



IN VITRO ANTAGONISTIC ACTIVITY OF BACILLUS THURINGIENSIS AGAINST FISH PATHOGENS PREVAILING IN AQUACULTURE

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Abstract:

Despite fish being highly nutritious, it is prone microbial attack, resulting in economic loss to the fish industry. Traditional application of chemical pesticides and antibiotics for control of microbial pathogens appear to be hazardous to the handlers and consumers as well, creating an imbalance in the ecosystem. As an alternative, the antagonistic activity of a known microbial biopesticide, *Bacillus thuringiensis*, was found to be effective against a good number of isolated pathogens from fish.

Keywords:

Antagonistic activity, Biopesticides, *Bacillus thuringiensis*, Fish pathogens

Introduction:

Fishes are well known for their nutritional value. Healthy fishes are prized for their table quality. However, this quality is influenced by several operational environmental factors. Often, they are prone to microbial and parasitic infections. A well known economic loss to the fish industry was the major outbreak of bacterial infection in major carps. The causative agents of the severe acute infectious abdominal dropsy outbreak in Indian major carps. *Cirrhinus mrigala* was reported Shome et al (1996). However, the first observation on diseases in Indian major carps was found in descending order of susceptibility on *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* (Gopalakrishnan 1981). Other well recorded cases have been the severe epidemic due to the diseased condition of European carps (Snieszko 1954; Van Dujin 1956).





Material and Method:

The antagonistic organisms used in the present investigation were *Bacillus turingiensis* strains B-17 and IPS -80 obtained from Vector Control Research Institute Pondicherry. *B.t.*(H-14) from the Department of Life sciences university of Mumbai and *Streptomyces antibioticus* (NCIM 2123) was obtained from National Chemical Laboratory (NCL) Pune. The fish pathogens were obtained from diseased fish collected from Chandrapur Dist. and identified using standard procedure. Thus, a total 50 bacterial culture were isolated from skin, gills, intestine operculum, tail region, liver etc. of the fish. Of these four common pathogens were identified are belonging to the genera viz., *Klebsiella* sp.(ANF1), *Pseudomonas* sp. (ANF2), *E. Coli* (ANF3) and *Salmonella* sp. (ANF4). The isolated fungal cultures were identified at center for higher learning and research in Microbiology, S.P. College Chandrapur (MS). A total 20 fungal culture were identified. The antagonistic activities of aforesaid biopesticides were tested against the isolated fish pathogens. The antagonistic organisms and fish pathogens were grown individually in sterile broth medium for about 7 days at 37°C with intermittent shaking and the titre inoculum was maintained around 10⁸ cfu/ml. The standard agar cup method was used for studying the interaction of antagonistic organisms with the fish pathogens. A basal layer of nutrient agar (6mm) was prepared in a 9 cm petriplates. After solidification, this layer was super-layered with a second layer of nutrient agar seeded with heavy suspension of the fish pathogen. The wells were made in the centre with the help of cork borer of 10 mm dia. and were filled with 0.2 ml broth culture of the antagonistic organisms in triplicate. In control plates, the wells were filled with sterile nutrient broth. In case of fungal pathogens, potato dextrose agar was used. After pre-diffusion time of 30 min petriplates were incubated for 48 hr at 37 °C. At the end of incubation, the diameter of the zone of inhibition was measured in mm with the help of zone reader, the averages were calculated.





Result and Discussion:

The inhibitory activities of isolates of *Bacillus thuringiensis* and *Streptomyces antibioticus* against *E. coli*, *Pseudomonas sp.*, *Proteus vulgaris* and *Saprolegnia sp.* are shown in Table 1. In the case of *E. coli* maximum growth was inhibited by *Streptomyces antibioticus* followed by B.t. (B-17), (H-14) and (IPS-80). On the other hand, in the case of *Pseudomonas sp.* The inhibition efficacy of B.t.(B-17) was found to be superior followed by B.t. (IPS-80) and (H-14). No antagonistic action of *Streptomyces antibioticus* was observed against *Pseudomonas sp.*, *Proteus vulgaris* and *Saprolegnia sp.* Maximum inhibition of both *Salmonella sp.* and *Proteus vulgaris* was observed with B. t. (B-17). No inhibition was noticed in case of *Rhodotorula sp.* against the tested pathogens. In the case of fungal pathogen, *Saprolegnia sp.*, only B.t.(H-14) was found to be effective. Overall, B.t.(B-17) a broad spectrum maximum inhibitory action against all the tested pathogens except *Saprolegnia sp.* followed by B.t. (H-14), whereas, B.t. (IPS-80) was inhibitory only to *E. coli* and *Pseudomonas sp.* The present investigation has shown that the inhibitory activity of microbial biopesticides against fish pathogens dignifies a viable approach for treating and controlling fish diseases by natural biological control phenomenon. This does have an edge over the traditional methods of the treatment as it is safe, economic and does not cause imbalance in the ecosystem. The reared healthy fingerlings of *Labeo rohita* were used as target system in the laboratory condition in order to check the effect of formulated biological control agent. All the fishes showed balance movement without any abnormal symptoms. Thus, the non-toxic effect biological control agent was confirmed. Similar buoyancy movement and histological examination of the hepatic region did not show any disintegration. The haemoglobin and glucose levels were taken at a fixed interval of one week each for one month. The weights of the experimental rats remained unchanged. The glucose level was suggestive of proper glucose metabolism and haemoglobin content confirmed that the erythrocytes were not damaged. These experiments for the effect of biological control agent on target





and non-target animal in the laboratory condition have proved that *Bacillus thuringiensis* (H-14) derived exotoxin does not have any toxic or harmful effect on fish.

Table:1. Antagonistic action of isolates of *Bacillus thuringiensis* and *Streptomyces antibioticus* against various isolated fish pathogens.

Antagonistic organism	Inhibition zone –mm (average of 3 replication)							
	Pseudomonas sp.	Salmonella sp.	E. coli	Proteus vulgaris	Saprolegnia sp.	Klebsiella sp.	Fusarium sp.	A. hydrophila
B.t.(B-17)	42	31	26	27	ND	41	38	36
B.t.(H-14)	17	27	23	26	26	53	ND	ND
B.t.(IPS-80)	26	ND	18	ND	ND	ND	ND	ND
S. antibioticus	ND	29	37	ND	ND	ND	ND	ND
Rhodotorula sp.	ND	ND	ND	ND	ND	ND	ND	

ND: Not Detected

Conclusion:

In conclusion *Bacillus thuringiensis* as a biocontrol agent against fish pathogen dignifies available approach for treating and controlling fish diseases by natural biological control phenomenon. This is a novel approach of treating fish infection over the other traditional methods.

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