IN VITRO ANTAGONISTIC ACTIVITY OF *BACILLUS THURINGIENSIS* AGAINST THE PATHOGENS OF FISH *CHANNA MORULIAS* FROM WAINGANGA RIVER OF GADCHIROLI DISTRICT (M.S) INDIA.

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ABSTRACT

The concept of biological control for health maintenance has received widespread attention during the last few years. This paper has a purpose to explain the experimental research society with the recent theoretical achievements in the research field of the biological control of the microbial fish diseases by using *Bacillus thuringiensis* (Bt) as a potent agent. The study is based on the fresh water fish *Channa morulias*. The main objective of this work was to look for active substances that could be used as antimicrobial agents in an efficient and safe manner. This different bacterial extracts were tested *in vitro* for their antimicrobial effects against Gram +ve and Gram -ve bacteria in addition to fungi, using agar well diffusion method. 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 *Bacillus thuringiensis*, was found to be effective against a good number of isolated pathogens from fish.

Keywords: Biological control, *Bacillus thuringiensis, Channa morulias,* microbial fish diseases, fish pathogens.

INTRODUCTION

Microbial fish diseases causes considerable economic losses in aquaculture yearly and it represents a worldwide problem. Members of the genus *Vibrio* are the causative agents of vibriosis, which can cause significant losses in fish culture. *Vibrio* spp. causes disease in many fish including the salmon, char and shellfish such as the shrimp (Eguchi et al., 2000; Kent and Poppe, 2002). *Escherichia coli* is a common human pathogen, contaminants of seafood in enormous number and fish usually acquire this pathogen through feeding on

food contaminated with feces causing serious life threatening illness within a very short time (Gomez et al., 2008).

Channa morulias is native to South Asia. In South India it is commonly found in reservoirs of eastern Vidarbha region. It is a faster growing fish than most of the other species of the genus. It is a carnivorous species. It is marketed live and fetches high prices in the market.

The fish *Channa morulias* is well known for its nutritional value. 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 Eropean carps (Snieszko 1954; Van Dujin 1956).

Microbial antagonism is a common phenomenon in nature and plays a major role in reducing or eliminating the incidence of opportunistic pathogens in the gastrointestinal tract of aquatic animals. Recently, the application of *Bacillus* sp. as a probiotic species for controlling aquatic pathogens shows promise. For example, Sugita et al. isolated a *Bacillus* strain that was antagonistic to 63% of the isolates from fish intestine. Sun et al. obtained two dominant gut *Bacillus* strains with antagonistic activity that could improve growth performance and immune responses of the isolated from fish sample (Y. Sun, H. Yang, R. Ma, and W. Lin).

In this study, we isolated a *Bacillus turingiensis* strains B-17 and IPS -80 antagonistic to different bacterial species, determined its taxonomic position, observed the physicochemical properties of its extracellular products, and assayed its *in vitro* growth inhibition effects on different bacterial species , and its antagonistic spectrum and pathogenicity.

MATERIAL AND METHOD

Sample Collection and Isolation of microorganisums

The fish pathogens were obtained from diseased fish collected from Wainganga river and identified using standard procedure. Thus, a total 40 bacterial culture were isolated from skin, gills, intestine operculum, tail region, etc. of the fish. Of these 6 common bacterial pathogens were identified, viz., *Klebsiella* sp., *Pseudomonas* sp., *E. Coli, Staphylococcus sp., Protius valgaris* and *Vibrio fluvialis.* The isolated fungal cultures were also identified at Centre for Higher Learning and Research in Microbiology, S.P. College Chandrapur (MS). A total 20 fungal culture were identified, of these 2 common pathogens were identified are *Fusarium sp.* and *Aspergillus sp.*

Testing antimicrobial activity by the agar-well diffusion method

The antagonistic activity of *Bacillus turingiensis* extracts was determined using cut–diffusion technique in which cut (5 mm) was punched upon the surface of agar plates previously inoculated with each of the above mentioned indicator strains. Each well bottom was sealed with two drops of sterile water agar About 0.2 ml of *Bacillus turingiensis* were transferred into each well. Wells loaded with the extracting solvents were used as controls, plates inoculated with bacteria were incubated at 37°C for 24 h and those inoculated with fungi were incubated for 3 days at 30°C. After incubation, the diameter of the inhibition zone was measured with callipers and the results were recorded in mm (Attaie et al., 1987). All tests were performed under sterile conditions in duplicate and repeated three times.

The antagonistic activities of abovementioned 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 37^0c with intermittent shaking and the titre inoculum was maintained around 10^8cfu/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 diameter 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 ^{o}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

Organisms and growth conditions

Total 8 different pathogens (6 Bacterial and 2 Fungal) were isolated from the fish *Channa morulias*, collected from Wainganga river, includes, *Klebsiella* sp., *Pseudomonas* sp., *E. Coli, Staphylococcus sp., Protius valgaris* and *Vibrio fluvialis*. Two common fungus pathogens identified are *Fusarium sp.* and *Aspergillus sp.* was predominantly present in the Wainganga river.

Microbial indicators and growth conditions

Eight microorganisms including Gram +ve and Gram -ve bacteria and fungi were used in this study *Staphylococcus sp.,* (Gram +ve), *E. coli* , *Pseudomonas sp.* and *Vibrio fluvialis, Protius valgaris* and *Klebsiella sp.* (Gram -ve), in addition to filamentous fungi *Fusarium sp.* and *Aspergillus sp.*

All bacterial strains were maintained on nutrient agar slants and incubated at 37°C. Each bacterial biomass was prepared by inoculating 100 ml of nutrient

broth medium. Bacterial cultures were shaken (250 rpm) at 37°C for 24 h. Different inocula were used at a logarithmic phase of growth (A550 = 1); the fungal strains were inoculated into glucose peptone broth (a wide spectrum antibiotic) for 5 days.

Antagonistic organism		**Inhibition zone –mm (average of 3 replication)**						
	E. coli	*Proteus vulgaris*	*Pseudomonas sp.*	*Klebsiella sp.*	*Staphylococcus sp.*	*Vibrio fluvialis*	*Fusarium sp.*	*Aspergillus sp.*
B.t.(IPS-80)	41	ND	24	ND	ND	ND	ND	ND
B.t.(B-17)	24	26	44	38	24	18	ND	ND
B.t(H-14)	22	24	28	44	22	17	32	28
S. antibioticus	32	ND	ND	22	ND	ND	ND	ND

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

ND: Not Detected

The inhibitory activities of isolates of *Bacillus thuringiensis* and *Streptomyces antibioticus Staphylococcus sp.*, *E. coli* , *Pseudomonas sp.* and *Vibrio fluvialis, Protius valgaris* and *Klebsiella sp.* in addition to filamentous fungi *Fusarium sp.* and *Aspergillus sp.* are shown in Table 1.

In case of *E. coli* maximum growth was inhibited by *Streptomyces antibioticus* followed by B.t.(IPS-80), (B-17), (H-14). On the other hand, in case of *Pseudomonas* sp. *coli* maximum growth was inhibited by B.t.(B-17).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, Vibrio fluvialis Klebsiella sp. Aspergillus niger* and *Aspergillus flavus*.

Maximum inhibition of both, *Pseudomonas sp,* and and *Klebsiella sp.* was observed with B.t.(B-17). However for fungal pathogens i.e. *Fusarium sp. and Aspergillus 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 bacterial pathogens but not effective on fungus species. Followed by B.t. (IPS-80) was inhibitory only to *E. coli* and *Pseudomonas* sp.

DISCUSSION

The present investigation shows that the inhibitory activity of microbial bipesticides 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 *Channa morulias* 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.

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.

CONCLUSION

In conclusion *Bacillus thuringiensis* as a biocontrol agent against fish pathogen dignifies aviable approach for treating and controlling fish diseases by natural biological control phenonmenon. This is a novel approach of treating fish infection over the other traditional methods. They are also safe for use in aquatic environments including drinking-water reservoirs for the control of mosquito, black fly and trouble insect larvae. However, it should be noted that vegetative *Bacillus thuringiensis* has the potential for the production of toxins, the significance of which as a cause of human disease is not known.

This paper deals with microbial pest control agents (MCPAs) based on *Bacillus thuringiensis* (Bt). This bacterium is also a key source of genes for transgenic expression to provide pest resistance in plants and microorganisms as pest control agents in so-called genetically modified organisms (GMOs). The potential effects on human health and the environment of GMOs involve several aspects that are only remotely or not at all related to Bt products, and they are therefore outside the scope of this study.

Thus from the above it is found that Bt could be used as a potent and safe biological control agent against the microbial diseases of fishes like *Channa morulias.*

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