0.378 ± 0.008 U/ml, respectively. However, a slightly better activity of 0.412 ± 0.010 and 0.437 ± 0.010 U/ml was noticed in *P. monodon* fed D3 and D5 diets.

Bacterial clearance efficiency recorded in the haemolymph samples of shrimp fed control and experimental diets is depicted in Fig. 1f. It was remarkably higher (81.0 ± 1.33%) in *P. monodon* fed D4 diet, whereas it was lower (52.0 ± 1.14%) in *P. monodon* fed control diet. The other probiotic treated groups displayed higher bacterial clearance efficiency when compared to shrimp fed control diet and it ranged between 64.0 ± 1.63 and 78.0 ± 1.77% in *P. monodon* fed D1, D2, D3 and D5 diets, respectively.

DISCUSSION

Probiotic application in aquaculture industry is a multidisciplinary process that requires fundamental research and feeding trial. Moreover, selection of a good probiotic is of much importance in aquaculture industry. The probiotic strains that contribute to the intestinal mucus of fish are considered a good strain to competitively exclude pathogens from the gut (Verschuere et al., 2000). *Bacillus* strains are widely used as probiotics in the sense that they are capable of spore formation, stable in the environment and are resistant to physical and chemical effects (Henriques and Moran, 2000). Further, they are also capable of producing exoenzymes (Moriarty, 1997). *Bacillus* bacteria are isolated from the intestines of crustaceans (Rengpipat et al., 2000), bivalves (Sugita et al., 1981) and marine fishes (Sugita et al., 1998). Likewise, in the present study, the probiotic strain *B. cereus* was isolated from the gut of estuarine shrimp *P. monodon* as mentioned in our earlier study (Navin Chandran et al., 2014). Further, reports are also available illustrating the beneficial role of *B. subtilis* and *B. licheniformis* as potent probiotics in shrimp culture (Moriarty, 1998; 1999, Moriarty et al., 2005).

Probiotics should be fed at an early stage of shrimp or fish for optimal improvement of gut microbiota because it may proliferate well in rearing water thereby decreasing the level of pathogenicity (Moriarty, 1998). Accordingly, in the present study, the low-cost probiotic product supplemented diets (D1-D5) were prepared and fed to the early postlarval (PL 15) stage of shrimp *P. monodon*. The aim of supplementing the probiotic strain in the dietary source is to obtain a good weight gain of shrimp within a short lifespan. Several authors have reported in this concern, i.e. *Bacillus* sp. supplemented in shrimp diet significantly improved the weight gain during 45 days of culture period (Gomez et al., 2008). Further, mixture of *Lactobacillus* sp. isolated from chicken gastrointestinal tract resulted in an improved growth performance and survival in juvenile *P. monodon* for a culture duration of 100 days (Reid, 2008). Similar effects were obtained in the present study that the *P. monodon* fed on probiotic diet D4 showed a better production of 11.99 ± 0.367 g, when compared to a lower production of 7.694 ± 0.345 g rendered by the shrimp fed control diet. Rosovitz et al. (1998) concluded that trout fry fed on probiotic *Bacillus* improved digestion of food, growth and resulted in a better FCR. The present findings also proved that a better food consumption of 13.82 ± 0.146 g, FCR of 1.15 ± 0.055 and SGR of 5.030 ± 0.171% in *P. monodon* fed D4 diet. The reason for improved growth performance in probiotic treated D4 diet may be attributed to the optimum production of digestive enzymes by *B. cereus*. Ochoa-Solano and Olmos-Soto (2006) also proved that the probiotic strains influenced such enzyme production that resulted in an improved digestibility of shrimp. Similar, effects were determined by Reid (2008) who proved that aquatic animals fed on probiotics had a positive role on digestive process.

Li et al. (2009) proved that probiotic bacteria *B. megeterism* elevated the immune response and suppressed pathogenicity in shrimp. Balcázar et al. (2006) suggested that probiotic application can trigger the non-specific immune system. Likewise, in the present study the immune response such as total haemocyte count (THC), phenoloxidase activity (PO), lysozyme activity and respiratory burst activity were elevated by *B. cereus* supplementation as a low-cost probiotic product through the diet. A higher THC of $273.66 ± 3.09 \times 10^5$ cells/ml was observed in *P. monodon* fed D4 diet, whereas it declined ($185.66 ± 3.29 \times 10^5$ cells/ml) in shrimp fed control diet. In the present study PO activity as well as RB activity displayed similar trend for shrimp fed D4 diet with maximum PO activity of 0.132 ± 0.007 OD and RB activity of 0.291 ± 0.02 OD when compared to the group fed control diet. Our results are consistent with the findings of Rengpipat et al. (2000) who inferred that *Bacillus* S11 isolated from healthy shrimp enhanced the immune response in shrimp, including phenoloxidase and antibacterial activity. The present study also showed that the bacterial clearance efficiency was notably high (81.0 ± 1.33%) when compared to a low clearance of 52.0 ± 1.14% observed in shrimp fed control diet. Further, Tseng et al. (2009) concluded that *B. subtilis* E20 was beneficial to shrimp against disease control and rendered a positive immune response including PO and bacterial clearance efficiency.

Several immune molecules such as lipopolysaccharide binding protein (Vargas-Albores et al., 1993), -glucan binding protein (BGBP) (Vargas-Albores et al., 1996) and clotting protein (Hall et al., 1995; Montano-Perez et al., 1998) have been identified in crustaceans, thus determining the plasma protein concentration as an important factor in the evaluation of shrimp health. In the present investigation,

shrimp fed on D4 diet displayed a higher plasma protein content (PPC) of 68.0 ± 1.14 mg/ml when compared to a lower PPC of 47.00 ± 1.67 mg/ml attained by the shrimp fed control diet. Lysozyme, an important component of innate immune system of invertebrates, plays a significant role as an antibacterial protein (Jolles and Jolles, 1984). The present findings proved that the lysoszyme activity was higher (0.483 ± 0.014 U/ml) in *P. monodon* fed on D4 diet, whereas a lower activity (0.221 ± 0.002 U/ml) was noticed in shrimps fed control diet. Similar effect was also observed in Bastard halibut *Paralichthys olivaceus* concerning plasma lysozyme activity that was higher in commercial probiotic treated group when compared to the control (Taoka et al., 2009). In conclusion, the present study suggested that the low-cost probiotic *B. subtilis* product at 4% concentration can be safely used in shrimp culture system with the view of enhancing the growth and immune status of shrimp.

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Sažetak

POTENCIJAL ZA POBOLJŠANJE IMUNOLOŠKOG SUSTAVA I POTICANJE RASTA UZ PROBIOTIČKI PROIZVOD NISKIH TROŠKOVA U SUSTAVU UZGOJA *Penaeus monodon*

Sposobnost probiotičkog proizvoda s niskim troškovima procijenjena je pri uzgoju vrste *Penaeus monodon* u vanjskom sustavu uzgoja tijekom 90 dana. Probiotski proizvod niske cijene formuliran je miješanjem bujonske kulture *Bacillus cereus* zajedno s komercijalnom praškastom kredom u omjeru 1: 1. Pripremljeni probiotički produkt uz dodatak praha inkorporiran je zajedno s hranom za rakove u obroke različite koncentracije (1 do 5%). Održivost *B. cereusa* u eksperimentalnoj prehrani testirana je jednom u 30 dana do 90 dana tijekom trajanja eksperimenta. Nakon 90 dana od početka hranidbenog istraživanja, određene su performanse rasta rakova. Utvrđena je maksimalna proizvodnja od 11,98 ± 0,367 g, SGR od 5,030 ± 0,171% i FCE od 86,63 ± 1,300% u *P. monodon* koji su hranjeni s D4 (4% dodatak probiotika). Imunološki parametri određeni na kraju eksperimenta pokazali su varijaciju među ishranama. Ukupni broj hemocita (273,66 ± 3,09 x 105 stanica / ml), aktivnost fenoloksidaze (0,132 ± 0,007 OD) i aktivnost respiratornog sustava (0,291 ± 0,020 OD) je bila visoka kod *P. monodon* koji su hranjeni s D4. Slični efekti zabilježeni su za koncentraciju proteina u plazmi (68,00 ± 1,41 mg / ml), aktivnost lizozima (0,483 ± 0,014 U / ml) i baktericidnu aktivnost (81,0 ± 1,33%) kod rakova hranjenih s D4. Zaključci ovog istraživanja su da je probiotički produkt u koncentraciji od 4% učinkovit za povećanje rasta i imunološke učinke kod rakova *P. monodon*.

Ključne riječi: probiotski proizvod s niskim troškovima, rast, fenoloksidaza, THC, poremećaj disanja

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