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Aquaculture or farming of aquatic animals is an important sector in the world which has involved a large number of people for earning of livelihood. Aquaculture and fisheries supply good quality protein enriched food. Different types of fishes- both inland and marine are concerned here. South Asian countries, one of the most fish diversity rich areas, contribute a major portion of world's total inland fisheries. *Clarias batrachus* (Linn.), an economically important catfish, is native to this region. The fish is exceptionally nutritive, easily digestive and has a delicious taste. It contains high-grade proteins, iron, minerals, good cholesterol, PUFA, vitamin A and vitamin D.

The Asian cat fish lives in water-logged low-land areas and rice fields. The exploitation of its natural habitat due to increased population, industrialization and other factors have made the species endangered. Cultivation of the species in semi-intensive manner is now focused to restore the indigenous fish, but the pathogenic diseases have again become constrains. Use of antibiotics in aquaculture to combat aquatic diseases has now been avoided globally and attention has been focused on the use of probiotics as an alternative. In this context, present thesis work has been focused on the isolation and establishment of new probiotics for the cultivation of *C. batrachus*.

The hydrobiological characters of a *C. batrachus* culture pond were studied to get a ready reference of the culture conditions of an ideal habitat of the species. This earthen pond is situated at Ramsagar (latitude 23˚06' and longitude 87˚15') of Bankura District, West Bengal, India. It is far away from the industrial sector and had no history of using probiotics. The physico-chemical parameters, planktonic diversity and bacterial flora of the culture pond were explored. The 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 recorded 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 zooplanktons and five groups of phytoplanktons were observed predominantly. The bacterial count in the culture water and sediment was low in winter in respect to the summer. The bacterial flora mainly contained Gram negative rods. The interactions of these hydro-biological characters were evaluated statistically. A contour surface plot was drawn to further validate the ecophysiology of limnological features. Each factor had its own influence as it showed separate divergent mode of distribution but they remained confined within a certain limit which signified the consistency of the obtained data. The study gives a launch-pad for successful application of probiotic organisms as they need to thrive in the aquatic environment and remain functional for a sustained period.

The intestinal flora of *C. batrachus* were isolated and subsequently validated for probiotic properties. The total viable intestinal bacterial count was $1.6\pm2.9 \times 10^{10}$ cfu/g. Thirty-two different bacterial isolates were obtained from the intestinal flora of *C. batrachus*. The antagonistic activity of the isolated bacterial strains was evaluated against fish pathogens. The intestinal isolates PKA17, PKA18 and PKA19 have shown maximum antagonism against pathogenic *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus* and were selected for further analysis. The biochemical characteristics and 16S rDNA sequencing identified the Gram positive rod-shaped motile strains (PKA17, PKA18 and PKA19) as *Lysinibacillus sphaericus* (Accession No. KX580190.1), *Bacillus cereus* (Accession No.

KX826079.1) and *Bacillus thuringiens*is (Accession No. MF139049.1) respectively. The isolates (PKA17, PKA18 and PKA19) were sensitive to common antibiotics, and non-haemolytic on blood agar. They exhibited high cell-surface hydrophobicity that ensures their capacity of adherence to the host intestine. The isolates have shown significant bile salt tolerance and hydrolysis property that are essential for survival and establishment at the intestinal tract of the host.

The bile salt hydrolase (bsh) protein of *L. sphaericus* is yet to be explored whereas there are available reports on bsh proteins of *Bacillus* species. Considering the importance of the bsh proteins to overcome the effect of bile salt, *in silico* structure prediction and catalytic interaction study was performed on bile salt hydrolase protein of *L. sphaericus*. *Lysinibacillus* sp. and *Bacillus* sp. possess diversity on bsh protein sequences due to their genus differentiation. However, this diversity has not been observed at their structural level. The analysis clearly indicated that, the sequential diversity is mainly attributed to the high percentage of similar amino acid substitution rather than identical substitution. The present findings will enrich the literature of functional protein evolution and further be used for protein engineering study to make more potent fish probiotic for *C. batrachus* cultivation. The investigation also emphasized on some inhibitors (carnosic acid, chrysophanol, epicatechin gallate, gossypetin, phenethyl caffeate and theaflavin) of this bsh protein that are generally used as ingredient in certain fish feed or fish growth related products. The study suggested that those inhibitors must 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 experimental feed (T1: 2×10^5 *L. sphaericus* PKA17; T2: 2×10^5 *B. cereus* PKA18;

T3: 2×10^5 *B. thuringiensis* PKA19 probiotic cell per 100 g feed) for 120 days. The growth of the test fishes were measured according to the increase in length and weight whereas the nutrient status was evaluated based on carcass composition and haematological parameters. The results were subsequently compared with the control set (fish reared without test probiotics). The fish fed T2 has displayed significantly ($P\leq0.05$) higher growth, specific growth rate and protein efficiency ratio. The FCR was significantly ($P\leq0.05$) lower in fish fed T1 (1.23±0.01) feed followed by T2 and T3. The carcass composition of the probiotic-supplemented fish revealed an apparent increase over the control fed fish. The carcass protein content was significantly ($P\leq0.05$) higher (164.9±0.23 g kg^{-1}) in T3 fed fish followed 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 groups. 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 ($P\leq0.05$) content of amino acids 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. The haematological analysis revealed that fish with probiotic-supplemented feed have considerably higher amount of HDL cholesterol and calcium than the control fed fish. The result recommended the successful establishment of the isolated strains as probiotics of *C. batrachus*. The obtained results were validated statistically with scattered diagram and mesh plotting where *L. sphaericus* PKA17 has shown the most consistent effectiveness among the three.

Finally, an intensive survey work was performed to study the present status of *C. batrachus* with reference to freshwater fish diversity in the Bankura district; the highest inland fish producing region of West Bengal. A total of 92 freshwater fish species were obtained through the study. The freshwater fisheries have considerable influence on community nutrition, rural economy and women empowerment. *C. batrachus* holds a good demand throughout the district but are extremely deficient in supply. The GIS-based study revealed the predominance of *C. batrachus* in Ranibundh, Hirbundh and Patrasayer blocks. This thorough survey has predicted that the semi-intensive mode of cultivation would be a better choice for conservation of the species. The study revealed massive infiltration of alien catfish *C. gariepinus* in local waterbodies that must be prohibited in order to restore biodiversity. The study also recommends scientific training and capacity building program for farmers for sustainable development of *C. batrachus* production. The rehabilitation of indigenous *C. batrachus* must be prioritized, before it becomes totally extinct.