List of Figures

List	Page No.
Fig. 1 (General Introduction): Adult healthy indigenous *Clarias batrachus*.	03
Fig. 1 (Review of Literature): Genes related to potential probiotic properties.	12
Fig. 2: Diagram for screening of autochthonous probiotic strain for *C. batrachus*.	21
Fig. 1.1: Geographical location of the study area and sample collection: (A) Map of India; (B) Map of West Bengal; (C) Map showing the position of Ramsagar in Bankura district; (D) Study pond; (E) Collection of water and mud samples; (F) Collection of plankton through hand plankton net.	24
Fig. 1.2: The contour plot showing impact of physico-chemical parameters on the bacterial load of *C. batrachus* culture pond.	38
Fig. 1.3: Photograph of zooplankton observed in *C. batrachus* culture pond.	40
Fig. 1.4: Photograph of phytoplankton observed in *C. batrachus* culture pond.	42
Fig. 2.A.1: Isolation of bacteria from the intestine of *C. batrachus*: (A) Dissection of the ventral surface; (B) Intestinal tract of *C. batrachus*; (C) Homogenization of the intestinal content.	48
Fig. 2.A.2: Antagonistic activity of bacterial isolates PKA17, PKA18 and PKA19 against pathogenic (A) *V. harveyi* (B) *V. vulnificus* (C) *V. parahaemolyticus*.	65
Fig. 2.A.3: Growth of selected bacterial isolates of *C. batrachus* at various temperatures in tryptone soya broth media at pH 7 under shaking (100 rpm) condition.	66
Fig. 2.A.4: Growth of selected bacterial isolates of *C. batrachus* at various pH in tryptone soya broth media at optimized temperature under shaking (100 rpm) condition.	67
Fig. 2.A.5: Growth of selected bacterial isolates of *C. batrachus* at various salt concentrations in tryptone soya broth media at optimized temperature and pH under shaking (100 rpm) condition.	67
Fig. 2.A.6: Effect of cultivation period on growth of PKA17, PKA18 and PKA19 in TSB at 37 °C at optimized pH under shaking (100 rpm) condition.	68
Fig. 2.A.7: Growth and clear zone production of bacterial isolates on Casein Hydrolysate agar medium.	71

Fig. 2.A.8: Light microscopic view: (A) PKA17; (B) PKA18; (C) PKA19 at 1000X total magnification. 71

Fig. 2.A.9: Agarose gel analysis of molecular weight of PCR products of 16S rDNA of PKA17, PKA18 and PKA19 bacterial isolates of *C. batrachus* (The three amplified 16S rDNA products were run separately parallel to the ladder lane and ladder lane contained standard molecular weight marker DNA). 73

Fig. 2.A.10: Phylogenetic analysis based on 16s rDNA sequencing of isolate PKA17 and related bacteria showing similarity with *Lysinibacillus sphaericus* OUG29GKBB (AccessionNo.KM972671.1). 73

Fig. 2.A.11: Phylogenetic analysis based on 16s rDNA sequencing of isolate PKA18 and related bacteria showing similarity with *Bacillus cereus* strain Gut16 (Accession No.KU156696.1). 74

Fig. 2.A.12: Phylogenetic analysis based on 16s rDNA sequencing of isolate PKA19 and related bacteria showing similarity with *Bacillus thuringiensis* Bt 53 (Accession No. KY784654.1). 74

Fig. 2.A.13: Scanning electron micrograph: (a) Isolated form of *L. sphaericus* PKA17; (b) Colonial growth of *L. sphaericus* PKA17; (c) Isolated form of *B. cereus* PKA18; (d) Colonial growth of *B. cereus* PKA18; (e) Isolated form of *B. thuringiensis* PKA19; (f) Colonial growth of *B. thuringiensis* PKA19. 76

Fig. 2.A.14: Antibiotic susceptibility test of bacterial isolates: (A) *L. sphaericus* PKA17 (B) *B. cereus* PKA18 and (C) *B. thuringiensis* PKA19 against 1. Streptomycin (SM); 2. Clindamycin (CD); 3. Gentamycin (GM); 4. Chloramphenicol (CH); 5. Ciprofloxacin (RC); 6. Tetracycline (TE); 7. Vancomycin (VA); 8. Nalidixic acid (NA). 77

Fig. 2.A.15: Bile salt tolerance activity of (A) *L. sphaericus* PKA17; (B) *B. cereus* PKA18; (C) *B. thuringiensis* PKA19 at 0.3% bile salt concentration in TSB media after overnight incubation. 79

Fig. 2.A.16: The bile salt hydrolase (bsh) activity of bacterial isolates grown on bile salt-TSA medium as manifested by the formation of precipitation zone around the colony. 80

Fig. 2.A.17: The test of mutual compatibility: (A) PKA18 and PKA19 isolates were cross-streaked against PKA17; (B) PKA17 and PKA19 isolates were cross-streaked against PKA18 on tryptone soya agar media. 81

Fig. 2.B.1: Multiple sequence alignment segment showing position of *Lysinibacillus sphaericus* bsh protein sequences with *B. anthracis* (Q81ST7) and *B. subterraneus* (A0A0D6Z9Q3). 88

Fig. 2.B.2: Neighbor-joined phylogenetic tree based on bsh protein sequences showing the relationships among *Lysinibacillus sphaericus* and *Bacillus* spp. 89

Fig. 2.B.3: Secondary structure comparison between predicted structure of *Lysinibacillus sphaericus* bsh protein (B1HPX8) and homologous structure of *Bacillus subtilis* (4wbdA) 91

Fig. 2.B.4: Structural alignment between predicted tertiary structure of *Lysinibacillus sphaericus* bsh protein (B1HPX8) and homologous structure of *Bacillus subtilis* (4wbdA). 92

Fig. 2.B.5: Ramachandran plot of predicted structure of *Lysinibacillus sphaericus* bsh protein (B1HPX8). 93

Fig. 2.B.6: Molecular docking between predicted *Lysinibacillus sphaericus* bsh protein (B1HPX8) and its substrate taurodeoxycholate. 95

Fig. 2.B.7: Amino acid involved in hydrogen bond formation with taurodeoxycholate at the catalytic site with specific H-bond length and binding energy. 96

Fig. 2.B.8: Molecular docking between predicted *Lysinibacillus sphaericus* bsh protein (B1HPX8) with substrate sodium taurocholate. 97

Fig. 3.1: Design of the cemented culture tank: (A) Rearing tank (195 × 105 × 90 cm^3); (B) Tank floor and walls, provided with cow dung; (C) Bottom mud of 5 cm; (D) Rearing fish. 103

Fig. 3.2: Weight of *C. batrachus* in relation to various feeds at different time interval. 113

Fig. 3.3.1: Effect of control feed on growth of *C. batrachus* fingerlings at different time interval. 114

Fig. 3.3.2: Effect of probiotic-supplemented feed (T1) on growth of *C. batrachus* fingerlings at different time interval. 115

Fig. 3.3.3: Effect of probiotic-supplemented feed (T2) on growth of *C. batrachus* fingerlings at different time interval. 116

Fig. 3.3.4: Effect of probiotic-supplemented feed (T3) on growth of *C. batrachus* fingerlings at different time interval. 117

Fig. 3.4: Regression line and scatter diagram of different growth parameters of *C. batrachus* with respect to (control, T1), (control, T2) and (control, T3) feed. 118

Fig. 3.5: Assessment of growth parameters of *C. batrachus* in T1 set in respect to control through mesh plot matrix. 119

Fig. 3.6: Assessment of growth parameters of *C. batrachus* in T2 set in respect to control through mesh plot matrix. 119

Fig. 3.7: Assessment of growth parameters of *C. batrachus* in T3 set in respect to control through mesh plot matrix. 120

Fig. 3.8: Weight of *C. batrachus* in relation to various feeds (supplemented with different concentration of *L. sphaericus* PKA17; control feed was not supplemented with probiotic) at different time interval. 129

Fig. 3.9: Weight of *C. batrachus* in relation to various feeds (supplemented with different concentration of *B. cereus* PKA18; control feed was not supplemented with probiotic) at different time interval. 130

Fig. 4.1: Geographical map of the study area (Bankura district with twenty-two blocks). 135

Fig. 4.2: Taxonomic order-wise occurrence of fishes in Bankura district. 136

Fig. 4.3: Spatial distribution of fish diversity of Bankura district (colours of the legend indicate the number of fish species found in the corresponding places). 141

Fig. 4.4: Spatial distribution map of Bankura district based on availability of *C. batrachus*. 148

Fig. 4.5: Substitution of *C. batrachus* with *C. gariepinus* in local fish markets: (A) Bankura-II; (B) Sarenga; (C) Ranibundh; (D) Bishnupur; (E) Raipur; (F) Bankura-I blocks. 149