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OPTIMIZATION OF EFFECTIVE DOSE OF A NEWLY ISOLATED PROBIOTIC BACTERIA FOR GROWTH AND DISEASE RESISTANCE OF *CLARIAS BATRACHUS* (LINN.)

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ABSTRACT: *Lysinibacillus sphaericus* PKA17, a probiotic bacteria was incorporated with basal feed to *Clarias batrachus* juveniles at different dose levels: control (without probiotic), 2×10^4 (C1), 2×10^5 (C2) and 2×10^6 (C3) probiotic cells per 100 g feed for 60 days. After the feeding trial, the fish were challenged with pathogenic *Vibrio harveyi* and percentage mortalities were enumerated upto 14 days of post challenge. The organism *L. sphaericus* PKA17 was found to produce extracellular digestive enzymes protease, phosphatase, xylanase and cellulase. It didn't show any visible haemolysis on blood agar which establishes its non-pathogenicity. The growth curve of the organism showed prolong stationary phase. Fish fed with C2 feed demonstrated significantly ($P \leq 0.05$) higher average daily growth, specific growth rate, protein efficiency ratio, percentage weight gain and lower feed conversion ratio than control. Highest survival percentage was observed in fish fed with feed C2 compared to control while challenging with *V. harveyi* which indicated the efficiency of the test organism in reducing vibriosis. The findings of this study suggests that the dose level of probiotic *L. sphaericus* PKA17 applied in feed C2 is the minimum effective dose to enhance growth performance and disease-resistance of *C. batrachus* juveniles.

KEYWORDS: *Clarias batrachus, Lysinibacillus sphaericus*, probiotic, dose-optimization.

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1. INTRODUCTION

Aquaculture is the sustainable mode of farming of aquatic organisms that provides nutritional security to a large biomass. Southern Asia, one of the mega biodiversity hotspots, is native to many indigenous freshwater fish species. *Clarias batrachus* (Linn.) is widely recognized in Indian sub-continent as a nutritious food by virtue of its richness in essential amino acids, minerals, trace elements, vitamins and polyunsaturated fatty acids. However, diseases have been posing a major setback to the sustainability of aquaculture. Juvenile fishes are not fully immunocompetent and often succumb to aquatic diseases. The emergence of vibriosis, caused by infectious *Vibrio harveyi, V. vulnificus, V. parahaemolyticus, V. alginolyticus*, is one of the major prevalent sources of mortality in aquaculture sector worldwide [1]. Appropriate prophylactic measures need to be taken to prevent frequent horizontal gene transfer of *Vibrio* pathogens [2]. Probiotic, a biocompatible alternative of antibiotic and chemical additive, is in increased use today to combat aquatic diseases. It confers protection against pathogens by production of bacteriocins, siderophores or lysozymes and stimulates immune responses [3]. Probiotics can be used as a functional feed additive to enhance feed digestibility and fecundity [4]. The present study has been designed to evaluate and optimize dietary doses of *Lysinibacillus sphaericus* PKA17 (Accession No.KM972671.1) as a putative probiotic in *C. batrachus* juveniles against *Vibrio harveyi* infection.

2. MATERIALS AND METHODS

Small-scale Study

C. batrachus juveniles obtained from a hatchery at Ramsagar (District of Bankura, West Bengal, India) were transferred to the aquaculture unit; Department of Microbiology (Vidyasagar University, India). The fish were acclimatized for two weeks at prevailing laboratory condition and then randomly separated in rectangular cemented tanks. Each tank (195 × 105 × 90 cm^3; 5 cm bottom mud) was stocked with 15 fish (mean weight 13.52 ± 0.05 g) and were reared under natural photoperiod. The tanks were connected to the flow-through water system with continuous aeration. The water quality of all the tanks was monitored at regular intervals [5]. Fifty percent of aged-water was replenished with fresh water at weekly interval. Fish were fed for 60 days at a daily rate of 5% of their body weight [6]. The unutilized feeds were sieved out 1 h after dispensing the feed into tanks.

Feed Preparation

Commercial floating feed (26% Crude protein, 5% Lipid, 5% fiber and 11% moisture) that is free from any probiotic was used as the basal diet. *L. sphaericus* PKA17 was grown in Tryptone Soya broth (TSB) medium (Pancreatic digest of casein 17g, Papaic digest of soyabean meal 3g, Sodium chloride 5g, Dextrose 2.5g, Dibasic potassium phosphate 2.5g; pH 7.3±0.2); centrifuged at 5000 g at 4ºC for 10 min, washed thrice with sterile 1.0% NaCl solution and resuspended in sterile saline water. Four different formulated diets were: (i) non-probiotic supplemented commercial diet

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(control group); (ii) diet supplemented with 2×10^4 *L. sphaericus* PKA17 (C1 feed); (iii) diet supplemented with 2×10^5 *L. sphaericus* PKA17 (C2 feed); (iv) diet supplemented with 2×10^6 *L. sphaericus* PKA17 (C3 feed).

Morphometric Measurement

Dietary performance indexes like live weight gain (LWG), average daily growth (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein intake (PI), percentage weight gain (PWG) and survival rate (SR) were precisely calculated [3].

Pathogen

Catfish pathogen *Vibrio harveyi* (MTCC 7954) was obtained from IMTECH (Chandigarh, India). It was used as target pathogens to detect *in vivo* antagonistic activity of the probiotic isolate. The survival percentage was enumerated taking 10 fish in each tank in triplicates upto 14 days of post challenge. The graphical representation and statistical analysis of data was carried out using Microsoft Office Excel software with 5% standard error.

Qualitative Assay of Enzymes

Protease

The efficacy of *L. sphaericus* PKA17 for production of extracellular protease enzyme was assessed by culturing on Casein Hydrolysate (pH 7.8±0.2) Agar (g^{-L}): Casein enzymic hydrolysate 5, Peptic digest of animal tissue 5, Yeast autolysate 1.5, Na_3PO_4 2.5, NaCl 5, Agar 15.

Cellulase

L. sphaericus PKA17 was streaked on carboxy-methylcellulose (CMC) agar plates (1% CMC, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.04% $MgSO_4 \cdot 7H_2O$, 0.005% NaCl, and 0.000125% $FeSO_4$, 1.8% Agar and pH 7.0) and incubated at 37°C for 24-48 h. The plates were then flooded with Gram's iodine solution for 3 to 5 minutes. The stain was then drained off and the plates were observed for appearance of distinct clear zone around the colony to identify cellulase producer [7].

Amylase

L. sphaericus PKA17 was grown on Starch Agar (g^{-L}) medium (Tryptone 2 g, KH_2PO_4 4 g, Na_2HPO_4 4 g, $MgSO_4 7H_2O$ 0.2 g, $CaCl_2$ 0.001 g, $FeSO_4 7H_2O$ 0.004 g, Starch 10 g; pH 7.0) to detect amylase activity through the appearance of clear zone around the colonies following addition of iodine solutions.

Phosphate Solubility

The Pikovskayas Agar (g^{-L}) medium (Yeast extract 0.500, Dextrose 10, $Ca_3(PO_4)_2$ 5, $(NH_4)_2SO_4$ 0.500, KCl 0.200, $MgSO_4$ 0.100, $MnSO_4$ 0.0001, $FeSO_4$ 0.0001, Agar 15.000) was sterilized and petriplated. *L. sphaericus* PKA17 was inoculated on Pikovskayas agar medium by continuous streaking method and incubated at 37°C for 24-48 h. The plates were then observed for appearance of clear zone around the colony which indicates the solubilization of phosphate and hence, confirms the production of extracellular alkaline phosphatase enzyme [8] by bacterial isolates.

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Xylanase

The composition of isolating medium (IM) was (g/l): $(NH4)_2SO_4$ 1.0; $MgSO_4$ 0.2; K_2HPO_4 0.2; $CaCl_2$ 0.2; $MnCl_2$ 0.02; yeast extract 0.1, xylan 10.0 and agar 20.0; pH 7. Before sterilization of the media, xylan was completely dissolved in water by sonication (7 hz, 2 min) [9]. The test isolates were streaked on respective agar plates and incubated. The plates were then observed for appearance of clear and transparent hollow zones.

Quantitative Assay of Xylanase and Cellulase

The sources of enzymes were the crude supernatant of submerged cultivation where TSB growth media of *L. sphaericus* PKA17 were not enriched with xylan or cellulose. Xylanase activity was assayed by measuring released reducing sugar from birch wood xylan (Fluka) with 3, 5-dinitrosalicylic acid [10]. The reaction mixture containing 0.4 ml phosphate buffer (0.2M, pH 7.0), 0.3ml of 5% (w/v) xylan and 0.3ml enzyme solution. The enzymatic reaction was carried out at 50ºC and after 30 min 1ml of DNS (3%w/v) was added to terminate the reaction. The solution was incubated in a boiling water bath for 15 min for colour development and the absorbency was measured at 540nm (Systronic spectrophotometer 105) against the enzyme blank. The enzyme activity was determined by using a standard calibration curve of D-Xylose (Sigma).Cellulose activity, using carboxymethylcellulose as substrate, was assayed in the same way as xylanase assay by measuring released reducing sugar from cellulose as substrates. The enzyme activity was determined by using a standard calibration curve of dextrose (Sigma).One unit of enzyme (xylanase and cellulose) activity (U/ml) was defined as the amount of enzyme required to produce 1 µmol of reducing sugars (xylose in case of xylanase, dextrose in case of cellulase) by hydrolyzing the respective substrates per minute under the above assay condition.

Haemolysis Test

The medium was prepared by adding 5% v/v sterile defibrinated sheep blood to sterile blood agar base that had been melted and cooled to 45°C. It was then thoroughly mixed avoiding formation of air bubbles. 15-20 ml of this blood agar was dispensed to sterile petriplates and solidified. Fresh overnight grown TSB cultures of the test organism were streaked on blood agar plates and incubated at 37°C for 24-48 h; thereafter, the plates were examined for the formation of any clear zones (β-haemolysis) or greenish hemolytic zone (α-haemolysis), or no such zone (γ-haemolysis) around the bacterial colonies [11]. The assay was repeated with defibrinated fish blood.

Growth Kinetics

The intestinal isolate *L. sphaericus* PKA17 was cultivated in TSB at 37°C with 100 g for 48 h. The viable cell counts were measured by spectrophotometer at 620 nm at regular time interval [12]. The test was done in triplicates.

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to analyze statistical difference among

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replicates. Significant differences (α=0.05) between the means of different groups in replicate were identified by Duncan Multiple Range Test (DMRT) using SPSS program version 19.

3. RESULTS AND DISCUSSION

Growth and Feed Efficiency

The growth parameters of Asian catfish *C. batrachus* juveniles fed with different dietary doses of probiotic are presented in Table 1. The application of probiotic in feed significantly increased growth (Fig.1) and feed utilization efficiency.

Table 1: Growth performance of *Clarias batrachus* juveniles fed with different diets

Parameters	Control	C1	C2	C3
Live weight gain (g)	11.88 ± 0.07^{c}	15.81 ± 0.24^{b}	17.02 ± 0.14^{a}	16.28 ± 0.20^{b}
Average daily growth (g)	0.20 ± 0^{c}	0.26 ± 0^{b}	0.28 ± 0^{a}	$0.27 \pm 0^{a,b}$
Specific growth rate (%)	1.02 ± 0.01^{c}	1.29 ± 0.01^{b}	1.35 ± 0.02^{a}	1.31 ± 0.02^{b}
Feed conversion ratio	2.90 ± 0.01^{a}	2.17 ± 0.02^{b}	2.07 ± 0.05^{b}	2.16 ± 0.06^{b}
Protein efficiency ratio	1.44 ± 0.02^{c}	1.86 ± 0.02^{b}	1.96 ± 0.03^{a}	1.88 ± 0.05^{b}
Percentage weight gain (%)	87.13 ± 0.91^{c}	118.23 ± 1.85^{b}	126.8 ± 2.41^{a}	121.25 ± 2.51^{b}
Protein intake (g)	8.21 ± 0.09^{c}	8.53 ± 0.06^{b}	8.67 ± 0.10^{a}	8.65 ± 0.12^{a}

Results are given as (Means ± SEM); Values in the same row with different superscripts denote a significant difference ($P \leq 0.05$); Mean initial length and weight were (9.32 ± 0.09) cm and (13.52 ± 0.05) g respectively; Survival Rate (%) was 100 % in all cases.

Fish feed C2 has displayed significantly ($P \leq 0.05$) higher LWG (17.02 ± 0.14), ADG (0.28 ± 0) and SGR (1.35 ± 0.02%) than control and other dose-treatments. FCR was significantly ($P \leq 0.05$) lower in all probiotic-fed fish where C2 (2.07 ± 0.05) has displayed maximum feed utilization. Significantly ($P \leq 0.05$) higher PI (8.67 ± 0.10g) and PER (1.96 ± 0.03) was obtained in fish fed with C2 indicating better utilization of protein for growth and metabolism. PWG (126.8 ± 2.41 %) was significantly ($P \leq 0.05$) higher in fish fed with C2 followed by C3 and C1 whereas the fish fed with control diet displayed lowest PWG (87.13 ± 0.91). The survival rate was (100 ± 0.00%) in all cases.

Challenge Test

After 14-days challenge trial, significantly higher survivability (90%) was observed in fish with C2 feed followed by C3, C1 and control (Fig.2). The cumulative mortality in the treatment groups was significantly ($P \leq 0.05$) lower compared to the control.

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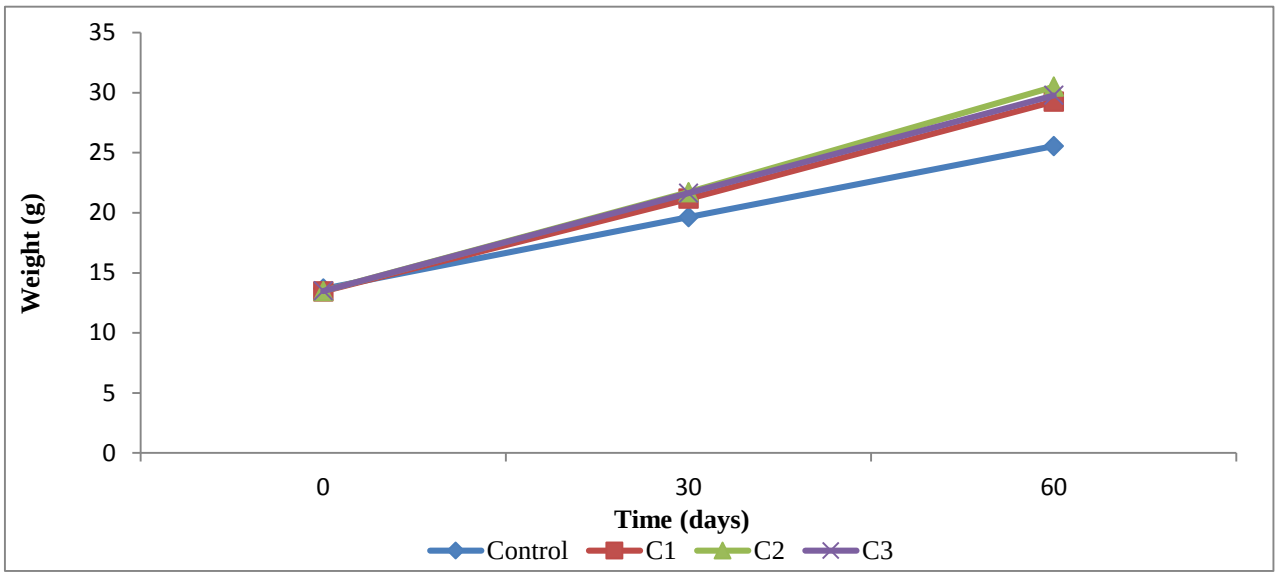

Fig 1. Weight of *Clarias batrachus* in relation to various feeds at different time interval

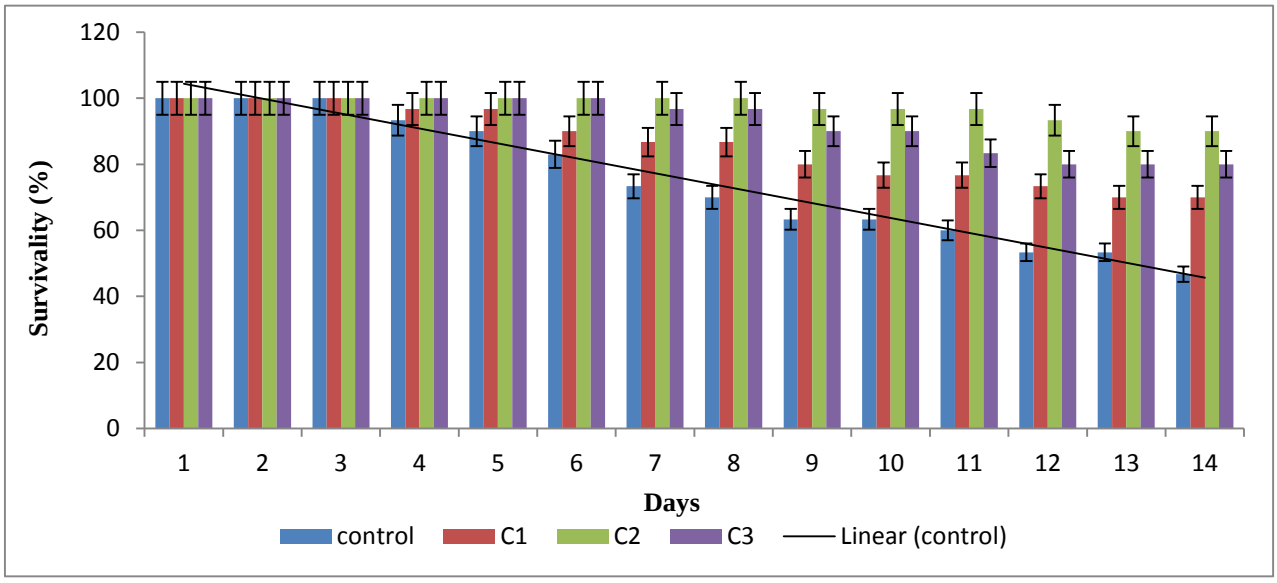

Fig 2.Survivability of *Clarias batrachus* in a 14-day challenge trial against *Vibrio harveyi*

Biochemical Tests

The present study showed the production of extracellular protease, cellulase, xylanase and phosphatase enzyme by *L. sphaericus* PKA17 strain through the appearance of distinct clear zone around the respective colonies (Table 2). However, the organism didn't exhibit amylolytic activity.

Table 2. Production of extracellular enzymes

	Protease	**Phosphatase**	**Amylase**	**Xylanase**	**Cellulase**
***L. sphaericus* PKA17**	+	+	−	+	+

(+) = Positive Reaction; (−) = Negative Reaction

The intensity of extracellular enzyme production by the bacterial strain *L. sphaericus* PKA17 was assayed both quantitavely and qualitatively. The bacterial strain *L. sphaericus* PKA17 is able to produce a very small amount of xylanase and cellulase (Table 3). The production of phosphatase was distinctly visible on Pikovskayas agar medium (Fig.3).

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Table 3. Enzymatic assay of xylanase and cellulase

Bacterial strain	Enzyme activity (U/ml)	
***L. sphaericus* PKA17**	Xylanase	Cellulase
	0.3	0.5
	0.4	0.52
	0.4	0.51
Mean ± SE	**0.37 ± 0.03**	**0.51 ± 0.01**

SE = Standard Error

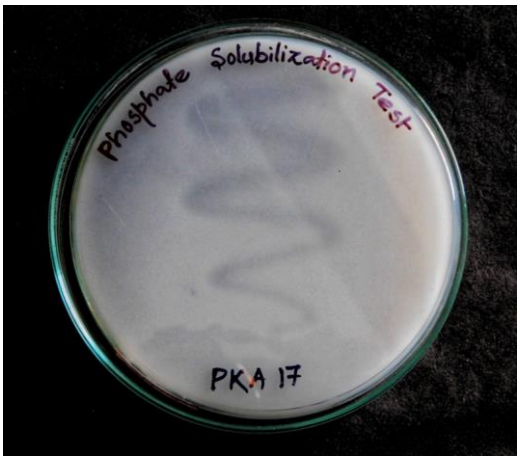

Fig 3. Phosphate solubilization test of *Lysinibacillus sphaericus* PKA17

Test of Haemolysis

The test isolate didn't exhibit any sign of haemolysis on blood agar irrespective of blood type (sheep, fish).

Growth Kinetics

The growth curve of a probiotic strain determines the most suitable time to harvest the cell. The growth kinetic study revealed that *L. sphaericus* PKA17 had prolonged stationary phase (Fig.4).

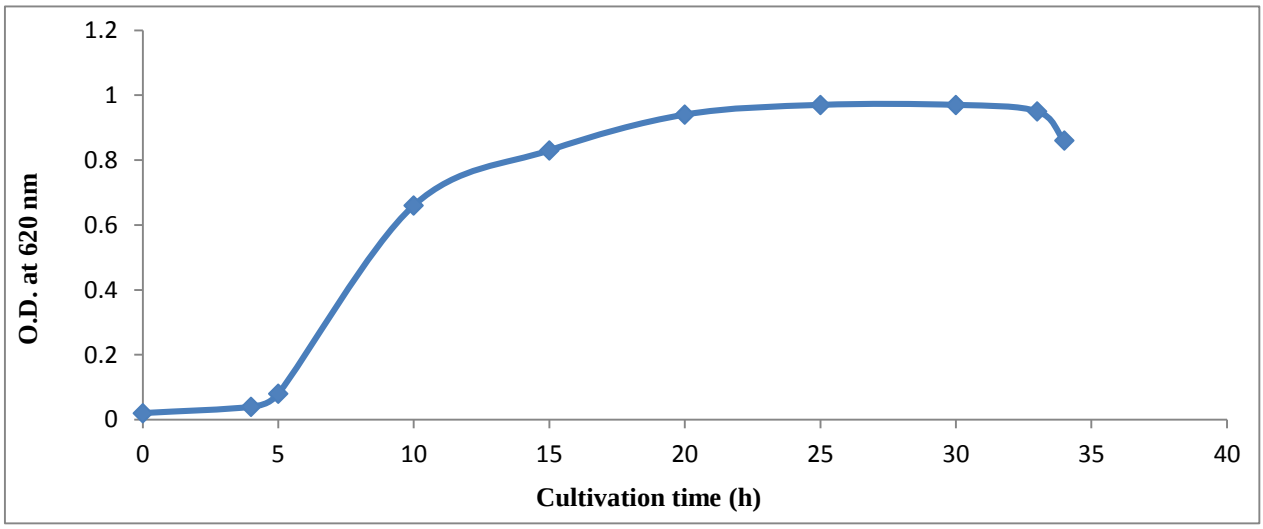

Fig 4.Effect of cultivation period on growth of *Lysinibacillus sphaericus* PKA17 in tryptone soya broth under shaking (100 rpm) condition

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DISCUSSION

The production of extracellular enzymes (protease, amylase, phosphatase, esterase, lipase, peptidase, cellulase etc.) by gastro-intestinal bacteria often contributes to digestive enzyme activity and subsequent feed utilization efficiency in fish [13]. *L. sphaericus* PKA17 strain secreted hydrolytic enzyme protease that formed clear zones around the colonies as a result of casein hydrolysis. Esakkiraj et al. [14] isolated protease-producing bacterial strain *Bacillus cereus* from brackish water fish *Mugil cephalus*. Cellulase activity may enable the fish species to utilize cellulose and similar fibrous carbohydrates [15]. In the present study, *L. sphaericus* PKA17, isolated from the intestine of *Clarias batrachus* has been shown to possess cellulolytic activity. The result was in accordance to Banerjee et al. [16] who reported high cellulase activity of the intestinal bacterial isolates of *C. batrachus.* Kar and Ghosh [17] reported the presence of cellulolytic bacteria in *Channa punctatus*. Xylan and cellulose are comparatively large molecules, and cannot enter the bacterial cell matrix. Hydrolysis of these molecules is done outside the cell by the constitutively produced xylanases [18] or cellulases [19]. The very little amount of constitutively xylanases [9] and cellulases hydrolyze the larger molecules into small oligosaccharides. These hydrolyzed products can enter directly into the cell matrix where they are degraded by intercellular respective enzymes. There are also reports that the small oligosaccharides induce the production of respective enzymes when particular substrates are present [20]. Thus the probiotic strains may help in digestion of the xylan (hemicelluloses) and cellulose containing feeds. Amylolytic activity was not detected in the intestinal bacterial strain *L. sphaericus* PKA17. Similarly, Bairagi et al. [19] reported absence of amylolytic bacterial strains in *Clarias batrachus*. Banerjee et al., [16] also observed trace amount of amylase in the gastrointestinal tract of *C. batrachus*. The solubilization of phosphate by test organisms confirms the production of extracellular alkaline phosphatase enzyme by bacterial isolates [21]. This phosphate solubilization property may consequence better feed utilization as well as accessibility of more phosphate to the host. Blood agar is useful to derive the hemolytic activity of an organism. Bacteria often excrete exoenzymes that lyse red blood cells and degrade hemoglobin. The degree to which the blood cells are haemolyzed is used to determine the pathogenicity of test organism. None of the test isolates exhibited any sign of haemolysis on blood agar irrespective of blood type (sheep, fish) which signifies non-pathogenicity of the test organism. Bacterial growth kinetics specifically determines *in vitro* proliferative activity of the putative probiotics which is often correlated to their colonizing ability in the GI tract of the host [22]. The prolonged stationary phase of *L. sphaericus* PKA17 has added advantage as it maintains constant number of viable bacterial cell throughout the phase. The study elucidated effect of *L. sphaericus* PKA17 on the growth performance, feed utilization efficiency and disease-resistance of *C. batrachus* juveniles. The findings of this study suggested faster growth of *C. batrachus* could be obtained through infusion of *L. sphaericus* PKA17 in basal diet at different dose level. The

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extracellular enzyme protease synthesized by *L. sphaericus* PKA17 strain may have contributed increased protein efficiency ratio [23]. The decrease of FCR also indicated proper dietary utilization of nutrients. The result corroborates with Falaye et al., [24] who reported improved growth rate, weight gain and FCR of *C. gariepinus* fingerlings using *Lactobacillus plantarum* supplemented diet. The probiotic *Bacillus* was reported to increase growth rate, PER and protein productive value of *C. gariepinus* [25]. The *in vivo* challenge with pathogenic *V. harveyi* revealed that, dietary administration of *L. sphaericus* PKA17 (C2 feed) for 14 days significantly increased survival rate of *C. batrachus* juveniles. The earlier studies have reported dietary administration of *L. plantarum* for 6 weeks in *Litopenaeus vannamei* resisted Vibriosis [26]. *Lysinibacillus sphaericus*, *Bacillus amyloliquefaciens* and *Bacillus cereus* have been reported to inhibit motile aeromonad septicaemia (MAS) in *C. gariepinus* by disintegrating quorum sensing pathway of pathogenic *Aeromonas hydrophila* [27]. The Biosurfactant produced by *Lysinibacillus fusiformis* S9 inhibited bacterial attachment and biofilm formation of pathogenic *Escherichia coli* and *Streptococcus mutans* [28].

4. CONCLUSION

The findings of this study suggest that the dose level of probiotic *L. sphaericus* PKA17 applied in feed C2 was able to enhance growth performance and disease-resistance of *C. batrachus* juveniles. The study provides a new perspective for the use of *L. sphaericus* PKA17 as a functional feed additive to enhance feed digestibility, growth performance and disease-resistance in *C. batrachus* juveniles. It will be helpful to the fish farmers to cultivate the indigenous cat fish to a large extent. However, pilot-scale study is still needed before commercialization of the process.

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CONFLICT OF INTEREST

There is no conflict of interest declared.

REFERENCES

1. Chatterjee S, Haldar S. *Vibrio* Related Diseases in Aquaculture and Development of Rapid and Accurate Identification Methods. J Marine Sci Res Dev. 2012; S:1
2. Osunla CA, Okoh AI. *Vibrio* Pathogens: A Public Health Concern in Rural Water Resources in Sub-Saharan Africa. Int J Environ Res Public Health. 2017; 14(10): 1188.
3. Bandyopadhyay P, Das Mohapatra PK. Effect of Probiotic Bacterium *Bacillus circulans* PB7 in the Formulated Diets: on Growth, Nutritional Quality and Immunity of *Catla catla* (Ham.). Fish Physiol Biochem. 2009; 35:467-478.
4. Hoseinifar SH, Dadar M, Ringo E. Modulation of Nutrient Digestibility and Digestive Enzyme Activities in Aquatic Animals: The Functional Feed Additives Scenario. Aquac Res. 2017; 48: 1–14.

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5. APHA-AWWA-WPCF Standard Methods for the Examination of Water and Wastewater, 20th edition. Washington DC, 1998.
6. Fagbenro O, Jauncey K, Haylor G. Nutritive Value of Diet Containing Dried Lactic Acid Fermented Fish Silage and Soybean Meal for Juvenile *Oreochromis niloticus* and *Clarias gariepinus*. Aquat Living Resour. 1994; 7(2):79-85.
7. Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. Curr Microbiol. 2008; 57:503–507.
8. Behera BC, Yadav H, Singh SK, Mishra RR, Sethi BK, Dutta SK, Thatoi HN. Phosphate Solubilization and Acid Phosphatase Activity of *Serratia* sp. Isolated from Mangrove Soil of Mahanadi River Delta, Odisha, India. J Gen Eng Biotech. 2017; 15:169-178.
9. Mandal A, Kar S, Das Mohapatra PK, Maity C, Pati BR, Mondal KC. Regulation of Xylanase Biosynthesis in *Bacillus cereus* BSA1. Appl Biochem Biotechnol. 2012; 167(5):1052-1060.
10. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal Chem. 1959; 31:426-428.
11. Forbes B, Sahm DF, Weissfeld AS. Bailey & Scott's Diagnostic Microbiology, 11th ed. Mosby, St. Louis, MO. ISBN 0-323-01678-2, 2002.
12. Prosser JI, Tough AJ. Growth Mechanisms and Growth Kinetics of Filamentous Microorganisms. Crit Rev Biotechnol. 2008; 10(4):253-274.
13. Ramirez RF, Dixon BA. Enzyme production by Obligate Intestinal Anaerobic Bacteria Isolated from Oscars *(Astronotus ocellatus*), Angelfish *(Pterophyllum scalare*) and Southern Flounder *(Paralichthys lethostigma*). Aquaculture. 2003; 227:417-426.
14. Esakkiraj P, Immanuel G, Sowmya SM, Iyapparaj P, Palavesam A. Evaluation of Protease-Producing Ability of Fish Gut Isolate *Bacillus cereus*. Food Bioprocess Technol. 2009; 2:383–390.
15. Chakrabarti I, Gani MA, Chaki KK, Sur R, Misra KK. Digestive Enzymes in 11 Freshwater TeleostFish Species in Relation to Food Habit and Niche Segregation. Comp Biochem Physiol Part A Mol Integr Physiol. 1995; 112A:167–177.
16. Banerjee G, Dan SK, Nandi A, Ghosh P, Ray AK. Autochthonous Gut Bacteria in Two Indian Air-Breathing Fish, Climbing Perch *(Anabas testudineus*) and Walking Catfish *(Clarias batrachus*): Mode of Association, Identification and Enzyme Producing Ability. Pol J Microbiol. 2015; 64(4):361–368.
17. Kar N, Ghosh K. Enzyme Producing Bacteria in the Gastrointestinal Tracts of *Labeo rohita* (Hamilton) and *Channa punctatus* (Bloch). Turk J Fish Aquat Sci. 2008; 8:115–120.
18. Wang P, Ali S, Mason JC, Sims PFG, Broda P. Xylanases from *Streptomycescyaneus*. In Visser J, Kusters-van-Someran MA, Voragen AGJ (eds) Xylans and Xylanases., Elsevier Science Publishers, Amsterdam BV, pp225, 1992.
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19. Bairagi A, Ghosh KS, Sen SK, Ray AK. Enzyme Producing Bacterial Flora Isolated from Fish Digestive Tracts. Aquacult Int. 2002; 10(2):109–121.
20. Polizeli MLTM, Rizzatti ACS, Monti R, Ternzi HF, Jorge A, Amorim Xylanase from Fungi: Properties and Industrial Applications. Appl Microbiol Biotechnol. 2005; 67:577-591.
21. Bhattacharjee M, Banerjee M, Mitra P, Ganguly A. Investigation of Alkaline Phosphatase Enzyme of a Novel *Bacillus* species Isolated from Rhizospheric Soil of Potato Field. Res J Life Sci Bioinform Pharm Chem Sci. 2018; 4(2):129-144.
22. Piątek J, Gibas-Dorna M. The Kinetics of Bacterial Growth in Selected Pro- and Synbiotics – Recommendations for Family Practitioners. Family Medicine & Primary Care Review. 2012; 14(4):566–572.
23. Allison SD, Lu L, Kent AG, Martiny AC. Extracellular Enzyme Production and Cheating in *Pseudomonas fluorescens* Depend on Diffusion Rates. Front Microbiol. 2014; 169(5):1-8.
24. Falaye A, Emikpe B, Ogundipe E. Influence of *Lactobacillus plantarum* Supplemented Diet on Growth Response, Gut Morphometry and Microbial Profile in Gut of *Clarias gariepinus* Fingerlings. J Coast Life Med. 2016; 4(8):597-602.
25. El-Haroun ER. Improved Growth Rate and Feed Utilization in Farmed African Catfish *Clarias gariepinus* (Burchell 1822) through a Growth Promoter Biogen[7] Supplementation. J Fish Aquat Sci. 2007; 2(5):319-327.
26. Kongnum K, Hongpattarakere T. Effect of *Lactobacillus plantarum* Isolated from Digestive Tract of Wild Shrimp on Growth and Survival of White Shrimp (*Litopenaeus vannamei*) Challenged with *Vibrio harveyi*. Fish Shellfish Immunol. 2012; 32(1):170–177.
27. Novita H, Rusmana I, Yuhana M, Pasaribu FH. Potential of *Bacillus* sp., as a Producer of AHL Lactonase and its Application as a Probiotic for the Prevention of Mas in Catfish (*Clarias gariepinus*). J Fish Aquat Sci. 2015; 10(6):464-476.
28. Pradhan AK, Pradhan N, Sukla LB, Panda PK, Mishra BK. Inhibition of Pathogenic Bacterial Biofilm by Biosurfactant Produced by *Lysinibacillus fusiformis* S9. Bioprocess Biosyst Eng. 2013; 37(2):139-149.

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