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Vibhuti Mishra
R & D Biotechnology, Tropilite Biosciences Private Ltd., Gwalior, Madhya Pradesh, India

Rohit Sharma
R & D Biotechnology, Tropilite Biosciences Private Ltd., Gwalior, Madhya Pradesh, India

Corresponding Author:
Vibhuti Mishra
R & D Biotechnology, Tropilite Biosciences Private Ltd., Gwalior, Madhya Pradesh, India

Impact of probiotic supplementation on water quality and behaviour parameters in *Cyprinus carpio*

Vibhuti Mishra and Rohit Sharma

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Abstract
The present study was carried out to study the effects of probiotic formulation product, Vapflex Aquafloc Pro on water quality in *Cyprinus carpio* fish aquaculture system. The observations were taken for a duration of four weeks during which the parameters of ammonia concentration, pH, temperature, total dissolved oxygen and behaviour were considered. All the parameters were greatly controlled in the probiotic treated group as compared to control, indicating beneficial effects of probiotics and efficient recycling of organic waste. The formulation of the product with a suitable carbon source could prove to be very efficient supplement for aquaculture systems.

Keywords: probiotics, ammonia concentration, pH, total dissolved oxygen, behaviour

1. Introduction
Probiotics are a vital component of the functional foods that have been studied widely in recent years for the wide range of functions [1,2]. The functions of probiotics are not limited to humans, they exhibit several functions in animals as well. They are used in poultry for better body weight increase and the feed conversion ratio. Apart from this the probiotics were also observed to control the pathogenic microorganisms in poultry [3]. The probiotic microorganisms have been known to enhance swine production. The probiotic *Lactobacillus plantarum* JDFM LP11 promotes beneficial microbial flora that helped in the development of intestine in weaned piglets [4]. The probiotics have recently been evaluated for their number of benefits in aquaculture. Antibiotics that are given with feed to aquatic animals, kill beneficial bacteria as well. Scientists have searched for suitable alternatives such as probiotics to be supplemented with diet, as these microorganisms have several functions and enhance healthy microflora in aquatic ecosystem [5]. The present study was aimed to study the effects of probiotics in common carp *Cyprinus carpio* since it plays a very important role in aquaculture production systems [6].

2. Materials and Methods
2.1 Study design
Common carp (10 ± 2 g) were purchased from a local commercial fish farm and transferred to aquarium located at R&D Biotech Lab of Bioprocessing Unit, Tropilite Foods Pvt. Ltd., in polyethene bags. Ten fishes were randomly distributed into each aquarium (2aquariums of 35 L each), which were filled with unchlorinated water. The tanks were labelled as control and treated. Both the tanks were maintained at a photoperiod of 12 h light/ 12 h dark during the period of trial. The feed was given to fishes twice per day for a period of four weeks and it was 2% of the total fish biomass. Vapflex Aquafloc Pro, a multistrain probiotic formulation was selected for the study. Each 100 g of the product contains probiotics (1200 Billion spores), consisting of *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus* polymyxa, *Bacillus megaterium*, *Bacillus licheniformis and Saccharomyces cerevisiae*. The product also contained Maltodextrin, Citric acid, Yucca Schidigera, Digestive Enzymes and other active ingredients. The probiotic was supplemented as per manufacturer's protocol. The required quantity of probiotic (dosage: 300g/10,000 litre of water) was calculated for fish tank containing 30 litres of water.

The calculated amount was weighed and dissolved in 100 ml of water with 10% jaggery (carbon source), mixed thoroughly and spread all over the tank [7, 8]. The probiotic starter was added along with carbon source every week.

2.2 Analysis of Water Quality Parameters

2.2.1 pH: The sampling of water was done every week for pH measurement from both control and treated tank. The pH was measured using Systronics µpH System 361 electrode.

2.2.2 Total dissolved oxygen: The total dissolved oxygen was measured using oxygen probe of Hamilton (Oxyferm FDA 225) PN 237452.

2.2.3 Total ammonia content: The ammonia content was measured using the method mentioned in FSSAI manual [9].

2.2.4 Temperature: The temperature was recorded using mercury thermometer.

2.3 Behaviour: Some behavioral changes were observed in fishes given probiotic treatment [10, 11].

Since we are a part of a corporate organization, we do not have an ethical committee to seek approval for the study. However, the experiments were carried out ethically to the best of our knowledge. Results are expressed as mean ± SE of six set of observations. Statistical analyses were performed using Sigma Stat Statistical software version 2.0. All the statistical analyses were performed using one-way analysis of variance with post hoc Bonferroni's multiple comparison test applied across the treatment groups. Significance was based on P value < 0.05.

3. Results and Discussion

In the present study, the major parameters being affected by probiotic supplementation were ammonia concentration and pH. These parameters play an important role in determining water quality [12]. From the experiments it was observed that the pH of water and ammonia concentration was well regulated in the probiotic supplemented group. During the first week the concentration of ammonia in the probiotic supplemented group was 25% less as compared to control. In the second week the decrease in ammonia levels was 30.84% in the probiotic supplemented group. During the third and fourth week, the decrease was 66.66% and 100% respectively as compared to control, thereby indicating efficient recycling of nitrogenous waste in the probiotic containing aquaculture system (Table 1).

Table 1: Effect of probiotic supplementation on ammonia concentration in fish tank

Group	Exposure period in Weeks - (mg/L)			
	I	II	III	IV
Control	0.150 ± 0.01	0.140 ± 0.01	0.170 ± 0.01	0.20 ± 0.01
Probiotic	0.120 ± 0.01***	0.107 ± 0.01***	0.102 ± 0.01***	0.10 ± 0.01***

$^{*}P<0.05$, $^{*}P<0.01$, $^{***}P<0.0001$ and $^{\#}P>0.05$ when compared with respective control.

Similarly, the pH was also controlled in the probiotic treated group. The pH was 2.9% less as compared to control during the first week, followed by a decrease of 6.59%, 3.89% and 7% during the second, third and fourth week respectively (Table 2).

Table 2: Effect of probiotics on pH of water in fish tank

Group	Exposure period in Weeks			
	I	II	III	IV
Control	8.08 ± 0.09	7.92 ± 0.02	8.01 ± 0.01	7.89 ± 0.01
Probiotic	7.85 ± 0.06$^{\#}$	7.43 ± 0.05**	7.71 ± 0.01*	7.37 ± 0.02*

$^{*}P<0.05$, $^{*}P< 0.01$, $^{***}P<0.0001$ and $^{\#}P>0.05$ when compared with respective control.

Similar results have been observed in previous studies in aquatic organisms involving probiotic supplementation. The effective combination that has been administered to fishes was *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei and Saccharomyces cerevisiae.* The treatment of probiotics in *Litopenaeus vannamei* (white shrimp) with a dose of 4L/h led to an improvement in specific growth rate and food conversion factor with respect to control. The same dose also increased survival in the fishes by 8.76%. The dose of 10 L/ha caused the removal of organic matter to a greater extent [13]. In another study involving *Litopenaeus vannamei*, a mixed culture of Bacillus strains was supplemented in the larval and post larval stages. The treatment helped in higher growth of post larvae as compared to control. The nitrite, ammonia and pH levels were decreased significantly as compared to control thereby exhibiting a positive effect on survival and growth of PL shrimp [14]. The *Bacillus subtilis* E20, that was isolated from a probiotic food significantly increased the survival of white shrimp larvae, that were supplemented with probiotic concentration of 10 (8) and 10 (9) cfu L(-1). The expression of the genes that were associated with the immune system i.e. prophenoloxidase I, propenoloxidase II, and lysozyme of larvae increased significantly in water treated with a probiotic concentration of 10(8) and 10(9) cfu L(-1) [15]. According to Banerjee *et al*., the supplementation of *Bacillus pumilus* with periphytic microalgae caused a significant reduction in total ammonia nitrogen in the tanks observed for post larval growth of *Penaeus monodon* [16]. The probiotics have proved to be very beneficial alternatives for antibiotics use, since they have several benefits, such as enhancement of immune system, improved digestion, protection from pathogens, improvement in water quality and promotion of growth and reproduction. Amongst the known probiotics, *Lactobacillus* and *Bacillus* have received prime focus due to their ability to exhibit gastric activities and produce extracellular enzymes in aquatic organisms [17]. In a study involving *Oreochromis niloticus* (*O. niloticus*), the supplementation of probiotic in diet that conatined 1 gm/Kg of *L.acidophilus* helped in decreasing genotoxicity in the subjects exposed to cadmium at 32 ppm, which was evident from the decreased micronucleus formation, as compared to the group that received just cadmium [18]. In a review focusing on the functions of probiotics, such as inflammatory bowel disease reduction,

cancer prevention, cholesterol reduction, Vitamin A synthesis, the ability of probiotics in enhancing the gastrointestinal microbiota of fishes is also mentioned [19]. In our study, the temperature was maintained in the range that is optimum for the growth and survival of *Cyprinus carpio* [20] (Table 3).

Table 3: Temperature of fish tank water during trial period

Group	Temperature ºC			
	I	II	III	IV
Control	27.03 ± 0.09	25.97 ± 0.03	25.0 ± 0.03	22.07 ± 0.03
Probiotic	26.90 ± 0.06 #	25.93 ± 0.07 #	24.97 ± 0.03 #	21.67 ± 0.33 #

*P<0.05, *P< 0.01, ***P<0.0001 and #P>0.05 when compared with respective control.

The levels of dissolved oxygen were closer to control values, indicating increased oxygen demand due to increased numbers of probiotic microorganisms in the fish tank (Table 4).

Table 4: Effect of probiotic supplementation on total dissolved oxygen

Group	Dissolved oxygen (%)			
	I	II	III	IV
Control	125 ± 0.58%	121.33 ± 0.88%	133.33 ± 0.88%	135 ± 0.58%
Probiotic	120.67 ± 0.88%*	118 ± 0.58%*	130.00 ± 0.58%*	130 ± 0.58%**

*P<0.05, *P< 0.01, ***P<0.0001 and #P>0.05 when compared with respective control.

In a study conducted by Reddy and Naik, it was observed that there were increased dissolved oxygen reduction rates in fishes receiving combination of probiotics with a carbon source i.e. molasses, wheat flour and rice flour, as compared to control [21]. Combination of our probiotic formulation with carbon sources other than jaggery could probably increase the dissolved oxygen concentration in aquaculture water. A remarkable difference was also observed in behavior of fishes given probiotics treatment. They were active in consuming feed and also swam freely whereas the control fishes were confined to a corner of the tank most of the time (Table 5).

Table 5: Comparison of behaviour in fishes given probiotic supplementation with control during duration of the study

	Rate of swimming	Corner Behaviour
Control	++	+++
Probiotic	+++	+

(-) None, (+) Mild, (++) Moderate, (+++) Strong

Similar findings have been reported in a study where a combination of *Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347 altered the swimming pattern in zebrafish model. Here the bottom dwelling behaviour of zebrafish was greatly reduced, indicating lower levels of anxiety due to probiotic consumption [22].

4. Conclusion

The present study demonstrates the ability of probiotic formulation Vapflex Aquafloc Pro in improving quality of water in fish tank, thereby making them an essential supplement for aquaculture systems. The property of probiotics to improve behavioral aspects of aquatic animals further makes them a beneficial dietary supplement.

5. Acknowledgement

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6. References

1. Day RL, Harper AJ, Woods RM, Davies OG, Heaney LM. Probiotics: current landscape and future horizons. Future science OA. 2019;5(4):FSO391.
2. Mishra V, Sharma R, Jaiswal M, Subramani SK, Yadav A, Rana S *et al*. A Review on Recently Explored Functional Aspects of Probiotics. Indian Journal of Pure and Applied Biosciences 2020;8(5):213-226.
3. Shin D, Chang SY, Bogere P, Won K, Choi JY, Choi YJ *et al*. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. PLoS One 2019;14(8):e0220843.
4. Markowiak P, Śliżewska K. The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens 2018;10:21.
5. Hasan KN, Banerjee G. Recent studies on probiotics as beneficial mediator in aquaculture: a review. Journal of Basic and Applied Zoology 2020;81**:**53.
6. Rahman MM. Role of common carp (*Cyprinus carpio*) in aquaculture production systems, Frontiers in Life Science 2015;8(4):399-410.
7. http://www.bioflocfishfarming.com/water-preparation-for-biofloc.
8. Putra I, Effendi I, Lukistyowati I, Tang UM, Fauzi M, Suharman I *et al*. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system [version 3; peer review: 2 approved]. F1000Research 2020;9:293.
9. https://old.fssai.gov.in/Portals/0/Pdf/Draft_Manuals/Water.pdf
10. Sharma M, Chadha P, Borah MK. Fish behaviour and immune response as a potential indicator of stress caused by 4-nonylphenol. American Journal of Biosciences 2015;3(6):278-283.
11. Sharma M, Thakur J. Effect of oxytetracyclin on behaviour of fish *Cyprinus carpio*. International Journal of Fisheries and Aquatic Studies 2020;8(6):187-188.
12. Zorriehzahra MJ, Delshad ST, Adel M, Tiwari R, Kartik K, Dhama K *et al*. Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Veterinary Quarterly 2016;36(4):228-241.
13. Melgar Valdes CE, Barba Macías E, Alvarez-González CA, Tovilla Hernández C, Sánchez AJ. Efecto de microorganismos con potencial probiótico en la calidad del agua y el crecimiento de camarón *Litopenaeus vannamei* (Decapoda: Penaeidae) en cultivo intensivo [Microorganisms effect with probiotic potential in water quality and growth of the shrimp Litopenaeus vannamei

(Decapoda: Penaeidae) in intensive culture]. Revista de Biologia Tropical 2013;61(3):1215-28.
14. Nimrat S, Suksawat S, Boonthai T, Vuthiphandchai V. Potential Bacillus probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (*Litopenaeus vannamei*). Veterinary Microbiology 2012;159(3, 4):443-50.
15. Liu KF, Chiu CH, Shiu YL, Cheng W, Liu CH. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish Shellfish Immunology 2010;28(5, 6):837-44.
16. Banerjee S, Khatoon H, Shariff M, Yusoff FM. Enhancement of *Penaeus monodon* shrimp postlarvae growth and survival without water exchange using marine *Bacillus pumilus* and periphytic microalgae. Fisheries Sciences 2010;76**:**481-487.
17. Banerjee G, Ray AK. The advancement of probiotics research and its application in fish farming industries. Research in Veterinary Science 2017;115:66-77.
18. Abu-Braka AZ, Zaki MS, Abbas HH, Ismail NEDA, Khalil R, Tanekhy M. *et al*. Filed studies on some probiotics to minimize hazard effects of prevailing heavy metals contamination for improving immunity and growth performance of *Oreochromis niloticus*. Electron Physician 2017;9(4):4138-4144.
19. Vijayaram S, Kannan S. Probiotics: The marvelous factor and health benefits. Biomedical and Biotechnology Research Journal 2018;2:1-8.
20. http://www.fao.org/tempref/FI/DOCUMENT/aquaculture/CulturedSpecies/file/en/encommoncarp.html
21. Reddy MS, Naik MK. Growth performance of *Litopenaeus vannamei* in the presence and absence of probiotics with Bioflocs of different carbon sources. International Journal of Fisheries and Aquatic Studies. 2020;8(5):349-354.
22. Valcarce DG, Martínez-Vázquez JM, Riesco MF, Robles V. Probiotics reduce anxiety-related behavior in zebrafish. Heliyon 2020;6(5):e03973.