Abstract

South Asian countries rear a variety of inland fish and fisheries and contribute to a major portion of the global demand. *Clarias batrachus* (Linn.), an indigenous fish of this region, is economically important due to its exceptional nutritional quality and taste.

Probiotics are now being used in farming of *C. batrachus* for better production and biological management of its diseases. In the present study, a considerable effort has been taken to discover specific probiotics for *C. batrachus* to rear the species in normal eco-friendly habitats. A non-polluted natural *C. batrachus* cultivation pond at Ramsagar (latitude 23˚06' and longitude 87˚15') of Bankura District, West Bengal, India was selected for studying the hydrobiological properties to get pre-hand information about the culture conditions. The average annual temperature of culture water ranged from 18±0.09 to 25±0.07 ºC, pH from 6.5±0 to 7.7±0.09, dissolved oxygen from 3.1±0 to 4.8±0.03 mg/l, alkalinity from 21±0.13 to 27±0.21 mg/l, salinity from 70±0.17 to 128±0.73 mg/l, total hardness from 78±0.33 to 128±0.88 mg/l, total dissolved solids from 176±0.58 to 260±1.15 mg/l. Ammonia, nitrate and nitrite contents were measured as 0.02±0 to 0.06±0.01 ppm, 0.1±0 to 0.4±0.01 ppm and 0.1±0 to 0.5±0.02 ppm respectively. Five groups of zooplankton and five groups of phytoplankton were obtained from the water. Gram negative bacteria were recorded to be predominant in occurrence. The bacterial count in the culture water and sediment was found to be greater in the summer. The consistency of the obtained eco-physiological features of the pond was validated statistically by contour surface plot.

The total viable intestinal bacterial count of *C. batrachus* was $1.6\pm2.9 \times 10^{10}$ cfu/g and thirty-two different bacterial strains were isolated. The intestinal isolates PKA17,

PKA18 and PKA19, on the basis of maximum antagonism against pathogenic *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus*, were selected for further analysis. These three Gram positive rod-shaped motile strains (PKA17, PKA18 and PKA19) were identified as *Lysinibacillus sphaericus* (Accession No. KX580190.1), *Bacillus cereus* (Accession No. KX826079.1) and *Bacillus thuringiens*is (Accession No. MF139049.1) respectively. The strains were sensitive to common antibiotics. They exhibited high cell-surface hydrophobicity, non-pathogenicity, bile salt tolerance and bile salt hydrolysis property that are crucial for colonization and growth at the host intestine. Considering the importance of the bile salt hydrolase (bsh) proteins to overcome the effect of bile of host intestine, *in silico* structure prediction and catalytic interaction study were performed on bsh protein of *L. sphaericus*. The sequential comparisons of bsh proteins between *Lysinibacillus* sp. and *Bacillus* sp were designed. The analysis clearly indicated that, the sequential diversity is mainly attributed to the high percentage of similar amino acid substitution. The investigation also put an insight on the inhibitory influence of some common inhibitors (theaflavin, gossypetin, phenethyl caffeate, chrysophanol, carnosic acid) on predicted bsh protein and those inhibitors should be avoided during the application of probiotic *L. sphaericus* in aquatic pond to overcome ineffectiveness.

C. batrachus fingerlings were cultivated in rearing tanks and provided with different supplemented experimental feed (T1: 2×10^5 *L. sphaericus* PKA17 cells per 100 g of basal feed; T2: 2×10^5 *B. cereus* PKA18 cells per 100g feed; T3: 2×10^5 *B. thuringiensis* PKA19 cells per 100 g feed) and control feed (without any probiotics). The T2 set displayed significantly ($P \leq 0.05$) higher growth, specific growth rate and protein efficiency ratio. The FCR was significantly ($P \leq 0.05$) lower in T1 (1.23±0.01) fed fish

followed by T2 and T3. The carcass composition of the probiotic-supplemented fish revealed an apparent better quality over the control fed fish. The protein content was significantly ($P \leq 0.05$) higher (164.9±0.23g kg^{-1}) in T3 fed fish followed by T1 and T2. The T1-fed fish produced considerably higher amount of carbohydrate (2.8±0.06 g kg^{-1}) and iron (2.21±0.01 mg $100g^{-1}$) than the other experimental set. The content of vitamin A (7.19±0.04 I.U. 100 g^{-1}) and vitamin E (0.34±0.01 I.U. 100 g^{-1}) was significantly ($P<0.05$) higher in T1 fed fish. The fatty acid profile revealed higher content of polyunsaturated ω-3 fatty acids (1.7±0.12 g kg^{-1}) in T3-fed fish followed by T1, T2 and control. The amino acid analysis of fish displayed significantly higher ($P \leq 0.05$) content of glycine, arginine, aspartic acid, glutamic acid, histidine, isoleucine, lysine, methionine, proline, threonine, tryptophan, tyrosine and valine in T1 fed fish than control and other test feeds. Fish with probiotic-supplemented feed showed higher amount of HDL cholesterol and calcium than the control fed fish. The effectiveness of the obtained results was statistically analyzed with scattered diagram and mesh plots where *L. sphaericus* PKA17 was found to be the most consistent. An intensive survey on freshwater fish diversity with special emphasis on the present status of *C. batrachus* in the Bankura district, West Bengal, India, was performed. A total of 92 freshwater fish species were recorded. The involvement of a considerable portion of the society in the indigenous fisheries has positive influence on rural economy and nutrition. *C. batrachus* holds good demand throughout the district but are extremely deficient in supply. The GIS-based analysis regarding the distribution of *C. batrachus* showed considerably better production in Ranibundh, Hirbundh and Patrasayer blocks. This study has predicted that the semi-intensive mode of cultivation would be the best strategy to increase the production and subsequent conservation of the species. The

study also pointed out the cultivation of forbidden alien catfish *C. gariepinus* in local water-bodies that must be prohibited. Specific training and awareness building programme for farmers for sustainable development of *C. batrachus* production are required to establish the rehabilitation of the species.