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EFFECTS OF PROBIOTICS-ENCAPSULATED LIVE FEED ON GROWTH AND SURVIVAL OF JUVENILE *Clarias batrachus* (Linnaeus, 1758) AFTER DIFFERENTIAL EXPOSURE TO PATHOGENIC BACTERIA

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ABSTRACT

Growth and survival of *Clarias batrachus* juveniles (10-day old) fed probiotic *Bacillus cereus* (KR809412) encapsulated live feed (chironomid larvae) have been evaluated after differential exposure to the pathogenic *Aeromonas hydrophila* (MTCC 1739). Catfish juveniles were stocked at a density of 30 fish per tank in five experimental groups (T1-T5) along with a control group in triplicate and fed twice @ 5% of body weight day^{-1} for four weeks. Groups T1 and T2 were fed probiotic-encapsulated (PR) or pathogen-inoculated (PGN) live feed respectively, for initial three weeks. During this period groups T3 (PGN-PR-PR), T4 (PR-PGN-PR), and T5 (PR-PR-PGN) were differentially exposed to the pathogen. Live feed without probiotic and pathogen was offered to the control group throughout the experimental period and all other treatment groups (T1-T5) during the 4th week. Continuous exposure to probiotics in group T1 resulted in significantly higher ($P<0.05$) specific growth rate (SGR, % d^{-1}) and survivability than other groups, whereas, pathogen exposed and probiotic deprived group (T2) noticed with the lowest SGR and the highest mortality. Among other treatment groups (T3, T4 and T5), group T4 resulted in improved SGR and survivability. The coefficient (r value) of 0.867 along with regression slope suggested a positive correlation (0.01 levels) between RNA: DNA and SGR. The study might suggest protective effects of probiotic *B. cereus* in pathogen exposed *C. batrachus* juveniles.

Keywords: *Clarias batrachus*, *Bacillus cereus*, probiotic, *Aeromonas hydrophila*, live feed

INTRODUCTION

Walking catfish, *Clarias batrachus* (commonly known as magur) is an economically important group of fish with high nutritional value and recuperation ability (Sahoo et

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al., 2010). High mortality of magur juveniles has been observed due to less availability of food and poor nutrient utilization (Sahoo et al., 2010) along with ecological imbalance in the breeding ground and habitat degradation (Ahmed et al., 2012). Moreover, magur juveniles are susceptible to the pathogen, majority of which are bacteria (Ikpi and Offem, 2011). Although antibiotics are traditionally used to solve this problem (Hu et al., 2007), the use of antibiotics has been criticized since they can alter the gut microbiota and might lead to develop resistant bacteria population (Verschuere et al., 2000). Alternatively, likely application of gut associated bacteria as probiotics in catfish has been apprehended in some of the recent investigations (Banerjee et al., 2015; Dey et al., 2016). Besides, magur juveniles require protein rich food to ensure proper growth and survival (Kiriratnikom and Kiriratnikom, 2012). Application of potent extracellular enzyme–producing and pathogen inhibitory autochthonous bacteria through protein rich live food might hold promise to supply nutritional support and limit pathogenic microbial load to reduce mortality in catfish juveniles as suggested elsewhere (Cruz et al., 2012).

Hence, this study made an effort to assess the role of probiotic-encapsulated live feed (chironomid larvae) in improving growth and survivability of juvenile *C. batrachus* after differential exposure to pathogenic *A. hydrophila*.

MATERIALS AND METHODS

Experimental fish

Ten day old juveniles of *C. batrachus* were procured from a reputed fish farm (Blutech Dynamics, Dakshin Bijoynagar, South 24 Parganas, West Bengal, India) and stocked in fiber reinforce plastic (FRP) tanks (45 L). Juveniles were acclimatized for 10 days with feeding of zooplanktons and/or chopped *Tubifex*. Water quality parameters, viz., temperature, pH, total dissolved solids (TDS) and dissolved oxygen content (mg l^{-1}) from each experimental set were monitored at regular intervals (APHA, 2005). Faecal matter and remains were siphoned out daily.

Experimental procedure

Autochthonous, extracellular enzyme-producing *Bacillus aryabhattai* KP784311, *B. flexus* KR809411 and *B. cereus* KR809412 were previously isolated from the gut of adult *C. batrachus* (Dey et al., 2016) and their probiotic features had been documented (Dey et al., 2017). Antibacterial activity of the putative probiotic strains was tested against pathogenic *Aeromonas hydrophila* (MTCC 1739), by cross-streaking and agar well-diffusion as described by Mukherjee et al. (2016). The pathogenic strain was obtained from the Microbial Type Culture Collection, Chandigarh, India. The efficient antagonistic strain was selected for the present study.

The experiment was conducted for 4 weeks (28 days) in rectangular fibre reinforced plastic (FRP) tanks (90 cm × 30 cm × 30 cm). Overall, experimental fish were distributed randomly at a stocking density of 30 fish per FRP tank with three replicates for each experimental set (altogether 6 sets; control and T1-T5), and exposed to probiotic and/or pathogenic bacteria through the live feed (12–15 day old chironomid larvae) following the experimental design depicted in table 1. Probiotic encapsulation (PR) and pathogen inoculation (PGN) of the live feed was done separately either with the selected probiotic strain (*B. cereus* KR809412) or the pathogenic *A. hydrophila* (MTCC 1739) as described in Dey et al. (2017).

Table 1. Experimental design showing combination of feed in control group and treatment groups for 4 weeks

Treatments	Duration			
	1st week	2nd week	3rd week	4th week
Control	LF	LF	LF	LF
T_1	PR	PR	PR	LF
T_2	PGN	PGN	PGN	LF
T_3	PGN	PR	PR	LF
T_4	PR	PGN	PR	LF
T_5	PR	PR	PGN	LF

PR = Probiotic encapsulated, PGN= Pathogen inoculated, LF= Live feed without any bacterial inoculation

Catfish juveniles were fed live feed twice (9.00h and 16.00h) @ 5% of body weight day^{-1} (Chepkirui–Boit et al., 2011). Fish juveniles were sampled prior to and after completion of the experiment. Growth was calculated as specific growth rate (SGR, % day^{-1}) = 100 $[(\ln W_f - \ln W_i)/T]$, where W_i and W_f are the initial and final wet weights of fish respectively; T is the trial period in days. RNA-DNA ratio was considered as an index of growth. DNA and RNA aliquots were prepared from the larval tissue (Esteves et al., 2000). DNA and RNA contents were determined following Bruton (1956) and Marham (1955), respectively.

Survivability was calculated as: (Final number of juveniles/ Initial number of juveniles) × 100. Growth (Length, weight and SGR) and survival rate were observed in each week and data analysed using one-way analysis of variance (ANOVA) and a post hoc analysis (Tukey HSD) followed by Zar (1999). To correlate RNA: DNA and SGR, ccorrelation coefficient (r value) and regression analysis were performed.

RESULTS

Among the three probiotic strains, *B. cereus* KR809412 was noticed to inhibit the growth of *A. hydrophila*. Thus, *B. cereus* KR809412 was used as the probiotic strain in this study. Water quality parameters; temperature (25-28^{0}C), pH (6.3-7.7), dissolved oxygen (5.8-6.8 mg l^{-1}) and TDS (1.21-1.25 ppm) varied within narrow range during the experiment. Feeding of *B. cereus* encapsulated midge larvae in group T1 resulted in significantly higher ($P<0.05$) specific growth rate (SGR, % d^{-1}) and maximum survivability in *C. batrachus* juveniles. While in contrast to the group T3, groups T4 and T5 exhibited improved growth rate and survivability. Group T2 displayed the lowest growth rate and the highest mortality. Weight gain, increment in total length, SGR (% day^{-1}) and survivability of the experimental fish at the end of 4th weeks have been presented in table 2. The RNA content in the carcass increased over the initial value in all treatment groups, whereas, the DNA content did not indicate any significant change. The coefficient (r value) of 0.867 along with regression slope suggested a positive correlation (0.01 levels) between RNA: DNA and SGR (Figure 1).

Table 2. Growth parameters and RNA: DNA of catfish juveniles

Treatments	Parameters						
	Weight (g)	Length (cm)	Survivability (%)	Specific Growth Rate (% day^{-1}/ fish)	RNA (μg ml^{-1})	DNA (μg ml^{-1})	RNA: DNA
Control	0.64± 0.01^c	3.10± 0.04^e	82.30± 2.36^d	1.10± 0.40^b	82	72.7	1.12^b
T1	0.72± 0.01^d	3.20± 0.07^e	91.70± 1.42^e	3.50± 0.05^e	88	71.4	1.23^d
T2	0.52± 0.01^a	2.52± 0.02^a	30.00± 2.86^a	0.80± 0.05^a	77	70.1	1.09^a
T3	0.57± 0.02^b	2.64± 0.01^b	69.3± 1.40^b	1.72± 0.01^c	84	71.2	1.14^b
T4	0.66± 0.02^c	2.77± 0.01^c	81.60± 2.30^d	2.58± 0.10^d	84.3	72.3	1.16^c
T5	0.60± 0.06^b	2.80± 0.01^d	73.00± 1.40^c	1.75± 0.06^c	81	71.2	1.13^b

Results are mean ± standard deviation of 3 determinations. Values with the same superscripts in the same vertical column are not significantly different ($P<0.05$).

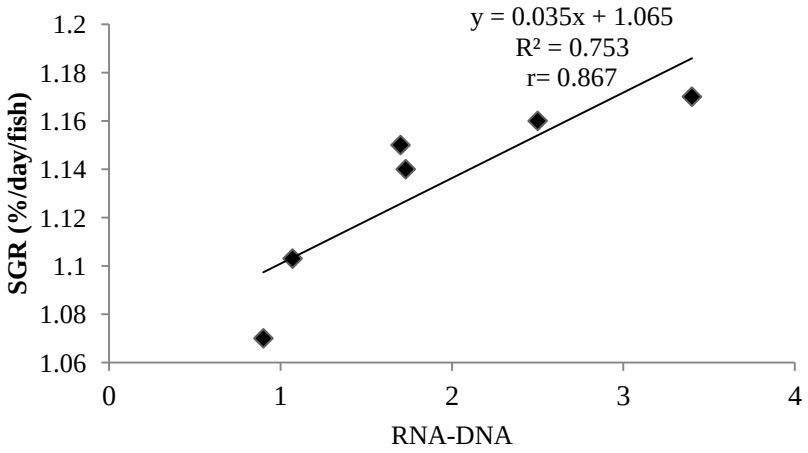

Figure 1. Relation between SGR (specific growth rate) and RNA-DNA ratio in catfish juveniles

DISCUSSION

The non-specific immune system of aquatic animals is developed with the application of probiotics that perhaps help the animals to resist infection from potential pathogens (Andani et al., 2012) and might explain the improvement in growth and survivability associated with the fish that received probiotics prior to exposure to the pathogenic *A. hydrophila* (T4 and T5) in the present study. Diverse strains of *Bacillus* spp. have been used as probiotics against the bacterial pathogens in fish (Aly et al., 2008). Probiotic *B. Subtilis* BT23 was noticed to antagonize *Vibrio harveyi* that reduced mortality in shrimps (Vaseeharan and Ramasamy 2003). The probiotic *B. cereus* used in the present investigation was antagonistic to *A. hydrophila*, which was in accordance with the reports portraying pathogen inhibitory activity of *B. cereus* and *B. circulans* isolated from the gut of different fish species (Lalloo et al., 2010; Geraylou et al., 2014). Administration of the probiotic *B. cereus* resulted in maximum growth and survival when catfish juveniles were not exposed to the pathogen. However, improvement in growth and survivability in catfish juveniles previously exposed to probiotic *B. cereus* prior to *A. hydrophila* exposure might indicate protective effects of the probiotics against the pathogen. Probiotic potential of *Bacillus* sp. in Indian major carp, *L. rohita* challenged with pathogenic *A. hydrophila* has been documented (Nandi et al., 2017). However, to the authors' knowledge, protective effects of probiotic *B. cereus* in pathogen exposed catfish, *C.*

batrachus juveniles has not been documented previously. Production of extracellular enzymes to assist digestion could be the reason behind improvement in growth performance of hosts with the administration of probiotics (Ray et al., 2012). Enzyme-producing ability of *B. cereus* used in the recent study has been documented earlier (Dey et al., 2016). Therefore, although not addressed in this pilot study, improved growth and survivability in the probiotic exposed groups might be attributed to the enhanced nutrient utilization and lower stressor levels as indicated elsewhere (Al-Dohail et al., 2009). RNA:DNA might be considered as a reliable indication of growth trend (Bandyopadhyay and Das-Mohapatra, 2009). The ratio was the greatest in the fish reared as T1 that continuously received probiotic encapsulated live feed and didn't expose to the pathogen. In conformity with the present study, Bandyopadhyay and Das-Mohapatra (2009) reported better growth as well as better RNA: DNA in an Indian major carp, *Catla catla* fed probiotic supplemented diet (*B. circulans* PB7; 2×10^5 cells 100 g^{-1}).

Furthermore, fish juveniles require good amount of nutrients because of their rudimentary digestive system (Govoni et al., 1986) and they use to prefer live food organisms that act as 'living capsules' of nutrition. Thus, administration of probiotics through bioencapsulated live feed might be an effective approach for introduction of large numbers of probiotic bacteria during rearing of the early stages (Ibrahem, 2015). However, more research is inevitable for a prolonged duration and with large number of replicates to assess the effects of mixed culture probiotics, as dominance of single strain in a continuous changing environment might be uncertain (Verschuere et al., 2000). Although higher survival against the pathogenic *A. hydrophila* exposure might specify immune-stimulatory property of the probiotic strain (Bandyopadhyay and Das-Mohapatra 2009), we need to look into stress and immune parameters to ascertain probiotic *B. cereus* as a potent immune-stimulant for likely use in aquaculture.

CONCLUSION

Controlled fish culture demands quality seed supply in optimum amount, which has now become difficult due to huge mortality and disease susceptibility. Inhibitory effect of a probiotic strain against one aeromonad fish pathogen was examined in the present investigation and the study pointed out that bacterial symbionts in fish may endow the host with some ecological benefits by enabling them to overcome the harmful effects of *Aeromonas* spp. *C. batrachus* juveniles were fed probiotic-encapsulated midge larvae and differentially exposed to pathogen inoculated midge larvae. Unfavourable effect of the pathogen was checked in those juveniles who were previously exposed to the probiotic bacteria.

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REFERENCES

Ahmed, R., Pandey, R.B., Arif, S.H., Nabi, N., Jabeen, N., and Hasnain, A. (2012). Polymorphic β and γ lens crystalline demonstrate latitudinal distribution of threatened walking catfish *Clarias batrachus* (Linn.) populations in North-Western India. *Journal of Biological Science,* 12, 98–104.

Akbar, I., Radhakrishnan, D.K., Venkatachalam, R., Sathrajith, A.T., and Sureshkumar, S. (2014).Standardization of the bioencapsulation of probiotics and oil emulsion in *Artemia parthenogenetica*. *International Journal of Research in Fisheries and Aquaculture,* 4, 122–125.

Al-Dohail, A.M., Hashim, R., and Aliyu-Paiko, M. (2009).Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clariasgariepinus*, Burchell 1822) fingerling. *Aquaculture Research,* 40, 1642-1652.

Aly, S.M., Abd-El-Rahman, A.M., John, G., and Mohamed, M.F. (2008).Characterization of some bacteria isolated from *Oreochromis niloticus* and their potential use as probiotics. *Aquaculture,* 277, 1–6.

Andani, H.R.R., Tukmechi, A., Meshkini, S., and Sheikhzade, N. (2012).Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology*, 28, 728–734.

APHA. (2005).Standard methods for the examination of water and wastewater (21st ed.), Greenberg, A.E., Clesceri, L.S., and Eaton, A.D. (eds). American Public Health Association, Washington DC. ISBN: 0-309-09122-5.

Bandyopadhyay, P. and Das Mohapatra, P.K. (2009). Effect of a probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: on growth, nutritional quality and immunity of *Catla catla* (Ham.). *Fish Physiology and biochemistry*, 35, 467–478.

Banerjee, G., Dan, S.K., Nandi, A., Ghosh, P., and Ray, A.K. (2015).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. *Polish Journal of Microbiology*, 64, 361–368.

Burton, K. (1956).A study condition mechanisms of diphenyl amine reaction for the estimation of DNA. *Journal of Biochemistry,* 62, 315.

Chepkirui–Boit, V., Ngugi, C.C., Bowman, J., Oyoo–Okoth, E., Rasowo, J., Bundi, J., and Cherop, L. (20110.Growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co–fed Artemia and a micro–

diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture nutrition*, 17, 82–89.

Cruz, P.M., Ibáñez, A.L., Hermosillo, O.A.M., and Saad, H.C.R. (2012).Use of probiotics in Aquaculture: Review article. *International Scholarly Research Network ISRN Microbiology* 1–14. Article ID 916845.

Dey, A., Ghosh, K., and Hazra, N. (2016).Evaluation of extra-cellular enzyme producing autochthonous gut bacteria in walking catfish *Clarias batrachus* (L.). *Journal of Fisheries*, 4, 345–352.

Dey, A., Ghosh, K., and Hazra, N. (2017).Improvement of growth and survival of the juvenile walking catfish, *Clarias batrachus* (L.) (Siluriformes:Clariidae) fed on probiotic encapsulated and ascorbic acid enriched chironomid larvae (Diptera:Chironomidae). *Proceedings of the Zoological Society*, doi: 10.1007/s12595-017-0236-9.

Esteves, E., Chícharo, M.A., Pina, T., Coelho, M.L., and Andrade, J.P. (2000). Comparison of RNA/DNA ratios obtained with two methods for nucleic acid quantification in gobiid larvae. *Journal of Experimental Marine Biology and Ecology*, 245, 43–55.

Fuller, R. (1989).Probiotics in man and animals. *Journal of Applied Bacteriology 66*, 365–378.

Ghosh, K., Roy, M., Kar, N., and Ringø, E. (2010).Gastrointestinal bacteria in rohu, *Labeo rohita* (Actinopterygii: Cypriniformes: Cyprinidae): scanning electron microscopy and bacteriological study. *Acta Ichthyologica et Piscatoria*, 40, 129–135.

Geraylou, Z., Vanhove-Maarten, P.M., Souffreau, C., Rurangwa, E., Buyse, J., and Ollevier, F. (2014). In vitro selection and characterization of putative probiotics isolated from the gut of *Acipenser baerii* (Brandt, 1869). *Aquaculture Research*, 45, 341–352.

Govoni, J.J., Boehlert, G.W., and Watanabe, Y. (1986).The physiology of digestion in fish larvae. *Environmental Biology of Fishes*, 61, 187–201.

Ibrahem, M.D. (2015). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospective. *Journal of Advanced Research*, 6, 765–791.

Ikpi, G., and Offem, B. (2011). Bacterial infection of mudfish *Clarias gariepinus* (Siluriformes: Clariidae) fingerlings in tropical nursery ponds. *Revista De Biologia Tropical*, 59, 751–759.

Islam, M.N., Islam, M.S., and Alam, M.S. (2007).Genetic structure of different populations of walking catfish *(C. batrachus* L.) in Bangladesh. *Biochemical Genetics*, 45, 647–662.

Kiriratnikom, S., and Kiriratnikom, A. (2012). Growth, feed utilization, survival and body composition of fingerlings of slender walking catfish, *Clarias nieuhofii*, fed diets containing different protein levels. *Songklanakarin Journal of Science and Technology*, 34, 37–43.

Lallo, R., Moonsamy, G., Ramchuran, S., Görgens, J., and Gardiner, N. (2010). Competitive exclusion as a mode of action of a novel *Bacillus cereus* aquaculture biological agent. *Letters in Applied Microbiology*, 50, 563–570.

Marham, R. (1955). *Nucleic acids, their components and related compounds*. In K. Paech,and M.V. Tracey (Eds.), Modern methods of plant analysis (pp. 264–350). Springer–Varlag, Berlin.

Mukherjee, A., Dutta, D., Banerjee, S., Ringø, E., Breines, E.M., Hareide, E., Chandra, G., and Ghosh, K. (2016). Potential probiotics from Indian major carp, *Cirrhinus mrigala*. Characterization, pathogen inhibitory activity, partial characterization of bacteriocin and production of exoenzymes. *Research in Veterinary Science*, 108, 76–84.

Nandi, A., Banerjee, G., Dan, S.K., Ghosh, K., and Ray, A.K. (2017).Probiotic efficiency of *Bacillus* sp. in *Labeo rohita* challenged by *Aeromonas hydrophila*: assessment of stress profile, haemato-biochemical parameters and immune responses. *Aquaculture Research*, 48, 4334-4345.

Sahoo, S.K., Giri, S.S., Chandra, S., and Sahu, A.K. (2010).Management in seed rearing of Asian catfish, *Clarias batrachus*, in hatchery conditions. *Aquaculture Asia Magazine*, 16, 23–25.

Vaseeharan, B., and Ramasamy, P. (2003).Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letters in Applied Microbiology*, 36, 83–87.

Verschuere, L., Heang, H., Criel, G., Dafnis, S., Sorgeloos, P. andVerstraete, W. (2000). Protection of *Artemia* against the pathogenic effects of *Vibrio proteolyticus* CW8T2 by selected bacterial strains. *Applied Environmental Microbiology*, 66, 1139–1146.

Zar, J.H. (1999).*Bio statistical analysis*. New Delhi: Pearson Education Inc. and Dorling Kindersley Publishing Inc.