DOI: 10.1111/are.13669

ORIGINAL ARTICLE

WILEY

Evaluation of growth, feed utilization efficiency and immune parameters in tiger shrimp (*Penaeus monodon*) fed diets supplemented with or diet fermented with gut bacterium *Bacillus* sp. DDKRC1. isolated from gut of Asian seabass (*Lates calcarifer*)

Debasis De[1] | **Ramalingam Ananda Raja**[2] | **Tapas Kumar Ghoshal**[1] | **Suchita Mukherjee**[1] | **Koyadan Kizhakedath Vijayan**[2]

[1]Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture, Kakdwip, West Bengal, India

[2]ICAR-Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu, India

Correspondence
Debasis De, ICAR-Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu, India.
Emails: debasiskrc@yahoo.com; dedebasis47@gmail.com

Funding information
Department of Biotechnology, Ministry of Science & Technology, Government of India

Summary

An experiment was conducted to assess the effect of supplementation of cellulolytic gut bacterium with plant protein-based diet on growth, digestibility and digestive enzyme status of tiger shrimp, *Penaeus monodon*. *Bacillus* sp. DDKRC1. (JN641289), a potential cellulolytic bacterium, isolated from the gut of Asian seabass was used as feed supplement in this experiment. The shrimps of group I, II and III were fed with control diet (without bacterial supplement, D1), diet supplemented with live *Bacillus* sp. DDKRC1. at 2.94×10^7 cfu per 100 g feed (D2) and diet fermented (48 hr) with same bacteria (D3) respectively. At the end of the 42 days experiment, shrimps of group II showed significantly better ($p < .01$) growth and protein efficiency ratio (PER) and lower ($p < .01$) feed conversion ratio (FCR) as compared to group I and III. Dry matter, cellulose, hemicellulose and lipid digestibilities were significantly ($p < .01$) higher in group II than other two groups. Cellulase, amylase and protease activities in the gastrointestinal (GI) tract were significantly ($p < .01$) higher in group II as compared to other two groups. Total heterotrophic count, amylolytic, cellulolytic and proteolytic bacterial counts in gut were significantly ($p < .01$) higher in group II as compared to other two groups. Haematological analysis indicated better ($p < .05$) immune response in groups II and III than control group. Diets D2 and D3 showed better effect on growth, digestibility, FCR, survival and immune response of *P. monodon* as compared to D1.

KEYWORDS
Bacillus sp. DDKRC1., digestibility, growth, gut bacteria, immune parameters, *Penaeus monodon*

1 | INTRODUCTION

The use of live bacterial supplementation (probiotics) in aquaculture is increasing day by day for more ecofriendly aquaculture practices (Gatesoupe, 1999). Probiotic agents exert a beneficial effect through enzymatic assistance to digestion and absorption of nutrients in gastrointestinal tract of host (Erasmus, Cook & Coyne, 1997; Krishnaprakash, Saravanan, Murugesan & Rajagopal, 2009; Sahu, Swarnakumar, Sivakumar, Thangaradjou & Kannan, 2008), competition for adhesion sites and resistance to colonization (Balcázar et al., 2006; Verschuere, Rombaut, Sorgeloos & Verstraete, 2000), competition for essential nutrients, reduction in gut pH, production of

 © 2018 John Wiley & Sons Ltd

antagonistic compounds against pathogens (De, 2014; Kesarcodi-Watson, Kaspar, Lategan & Gibson, 2008; Ringø & Gatesoupe, 1998; Verschuere et al., 2000), enhancement of the immune response and disease resistance (Balcázar et al., 2007; Rengpipat, Runkpratanporn, Piyatiratitivorakul & Menasaveta, 2000; Sahu et al., 2008). In addition, supplementation of beneficial bacteria improve enzyme activity feed digestibility and feed utilization, health and performance in aquatic animals (Balcázar et al., 2006; De, Ghoshal, Ananda Raja & Kumar, 2015; Kesarcodi-Watson et al., 2008; Ringø & Gatesoupe, 1998). *Bacillus* has been widely used as potential probiotics (Ziaei-Nejad, 2004), since they secrete a variety of antimicrobial compounds and exoenzymes (Moriarty, 1996, 1998). Earlier studies showed that administration of *Bacillus* spp. during a long period could enhance the digestive enzyme activity, leading to enhanced weight gain of *Litopenaeus vannamei* (Gómez, Geovanny & Shen, 2008) and pathogenic protection by activating both cellular and immune defenses of black tiger shrimp, *Penaeus monodon* (Rengpipat et al., 2000). There is no united stand to conclude the best source of probiotics for any fish or shrimp. Probiotics from terrestrial environment confer many beneficial effects to the fish or shrimp but probiotics isolated from aquatic environment offer little more advantage when used for any aquaculture species (Zorriehzahra Mohammad et al., 2016). Very few studies were conducted to assess the effect of supplementation of probiotic bacteria isolated from gut of aquatic animal, on performance of shrimp of similar environment (Sánchez-Ortiz et al., 2015). A study was conducted to isolate and characterize the potential cellulolytic bacterium from the gut of brackiswater fish and shrimp. *Bacillus* sp. DDKRC1. isolated from the gut of *Lates calcarifer* was found to be the most potential cellulolytic bacterium (De, Ghoshal & Ananda Raja, 2014). Hence, the present study was aimed to investigate the influence of *Bacillus* sp. DDKRC1. on weight gain, apparent nutrient digestibility and haematological parameters of economically important black tiger shrimp, *P. monodon* in brackishwater environment and to evaluate and compare the efficacy of approaches for using bacterial culture (*Bacillus* sp. DDKRC1.), either for fermentation or as feed supplement (probiotics), for better utilization of diet in tiger shrimp.

2 | MATERIALS AND METHODS

This study on the effect of cellulolytic gut bacteria as a feed supplement on growth performance, apparent nutrient digestibility and digestive enzyme activity of tiger shrimp, *P. monodon* was conducted in fish nutrition laboratory at Kakdwip Research Centre of CIBA, Kakdwip (Lat. 21°51′15.01″–21°51′30.77″N, Long. 88°10′58.44″ 88°11′12.09″E), West Bengal, India.

2.1 | Experimental diets preparation

Bacillus sp. DDKRC1. (JN641289), a potential cellulolytic bacterium was previously isolated from the gut of adult *L. calcarifer* and identified through 16s rDNA sequencing (De et al., 2014). The same isolate was used as feed supplement with plant protein rich formulated feed (comprised of fish meal, soybean meal, sesame cake, mustard cake, azolla, amino acid mixture, mineral and vitamin mixture) containing crude protein (CP) ~35% and lipid ~5% (Table 1). For preparation of control diet, all the feed ingredients, except mineral–vitamin mixture and amino acid mixture, were mixed with water and cooked in autoclave for 20 min with exposure at 15 psi, 121°C for 5 min. The remaining ingredients were mixed after cooling the dough. Dough was passed through a mincer with a die (2 mm diameter) to get spaghetti-like strings. Feed strings were air-dried for 1 hr at ambient temperature and fed to shrimp. Experimental diet D2 was prepared as of control diet but supplemented with live *Bacillus* sp. DDKRC1. at 2.94×10^7 cfu per 100 g of feed. Bacterial culture was added after the autoclaving of major ingredients and cooling the dough. Feed was prepared twice daily before feeding to maintain the efficacy of bacterial culture. Diet D3 was prepared in a similar way as of control diet and was incubated with the same bacterial strain at 2.94×10^7 cfu per 100 g of feed at 34°C (De et al., 2014) with 50% moisture content for 24 hr, 48 hr, 72 hr, 96 hr and 120 hr to know the optimum incubation period in regards to nutrient enrichment of feed. After 48 hr incubation, the brittle feed was re-pelletized and the diet D3 was prepared.

2.2 | Experimental set up

The feeding trial was conducted for 42 days under laboratory condition in fibre reinforced plastic tank (Length × Breadth × Height: 0.65 × 0.50 × 0.48 m) containing dechlorinated brackishwater with continuous aeration. Hatchery-bred and PCR tested healthy *P. monodon* (mean individual weight 2.73 ± 0.01 g) were randomly distributed in tanks with 10 shrimps per tank for three groups in triplicates (Ananda Raja et al., 2017a). Groups I, II and III were fed with diets D1, D2 and D3 respectively. Feed was adjusted at 10 days interval after calculating the biomass through intermediate sampling and counting the numbers of shrimp survived. The daily ration was offered twice daily at 10% (w/w) of the total body weight. The left-over feed was siphoned off after 4 hr of feeding, dried in an oven at 105°C for 16 hr. Daily feed consumption was

TABLE 1 Proximate composition (% of dry weight) of the experimental diets

Parameters	Diets D1	D2	D3
Moisture	8.87 ± 0.04	8.71 ± 0.11	12.20 ± 0.26
Crude protein	34.87 ± 0.03	34.59 ± 0.29	35.93 ± 0.14
Crude fibre	2.72 ± 0.06	2.74 ± 0.12	2.52 ± 0.07
Lipid	5.28 ± 0.07	5.28 ± 0.05	5.19 ± 0.05
Ash	12.07 ± 0.14	12.16 ± 0.03	12.78 ± 0.03
NFE	36.19 ± 0.05	36.52 ± 0.15	31.38 ± 0.08

D1 - control diet, D2- live bacterial-supplemented diet, D3 - fermented diet.
NFE, nitrogen-free extract; values are mean ± *SD*. Error of 3 determination.

estimated by the difference between feed offered and uneaten feed in dry weight. Water of the experimental tanks was replaced with 50% of fresh dechlorinated brackishwater at 2-day interval. The faecal samples were collected daily from each tank by pipetting (Spyridakis, Metailler, Gabaudan & Riaza, 1989). The oven dried (60°C) faecal samples were analysed for digestibility estimation. During the experiment water pH, temperature, salinity and dissolved oxygen were 7.84–7.94, 30–32°C, 7.10–7.23 g/L and 5.33–5.47 mg/L respectively (Table 2). Five shrimps from each tank were collected at the end of the experiment for gut bacterial population, intestinal enzyme and haematological study.

2.3 | Sample collection, chemical analysis and data collection

The proximate principles of feed, faecal and shrimp tissue were determined following Association of Official Analytical Chemists (AOAC) (1995). Hemicellulose and cellulose content of feed and faecal were measured according to the Van Soest (1963, 1967) method. Chromic oxide in the diets and faecal samples were estimated by wet digestion method of Furukawa and Tsukahara (1966). Water quality parameters were measured by APHA (1998). Total microbial count and *Vibrio* count of water was done using tryptone soya agar (TSA [g/L]: Pancreatic digest of casein, 15; papaic digest of soybean meal, 5; NaCl, 5; agar, 15; pH, 7.3) and thiosulphate citrate bile salts sucrose (TCBS) agar (TCBS agar [g/L]: Protease peptone,10; yeast extract,5; sodium thiosulphate, 10; sodium citrate, 10; Oxgall, 8; sucrose, 20; NaCl, 10; ferric citrate, 1; bromothymol blue, 0.04; thymol blue, 0.04; agar, 15; pH, 8.6 ± 0.2 at 25°C) respectively. Average live weight gain (%), specific growth rate (SGR; % day^{-1}), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using standard methods (Steffens, 1989).

The apparent digestibility coefficient (ADC) of nutrients was calculated according to (De Silva & Anderson, 1995), using the following formula:

$$\text{Digestibility coefficient}\,(\%) = 100 - \frac{\%\,Cr_2O_3\,\text{in diet}}{\%\,Cr_2O_3\,\text{in faeces}} \times \frac{\%\,\text{nutrient in faeces}}{\%\,\text{nutrient in diet}} \times 100$$

2.4 | Intestinal microbial analysis

Shrimps from each experimental set were dissected on an ice tray to remove the gastrointestinal (GI) tract in order to determine the intestinal microbial population at the end of the feeding trials. The entire GI tract was homogenized with five times (w/v) of sterile chilled phosphate-buffered saline with 0.9% NaCl (pH 7.2). The homogenate of the intestine of each shrimp was tenfold serially diluted and 0.1 ml of each dilution was poured aseptically under laminar flow on sterilized TSA, carboxy methyl cellulose agar (CMC-agar [g/L]: CMC, 10; KH_2PO_4, 4; Na_2HPO_4, 4; tryptone, 2; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.001; $FeSO_4.\ 7H_2O$, 0.001; agar, 15; pH 7), starch agar (SA [g/L]: Peptone, 5; yeast extract, 1.5; beef extract, 1.5; starch soluble, 2.0; sodium chloride, 5; agar, 15; pH 7.4.) and peptone gelatin agar (PGA [g/L]: Peptone, 5; gelatin, 4; beef extract, 3; agar, 20; pH 7) media containing plates, in duplicate for total cultivable microbial population, cellulolytic, amylolytic and proteolytic bacterial count respectively. These culture plates were incubated at 30°C for 48 hr and examined for the development of bacterial colonies (De et al., 2014). By multiplying the number of colonies formed on each plate by the reciprocal of dilution, colony numbers per unit sample volume of gut homogenate were determined (Rahmatullah & Beveridge, 1993).

2.5 | Digestive enzyme assay

Shrimps from each experimental set were dissected and GI tract was homogenized as mentioned in section 2.3. Homogenate was centrifuged at 10,621 *g* for 1 hr at 4°C and the supernatant was collected and used for enzyme assay. Cellulase activity was assayed using 1% carboxy methyl cellulose in citrate buffer (0.1 M, pH 6.75) as substrate (Denison & Koehn, 1977). Amylase

TABLE 2 Water quality parameters in the rearing tanks during feeding trial

	Measurements (mean ± *SD*)		
Water parameters	**Group I**	**Group II**	**Group III**
Dissolved oxygen (mg/L)	5.47 ± 0.07	5.33 ± 0.07	5.47 ± 0.18
pH	7.84 ± 0.03	7.94 ± 0.04	7.85 ± 0.03
Temperature°C	31.00 ± 0.57	30.80 ± 0.57	32.33 ± 0.88
Salinity (g/L)	7.10 ± 0.00	7.23 ± 0.03	7.13 ± 0.03
Alkalinity (mg/L)	130.67 ± 1.33	136.00 ± 2.31	129.33 ± 0.53
Nitrate-(N) (mg/L)	0.181 ± 0.02	0.163 ± 0.01	0.151 ± 0.02
Nitrite-(N) (mg/L)	0.027 ± 0.007	0.028 ± 0.009	0.024 ± 0.006
Total Ammonia (mg/L)	0.378 ± 0.012	0.326 ± 0.022	0.366 ± 0.011
Total microbial count (×10^4 cfu/ml)*	2.51 ± 0.03^a	2.87 ± 0.03^c	2.67 ± 0.02^b
Total *Vibrio* count (×10^2 cfu/ml)*	1.15 ± 0.02^b	0.88 ± 0.04^a	1.12 ± 0.02^b

Group I: fed diet D1, Group II: fed diet D2 and Group III: fed diet D3.
Values bearing different superscript letters (a,b,c) in a row differ significantly.
*$p < .05$.

activity was measured using 1% soluble starch in phosphate buffer (0.02 M; pH 6.9 containing 0.0067 M NaCl) as substrate (Bernfeld, 1955). Protease activity was detected by caseinase assay method using 0.5% casein in Tris-HCl buffer (0.02 M, pH 7.0) as substrate (Walter, 1984).

2.6 | Analysis of haematological parameters

2.6.1 | Total Haemocyte count

Haemolymph was collected individually from five shrimps of each group at the end of the trial and prepared for total haemocyte count (THC), granular haemocyte (GH) and nongranular haemocyte (NGH) count (Ananda Raja et al., 2012). Haemolymph (0.1 ml) was collected from the ventral sinus of the first abdominal segment of shrimp into a syringe containing equal volume of fixative (10% formalin in 0.45 M NaCl) and transferred to a micro-centrifuge tube. After 10 min, 20 μl of the fixed haemocyte suspension was mixed with same volume of Rose Bengal solution (1.2% Rose Bengal in 50% ethanol) and incubated at ambient temperature (27–35°C) for 20 min, before being used to determine THC. Haemocytometric (improved Neubauer, Marienfeld, Germany) counts were made for 5/25 squares (vol. of one square = 0.2 × 0.2x0.1 mm^3) in triplicate. THC was calculated as:

$$\text{THC ml}^{-1}\text{ of haemolymph} = 5 \times \text{count} \times 10^4 \times \text{dilution factor}$$

2.6.2 | Granular and nongranular haemocyte count

For GH counts, smears were prepared from the fixed and Rose Bengal stained haemocyte suspension. The smears were completely dried before counterstaining with haematoxylin solution (50 g aluminium or potassium alum, 1 g haematoxylin crystals, 0.2 g sodium iodate, 1 g citric acid, 50 g chloral hydrate and distilled water to 1 L) for 7–10 min. The slides were then rinsed with tap water for 10 min followed by dehydration with ascending grades of ethanol (10 dips each). After dehydration, the slides were cleared in xylene (3 times for 3 min each) before being mounted with DPX mountant (Merck) and covered with a coverglass. The proportions of GH that included both large-granular and small-granular/semigranular haemocytes in 200 total haemocytes were recorded and these proportions were used to calculate the total number of GH (i.e. GH count/200 × THC). The same procedure was adopted for counting the translucent smooth NGH (Ananda Raja et al., 2012).

2.6.3 | Phenoloxidase activity

Phenoloxidase (PO) activity of serum was measured spectrophotometrically with the formation of dopachrome from L-dihydroxyphenylalanine (L-DOPA) as described by Pan Lu-Qing, Bo, Ling-Xu and Jing (2007). A 0.01 M of L-DOPA was prepared in 0.1 M phosphate buffer (12.3 ml 0.2 M Na_2HPO_4, 87.7 ml 0.2 M NaH_2PO_4, pH 6.0). One hundred microlitres of serum and 100 μl of L-DOPA were added to a spectroscopy cell (10.0 mm) containing 3 ml of phosphate buffer. After the mixture was agitated, the optical density (OD) at 490 nm was measured at 2 min intervals for 60 min. Phenoloxidase activity was determined from the increase in OD per minute at the environmental condition. Total haemolymph protein was determined spectrophotometrically based on the methods of Lowry, Rosebrough, Farr and Randall (1951) using bovine serum albumin as a standard.

2.6.4 | Antibacterial activity

Antibacterial activity of shrimp plasma was measured against *Vibrio mimicus* (Pan Lu-Qing et al., 2007). *Vibrio mimicus* was isolated and characterized based on the morphological, physiological and biochemical characters as reported elsewhere (Ananda Raja, Panigrahi, De & Kumar, 2017; Ananda Raja et al., 2017b). An 18-hr shake culture was centrifuged at 4800 × *g* for 15 min. The cell pellet was suspended with 0.1 M phosphate buffer (pH 6.4) to adjust the OD value of 0.3 at 570 nm. The bacterial suspension (3 ml) was mixed with 50 μl of plasma in tubes in ice water (0°C), and the OD at 570 nm (A_o) was measured. The tubes were then transferred to a water bath at 37°C for 30 min, then returned to ice water for 10 min to stop the reaction and the OD at 570 nm (A) was measured again.

The antibacterial activity was calculated as follows:

$$U = \frac{A_o - A}{A}$$

2.6.5 | Phagocytic activity assay

Phagocytic activity was measured following the method described by Li et al. (2008). A suspension containing 10^7 cells/ml of yeast (*Saccharomyces cerevisiae*) was prepared in phosphate salt buffer (pH 7.0), and 0.1 ml of this solution was added to the well of a sterile microplate containing 0.1 ml of haemolymph. The haemocyte yeast suspension was gently mixed, then kept at 25°C in a water bath for 30 min and shaken once in 10 min. Following incubation, 50 μl was placed on three glass slides from which smears were made. After air-dried smears were fixed with methanol for 3 min, slides were washed with anticoagulant solution (580 mM NaCl, 13 mM $CaCl_2.6H_2O$, 0.54 mM $Na_2HPO_4.12H_2O$, 12.6 mM KCl, 28 mM $MgCl_2.6H_2O$, 50 mM Tris, pH 7.3) to remove nonadherent haemocytes and air-dried and then stained with Giemsa stain (HiMedia, Mumbai) for 5 min. The numbers of phagocytosis and unphagocytosis haemocytes were counted under the microscope. Phagocytosis ratio was calculated by the formula:

$$\text{Percentage phagocytosis} = \frac{\text{number of cells ingesting yeasts}}{\text{number of cells observed}} \times 100$$

2.6.6 | Haemolymph microbial count

At the end of the experiment, total plate count (TPC) and total *Vibrio* count (TVC) in haemolymph were estimated by serial dilution spread

plate method using sterile TSA and TCBS agar plates respectively (Ananda Raja et al., 2017a).

2.7 | Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) to test the significance among the treatments. One-way analysis of variance, followed by Duncan's multiple range test (Duncan, 1955) was applied to find out the significant difference between the treatments, using IBM SPSS, Version 20 for Windows (IBM SPSS Inc., Chicago, IL, USA).

3 | RESULTS

The proximate compositions of three formulated diets are presented in the Table 1. Diets were isonitrogenous (35% crude proteins). Crude fibre, lipid and ash contents were within a close range of 2.52–2.74, 5.19–5.28 and 12.07–12.78 per cent, respectively and did not differ significantly ($p > .05$).For preparation of diet D3, fermentation condition was optimized and it was found that after 48 hr of incubation at pH of 5.81, crude protein content, free glucose concentration and microbial count was maximum (21.72×10^7 CFU/g) in the feed and dry matter loss was less (Figure 1). In addition, crude fibre content was reduced as fermentation progressed (Figure 1). Therefore, it was concluded that 48 hr of incubation is optimum in regard to nutrient enrichment of feed.

The shrimps of group I, II and III were fed with D1, D2 and D3 respectively. Total weight gain (3.79 g) and PER were significantly ($p < .01$) higher and FCR (1.64) was significantly ($p < .01$) lower in group II as compared to that of group I and III (Table 3). Dry matter (DM) intake (g/d) was significantly higher ($p < .01$) in group III followed by group I and II. Survival (%) was significantly higher ($p < .05$) in group II and III as compared to that of group I. Dry matter, organic matter, hemicelluloses, cellulose and lipid digestibilities were significantly ($p < .01$) higher in group II than that of group III and I (Table 4). Again hemicellulose and cellulose digestibilities were significantly ($p < .01$) higher in group III than that of group I. Protein digestibility was also significantly ($p < .01$) higher in group III

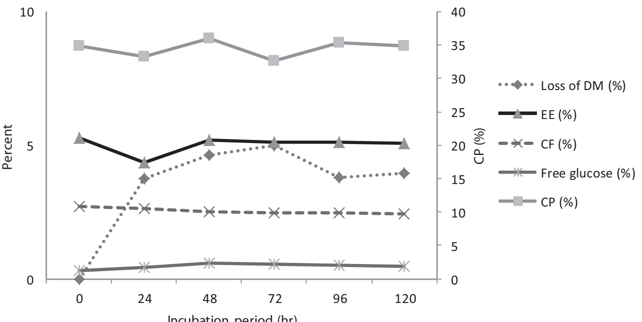

FIGURE 1 Change in nutrient content of diet after different hours of fermentation with *Bacillus sp.* DDKRC1.

followed by group II and I. Cellulase activity in gut was significantly ($p < .01$) higher in group II and III as compared to that of group I (control) (Table 4). Gut amylase and protease activity were significantly ($p < .01$) higher in group II as compared to that of other two groups. Total heterotrophic count, amylolytic, cellulolytic and proteolytic bacterial count in the GI tract were significantly ($p < .01$) higher in group II as compared to that of other two groups (Figure 2). In tank water total heterotrophic count was higher ($p < .01$) in group II followed by III and I, whereas, *Vibrio* count was significantly lower ($p < .01$) in tank water of group II as compared to that of group I and III (Table 2).

Carcass composition of shrimps at the end of the experiment (Table 5) revealed that body protein and lipid content of group II animals were significantly ($p < .01$) higher than that of other two groups, whereas fibre content was higher in group I animals.

Total haemocyte count was significantly ($p < .05$) higher in groups II and III as compared to group I (Table 6). GH count was significantly ($p < .05$) higher in group II as compared to group I but no significant difference ($p > .05$) was observed between group II and III, and group I and III. NGH count and serum PO activity were significantly ($p < .05$) higher in group III as compared to other groups. NGH count and serum PO activity of group II was significantly ($p < .05$) higher as compared to group I. Antibacterial activity and phagocytic activity were significantly ($p < .05$) higher in group II followed by group III and I. Total microbial count in haemolymph was significantly ($p < .05$) lower in group II and III than that of control and total *Vibrio* count in haemolymph did not differ significantly ($p > .05$) among the groups.

TABLE 3 Growth performance of *P. monodon*-fed experimental diets

	Treatment groups		
Parameters	Group I	Group II	Group III
Initial body wt. (g)	2.73 ± 0.003	2.73 ± 0.01	2.73 ± 0.04
Final body wt. (g)**	6.10 ± 0.01^a	6.52 ± 0.03^c	6.33 ± 0.02^b
Total wt. gain (g)**	3.37 ± 0.02^a	3.79 ± 0.02^c	3.60 ± 0.04^b
ADG (mg/d)**	80.15 ± 0.48^a	90.23 ± 0.63^c	85.71 ± 1.03^b
Weight gain (%)**	123.17 ± 0.88^a	138.48 ± 0.79^b	131.96 ± 3.46^b
DM intake (g/d)**	0.1517 ± 0.00^b	0.1486 ± 0.00^a	0.1536 ± 0.00^c
FCR **	1.89 ± 0.00^c	1.64 ± 0.00^a	1.78 ± 0.02^b
SGR (%)	1.91 ± 0.008	1.99 ± 0.07	2.00 ± 0.03
PER**	1.47 ± 0.00^a	1.75 ± 0.01^c	1.60 ± 0.01^b
Survival (%)*	70.83 ± 4.16^a	87.50 ± 0.00^b	83.33 ± 4.16^b

Group I: fed diet D1, Group II: fed diet D2 and Group III: fed diet D3. Values bearing different superscript letters (a,b,c) in a row differ significantly.
*$p < .05$.
**$p < .01$.

TABLE 4 Apparent nutrient digestibility and digestive enzyme status of *P. monodon*-fed experimental diets

Digestibility (%)	Treatment groups Group I	Group II	Group III
Digestibility (%)			
Dry matter**	85.01 ± 0.10[a]	87.37 ± 0.20[b]	85.41 ± 0.20[a]
Organic matter**	87.69 ± 0.08[a]	90.05 ± 0.16[b]	87.39 ± 0.17[a]
Hemicellulose**	87.25 ± 0.80[a]	90.03 ± 0.10[c]	88.54 ± 0.08[b]
Cellulose**	69.79 ± 0.63[a]	77.27 ± 0.08[c]	75.54 ± 0.37[b]
Crude protein **	88.27 ± 0.08[a]	90.62 ± 0.15[b]	92.52 ± 0.10[c]
Lipid**	91.94 ± 0.05[a]	95.81 ± 0.06[b]	92.19 ± 0.10[a]
Gut enzyme activity			
Specific Amylase activity**x	85.87 ± 0.12[a]	93.48 ± 0.69[b]	86.40 ± 0.09[a]
Specific Cellulase activity**y	38.52 ± 0.17[a]	40.68 ± 0.74[b]	38.92 ± 0.24[ab]
Specific Protease activity**z	3.73 ± 0.15[a]	4.98 ± 0.15[b]	3.99 ± 0.08[a]

Group I: fed diet D1, Group II: fed diet D2 and Group III: fed diet D3.
Values bearing different superscript in a row differ significantly (a,b,c).
x = µg of maltose liberated/mg of protein/min.
y = µg of D-glucose liberated/mg of protein/min.
z = µg of L-tyrosine liberated/mg of protein/min.
**$p < .01$.

4 | DISCUSSION

In the present study shrimps fed with live *Bacillus* sp. DDKRC1. (JN641289) were found to have increased growth and survival than that of shrimp without microbial supplementation. The increased survival of shrimp might be due to a bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host (Verschuere et al., 2000). *Bacillus* used as a probiotic was able to colonize both in the tank water and shrimp digestive tract because they

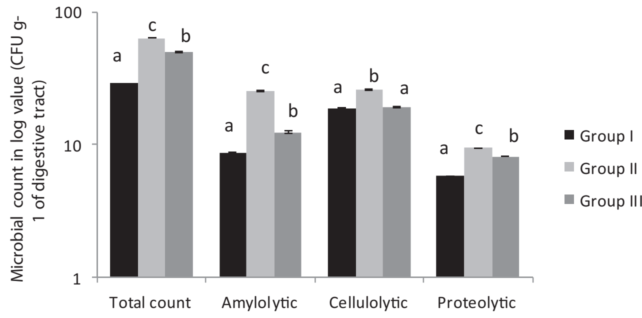

FIGURE 2 Microbial count in digestive tract of *P. monodon* fed experimental diets for 42 days. Mean values of different groups are significantly different ($p < .01$)

TABLE 5 Carcass composition (% of dry weight) of *P. monodon*-fed experimental diets

Parameter	Treatment groups Group I	Group II	Group III
Crude protein**	62.19 ± 0.09[a]	65.61 ± 0.14[b]	62.27 ± 0.06[a]
Crude fibre**	9.62 ± 0.06[b]	9.28 ± 0.07[a]	9.44 ± 0.21[a]
Ether extract**	2.80 ± 0.06[a]	3.39 ± 0.04[c]	3.06 ± 0.04[b]
Ash**	16.39 ± 0.11[b]	16.14 ± 0.05[b]	14.43 ± 0.07[a]

Group I: fed diet D1, Group II: fed diet D2 and Group III: fed diet D3.
Values bearing different superscript letters (a,b) in a row differ significantly.
**$p < .01$.

TABLE 6 Haematological parameters of *P. monodon*-fed experimental diets

Parameter	Treatment groups Group I	Group II	Group III
THC/ml of haemolymph ($\times 10^6$)*	13.90 ± 0.06[a]	17.40 ± 0.35[b]	17.90 ± 0.17[b]
GH count/ml of haemolymph ($\times 10^6$)*	2.64 ± 0.09[a]	4.28 ± 0.29[b]	2.96 ± 0.18[ab]
NGH count/ml of haemolymph ($\times 10^6$)*	11.26 ± 0.03[a]	13.13 ± 0.06[b]	14.94 ± 0.01[c]
Serum PO activity U/min./mg protein	0.29 ± 0.01[a]	0.53 ± 0.01[b]	0.66 ± 0.03[c]
Antibacterial activity	0.06 ± 0.00[a]	0.14 ± 0.00[c]	0.11 ± 0.00[b]
Phagocytic activity (%)	17.75 ± 0.43[a]	25.75 ± 0.14[c]	23.75 ± 0.14[b]
Total microbial count in haemolymph (cfu/ml $\times 10^3$)*	2.11 ± 0.00[b]	1.52 ± 0.03[a]	1.57 ± 0.02[a]
Total *Vibrio* count in haemolymph (cfu/ml)	<30	<30	<30

Values bearing different superscript letters (a,b,c) in a row differ significantly.
*$p < .05$.

have a higher multiplication rate than the rate of expulsion. As a bacterial supplement was constantly added to the experimental tank water through feed, they adhered to the intestinal mucosa of shrimp, multiplied and exercised their multiple benefits (Balcázar et al., 2006) and were also able to replace *Vibrio* spp in the gut of shrimp, thereby increasing shrimp survival (Rengpipat, Phianphak, Piyatiratitivorakul & Menasveta, 1998). Furthermore, the bacterial supplemented group had significantly higher ($p < .01$) PER, improved nutrient digestibility and significantly lower ($p < .01$) FCR compared with control. Improved survival and growth were reported after supplementation of probiotics in different shrimp and fish species like Indian white shrimp, *Fenneropenaeus indicus* (Ziaei-Nejad et al., 2006), American white shrimp, *Litopenaeus vannamei* (Wang, 2007), Asian seabass (De et al., 2015), common carp (Wang & Xu, 2006), rohu (Ghosh, Sen & Ray, 2003), Japanese flounder (Taoka et al., 2006), gilthead seabream (Suzer et al., 2008) and Pursean sturgeon and beluga (Askarian, Kousha, Salma & Ringø, 2011). However, Shariff, Yosoff, Vevaraja and Srinivasa Rao (2001) found that treatment of commercial *Bacillus* probiotics in *L. vannamei* did not significantly increase either the survival or the growth. In the present study total microbial, cellulollytic, amylolytic and proteolytic bacterial count in the gut were higher in live *Bacillus* sp. DDKRC1.-supplemented shrimp (group II) as compared to shrimp fed control diet and fermented diet (group III). Increased beneficial bacterial population in gut of the bacterial supplemented group leads to increased digestive enzyme (amylase and protease) and degradation enzyme (cellulase) activity due to extracellular secretion in the intestinal tract, better nutrient digestibility and nutrient absorption (Al-Dohail, Hashim & Aliyu-Paiko, 2009; De et al., 2015), which in turn contributed to the improved weight gain and improved FCR ($p < .01$) in *P. monodon*. Nutrient digestibility of shrimp fed with live *Bacillus* sp. DDKRC1.-supplemented feed or feed fermented with *Bacillus* sp. DDKRC1. was higher as because the extracellular enzyme produced by the bacteria complement the digestive enzyme of shrimp (Ochoa-Solano & Olmos-Soto, 2006) and in case of fermented feed, during fermentation bacteria could produce enzymes which could help to reduce cellulose level and facilitated digestion of feed (De et al., 2014). Apart from digestive enzymes, supplementation of cellulolytic bacteria provides additional nutrients such as vitamins, essential amino acid and fatty acids (Ray, Ghosh & Ringø, 2012). Overall, increased digestive enzyme activities and availability of additional nutrients from microbes may have enhanced the nutrient digestibility and growth performance in microbial supplemented groups. The bacteria could also have improved digestive activity via synthesis of vitamins and cofactors or via enzymatic improvement (Gatesoupe, 1999). Also, the probiotic enzymes have a broader pH range than the shrimp enzymes, which would prolong the digestion period. The increased population of these favourable cellulolytic, amylolytic and proteolytic bacteria in the gut, apart from secreting the digestive enzymes and essential nutrients, colonize within the gut and so prevent the colonization by pathogenic microbes (Vine, Leukes & Kaiser, 2006). This may have caused the better survival in microbe supplemented groups (De et al., 2015).

Supplementation of *Bacillus* to the diet of shrimp in the present study improved the survival by activating cellular immune defences of shrimp, which was manifested by increased THC, GH and NGH count. The higher ($p < .05$) serum PO activity, antibacterial activity and phagocytic activity in *Bacillus* sp. DDKRC1.-supplemented shrimp indicated their higher immune response as compared to the group without bacterial supplement. Increased immunity and disease resistance of the black Tiger shrimp, *P. monodon* was reported against *Vibrio harveyi* after supplementation of *Bacillus* S11 probiont with the feed (Rengpipat et al., 2000). Gullian, Thompson and Rogriguez (2004) also reported better growth and improved disease resistance in *P. vannamei* immersed in a suspension of *Bacillus* strain P64 in cultured water. The colonization of the shrimp gut by *Bacillus* sp. presumably protected *P. monodon* against pathogenic bacterial infection through competitive exclusion (Rengpipat et al., 2000). *Vibrio* is dominant in the gut and aquatic environment of crustaceans (Moriarty, 1990) and is also reported as a major bacterial pathogen in the brackishwater fish and shrimp (Gatesoupe, 1999). In the present study, *Vibrio* count of water in bacteria supplemented group was significantly lower ($p < .01$).Though count of supplemented cellulolytic bacteria was not measured in tank water of bacteria-supplemented groups but it seems that reduced number of *Vibrio* resulted from the competitive exclusion by bacteria fed to shrimp (De et al., 2015).

Carcass composition of experimental shrimp revealed that the protein and lipid content was significantly ($p < .01$) higher in the bacterial-supplemented group compared with the control. This might be due to higher conversion of feed nutrients to the carcass nutrient as reflected by lower FCR, better protein digestibility and protein efficiency ratio (De, Ghoshal & Kundu, 2012; De, Ghoshal, Kundu & Ali, 2011; De et al., 2015).

In the present study, it was clear that supplementation of live *Bacillus* sp. DDKRC1. in feed augmented the growth and feed utilization in shrimp. Moreover, improved performance regarding growth and FCR was also observed when the feed was fermented with *Bacillus* sp. DDKRC1. for 48 hr and fed to shrimp.

5 | CONCLUSION

Supplementation of *Bacillus* sp. DDKRC1. (JN641289), with plant protein-based diet and fermentation of diet with the same bacterium showed better effect on growth, digestibility, FCR, survival and immune response of *P. monodon* as compared to diet without bacterial supplement. Diet supplemented with live *Bacillus* sp. DDKRC1. had a better effect than diet fermented with the same bacteria on FCR, PER and gut digestive enzyme activity of *P. monodon.* The findings of this study have practical significance towards the development of feed probiotic for brackishwater aquaculture. However, our finding should be confirmed through pond trials before they are applied commercially. There is a scope for refinement with regard to dose and form of supplementation and working out the economic return.

ACKNOWLEDGMENTS

The authors are grateful to Department of Biotechnology, Ministry of Science & Technology, Government of India for financial support. The authors are also grateful to the Director, ICAR-CIBA for providing required facilities to conduct the experiment. Support and help received from the staff of the Kakdwip Research Centre of CIBA are duly acknowledged.

ORCID

Debasis De http://orcid.org/0000-0001-6062-2651
Ramalingam Ananda Raja http://orcid.org/0000-0001-9006-9260

REFERENCES

Al-Dohail, M. A., Hashim, R., & Aliyu-Paiko, M. (2009). Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin. *Aquaculture Research*, *40*, 1642–1652. https://doi.org/10.1111/j.1365-2109.2009.02265.x

Ananda Raja R., Panigrahi, A., De, D., & Kumar, S. (2017). Investigation on white spot disease outbreak in *Penaeus monodon* (Fabricius, 1798) in association with *Vibrio mimicus* infection in the Sunderbans, West Bengal, India. *Indian Journal of Fisheries*, *64*, 56–60.

Ananda Raja, R., Kumar, S., Sundaray, J. K., De, D., Biswas, G., & Ghoshal, T. K. (2012). Hematological parameters in relation to sex, morphometric characters and incidence of white spot syndrome virus in tiger shrimp *Penaeus monodon* Fabricius, 1798 from Sunderban, West Bengal. *Indian Journal of Fisheries*, *59*, 169–174.

Ananda Raja, R., Sridhar, R., Balachandran, C., Palanisammi, A., Ramesh, S., & Nagarajan, K. (2017a). Pathogenicity profile of *Vibrio parahaemolyticus* in farmed Pacific white shrimp, *Penaeus vannamei*. *Fish and Shellfish Immunology*, *67*, 368–381. https://doi.org/10.1016/j.fsi.2017.06.020

Ananda Raja, R., Sridhar, R., Balachandran, C., Palanisammi, A., Ramesh, S., & Nagarajan, K. (2017b). Prevalence of *Vibrio* spp. with special reference to *Vibrio parahaemolyticus* in farmed penaeid shrimp *Penaeus vannamei* (Boone, 1931) from selected districts of Tamil Nadu, India. *Indian Journal of Fisheries*, *64*, 122–128.

APHA (1998). *Standard Methods for the analysis of water and wastewater*, 20th ed. VA: American Public Health Association Alexandria.

Askarian, F., Kousha, A., Salma, W., & Ringø, E. (2011). The effect of lactic acid bacteria administration on growth, digestive enzyme activity and gut microbiota in Persian sturgeon (Acipenser persicus) and beluga (Huso huso) fry. *Aquaculture Nutrition*, *17*, 488–497. https://doi.org/10.1111/j.1365-2095.2010.00826.x

Association of Official Analytical Chemists (AOAC) (1995). *Official Methods of Analysis*, 16th ed. Arlington, Virginia: Association of Official analytical chemists.

Balcázar, J. L., de Blas, I., Ruiz-Zarzuela, D. C., Cunningham, D., Vendrell, D., & Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary Microbiology*, *114*, 173–186. https://doi.org/10.1016/j.vetmic.2006.01.009

Balcázar, J. L., de Blas, I., Ruiz-Zarzuela, D. C., Vendrell, D., Calvo, A. C., & Marquez, I. (2007). Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (*Salmo trutta*). *British Journal of Nutrition*, *97*, 522–527. https://doi.org/10.1017/S0007114507432986

Bernfeld, P. (1955). Amylases, alpha and beta. *Methods in Enzymology*, *1*, 149–158. https://doi.org/10.1016/0076-6879(55)01021-5

De, D. (2014). Immunostimulants probiotics and prebiotics. In J. K. Sundaray, S. Munilkumar, R. K. Mohanty, & S. K. Otta (Eds.), *Frontiers in Aquaculture* (pp. 213–222). Delhi: Narendra Publishing House.

De, D., Ghoshal, T. K., & Ananda Raja, R. (2014). Characterization of enzyme producing bacteria isolated from the gut of Asian seabass, Lates calcarifer and milkfish Chanos chanos and their application for nutrient enrichment of feed ingredients. *Aquaculture Research*, *45*, 1573–1580. https://doi.org/10.1111/are.12099

De, D., Ghoshal, T. K., Ananda Raja, R., & Kumar, S. (2015). Growth performance, nutrient digestibility and digestive enzyme activity in Asian seabass, *Lates calcarifer* juveniles fed diets supplemented with cellulolytic and amylolytic gut bacteria isolated from brackishwater fish. *Aquaculture Research*, *46*, 1688–1698. https://doi.org/10.1111/are.12325

De, D., Ghoshal, T. K., & Kundu, J. (2012). Effect of feeding different levels of protein on growth performance, feed utilization and digestive enzyme of grey mullet (Mugil cephalus L). *Animal Nutrition and Feed Technology*, *12*, 179–186.

De, D., Ghoshal, T. K., Kundu, J., & Ali, S. A. (2011). Optimal dietary lipid requirement for grey mullet, Mugil cephalus. *Indian Journal of Animal Nutrition*, *28*, 433–439.

De Silva, S. S., & Anderson, T. A. (1995). *Fish nutrition in aquaculture*. NY: Springer.

Denison, D. A., & Koehn, R. D. (1977). Cellulase activity of Poronia oedipus. *Mycologia*, *69*, 592–603. https://doi.org/10.2307/3758562

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*, 1–42. https://doi.org/10.2307/3001478

Erasmus, J. H., Cook, P. A., & Coyne, V. E. (1997). The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. *Aquaculture*, *155*, 377–386. https://doi.org/10.1016/S0044-8486(97)00112-9

Furukawa, A., & Tsukahara, H. (1966). On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feeds. *Bulletin of the Japanese Society for the Science of Fish*, *32*, 502–506. https://doi.org/10.2331/suisan.32.502

Gatesoupe, F. J. (1999). The use of probiotics in aquaculture. *Aquaculture*, *180*, 147–165. https://doi.org/10.1016/S0044-8486(99)00187-8

Ghosh, K., Sen, S. K., & Ray, A. K. (2003). Supplementation of an isolated fish gut bacterium, Bacillus circulans, in formulated diets for rohu, Labeo rohita, fingerlings. *Israeli Journal of Aquaculture- Bamidgeh*, *55*, 13–21.

Gómez, R., Geovanny, D., & Shen, M. A. (2008). Influence of probiotics on the growth and digestive enzyme activity of white pacific shrimp (*Litopenaeus vannamei*). *Journal of Ocean University of China*, *7*, 215–218. https://doi.org/10.1007/s11802-008-0215-x

Gullian, M., Thompson, F., & Rogriguez, J. (2004). Selection of probiotic bacteria and study of their immunostimulatory effect in Penaeus vannamei. *Aquaculture*, *233*, 1–14. https://doi.org/10.1016/j.aquaculture.2003.09.013

Kesarcodi-Watson, A., Kaspar, H., Lategan, M., & Gibson, L. (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*, 1–14. https://doi.org/10.1016/j.aquaculture.2007.11.019

Krishnaprakash, R., Saravanan, R., Murugesan, P., & Rajagopal, S. (2009). Usefulness of probiotics in the production of high quality shrimp (*Penaeus monodon*) seeds in hatcheries. *World Journal of Zoology*, *4*, 144–147.

Li, J., Tan, B., Mai, K., Ai, Q., Zhang, W., Liufu, Z., & Wei, X. (2008). Immune responses and resistance against vibrio parahaemolyticus induced by probiotic bacterium Arthrobacter XE-7 in pacific white shrimp, *Litopenaeus vannamei*. *Journal of World Aquaculture Society*, *39*, 477–489. https://doi.org/10.1111/j.1749-7345.2008.00188.x

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal Biological Chemistry*, *193*, 265–275.

Lu-Qing, P., Bo, F., Ling-Xu, J., & Jing, L. (2007). The effect of temperature on selected immune parameters of the white shrimp, Litopenaeus vannamei. *Journal of World Aquaculture Society*, *38*, 326–332. https://doi.org/10.1111/j.1749-7345.2007.00105.x

Moriarty, D. J. W. (1990). Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. In R. Lesel (Ed.), *Microbiology in Poecilotherms* (pp. 217–222). Amsterdam: Elsevier.

Moriarty, D. J. W. (1996). Microbial Biotechnology: A key ingredient for sustainable aquaculture. *Infofish International*, *4*, 29–33.

Moriarty, D. J. W. (1998). Control of luminous *Vibrio* species in penaid aquaculture ponds. *Aquaculture*, *164*, 351–358. https://doi.org/10.1016/S0044-8486(98)00199-9

Ochoa-Solano, L. J., & Olmos-Soto, J. (2006). The functional property of *Bacillus* for shrimps feeds. *Food Microbiology*, *23*, 519–525. https://doi.org/10.1016/j.fm.2005.10.004

Rahmatullah, S. M., & Beveridge, M. C. M. (1993). Ingestion of bacteria in suspension by Indian major carps (*Catla catla*, *Labeo rohita*) and Chinese carps (*Hypophthalmichthys molitrix*, *Aristichthys nobilis*). *Hydrobiologia*, *264*, 79–84. https://doi.org/10.1007/BF00014095

Ray, A. K., Ghosh, K., & Ringø, E. (2012). Enzyme-producing bacteria isolated from fish gut: A review. *Aquaculture Nutrition*, *18*, 465–492. https://doi.org/10.1111/j.1365-2095.2012.00943.x

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S., & Menasveta, P. (1998). Effect of probiotics on Black Tiger shrimp, Penaeus monodon, survival and growth. *Aquaculture*, *167*, 301–313. https://doi.org/10.1016/S0044-8486(98)00305-6

Rengpipat, S., Runkpratanporn, S., Piyatiratitivorakul, S., & Menasaveta, P. (2000). Immunity enhancenment on black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). *Aquaculture*, *191*, 271–288. https://doi.org/10.1016/S0044-8486(00)00440-3

Ringø, E., & Gatesoupe, F. J. (1998). Lactic acid bacteria in fish: A review. *Aquaculture*, *160*, 177–203. https://doi.org/10.1016/S0044-8486(97)00299-8

Sahu, M. K., Swarnakumar, N. S., Sivakumar, K., Thangaradjou, T., & Kannan, L. (2008). Probiotics in aquaculture: Importance and future perspectives. *Indian Journal of Microbiology*, *48*, 299–308. https://doi.org/10.1007/s12088-008-0024-3

Sánchez-Ortiz, A. C., Luna-González, A., Campa-Córdova, A. I., Escamilla-Montes, R., Flores-Miranda María del, C., & Mazón-Suástegui, J. M. (2015). Isolation and characterization of potential probiotic bacteria from pustulose ark (*Anadara tuberculosa*) suitable for shrimp farming. *Latin American Journal of Aquatic Research*, *43*, 123–136. https://doi.org/10.3856/vol43-issue1-fulltext-11

Shariff, M., Yosoff, F. M., Vevaraja, T. N., & Srinivasa Rao, S. P. (2001). The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius), ponds. *Aquaculture Research*, *32*, 181–187. https://doi.org/10.1046/j.1365-2109.2001.00543.x

Spyridakis, P., Metailler, R., Gabaudan, J., & Riaza, A. (1989). Studies on nutrient digestibility in European sea bass (*Dicendrarchus labrax*) Methodological aspects concerning faeces collection. *Aquaculture*, *77*, 61–70. https://doi.org/10.1016/0044-8486(89)90021-5

Steffens, W. (1989). *Principles of Fish Nutrition* (p. 384). Chichester: Ellis Horwood.

Suzer, C., Coban, D., Kamaci, H. O., Saka, S., Firat, K., Otgucuoğlu, O., & Küçüksari, H. (2008). Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata, L.) larvae: Effects on growth performance and digestive enzyme activities. *Aquaculture*, *280*, 140–145. https://doi.org/10.1016/j.aquaculture.2008.04.020

Taoka, Y., Maeda, H., Jo, J. Y., Jeon, M. J., Bai, S. C., Lee, W. J., ... Koshio, S. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. *Fisheries Science*, *72*, 310–321. https://doi.org/10.1111/j.1444-2906.2006.01152.x

Van Soest, P. J. (1963). Use of detergents in the analysis of fibrous feeds. I. Preparation of fiber residues of low nitrogen content. *Journal of Association of Official Agricultural Chemists*, *46*, 825–835.

Van Soest, P. J. (1967). Development of a comprehensive system of feed analysis and its appli- cation to forages. *Journal of Animal Science*, *26*, 119–128. https://doi.org/10.2527/jas1967.261119x

Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbial Molecular Biological Review*, *64*, 655–671. https://doi.org/10.1128/MMBR.64.4.655-671.2000

Vine, N. G., Leukes, W. D., & Kaiser, H. (2006). Probiotics in marine larviculture. *FEMS Microbiology Reviews*, *30*, 404–427. https://doi.org/10.1111/j.1574-6976.2006.00017.x

Walter, H. E. (1984). Proteinases: Methods with hemoglobin, casein and azocoll as substrates. *Methods of Enzymatic Analysis*, *5*, 270–277.

Wang, Y.-B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*, *269*, 259–264. https://doi.org/10.1016/j.aquaculture.2007.05.035

Wang, Y. B., & Xu, Z. R. (2006). Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. *Animal Feed Science and Technology*, *127*, 283–292.

Ziaei-Nejad, S. (2004) *The effect of Bacillus spp. bacteria as a probiotic on growth, survival and digestive enzyme activity of Indian white shrimps, Fenneropenaeus indicus, larvae and post-larvae*. M.Sc dissertation. Tehran University, pp. 100.

Ziaei-Nejad, S., Rezaei, M. H., Takami, G. A., Lovett, D. L., Mirvaghefi, A. R., & Shakouri, M. (2006). The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture*, *252*, 516–524. https://doi.org/10.1016/j.aquaculture.2005.07.021

Zorriehzahra Mohammad, J., Delshad, S. T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., & Lazado, C. C. (2016). Probiotics as beneficial microbes in aquaculture: An update on their multiple modes of action: A review. *Veterinary Quarterly*, *36*, 228–241. https://doi.org/10.1080/01652176.2016.1172132

How to cite this article: De D, Ananda Raja R, Ghoshal TK, Mukherjee S, Vijayan KK. Evaluation of growth, feed utilization efficiency and immune parameters in tiger shrimp (*Penaeus monodon*) fed diets supplemented with or diet fermented with gut bacterium *Bacillus* sp. DDKRC1. isolated from gut of Asian seabass (*Lates calcarifer*). *Aquac Res*. 2018;00:1–9. https://doi.org/10.1111/are.13669