BACILLUS THURINGIENSIS AS A BIOPESTICIDES AGAINST FISH PATHOGENS OF CHANNA MORULIAS FROM WAINGANGA RIVER OF GADCHIROLI DISTRICT OF MAHARASHTRA STATE, INDIA.

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Abstract:

The concept of biological control for health maintenance has received widespread attention during the last few years. This review has a purpose to explain the experimental research society with the recent theoretical achievements in the research field of the biological control the microbial fish diseases by using Bacillus thuringiensis (Bt) as a potent agent. The study is based on the fresh water fish Channa morulias. It is well-known that Bacillus thuringiensis is biological control agent against the various insects and pests. The biological control methods are very safest and handy. Therefore, 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 positive and Gram negative 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 a known microbial biopesticide, Bacillus thuringiensis, was found to be effective against a good number of isolated pathogens from fish.

Keywords:

Antagonistic activity, Channa morulias, Biopesticides , Bacillus thuringiensis, Fish pathogens.

Introduction:

Fish diseases caused by micro-organisms 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 are 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. 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 craps. 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 Bt-2 and Bt-14 antagonistic to the eel-pathogenic of 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. The data could establish its potential as an environmentally friendly probiotic for eel aquaculture.

Material and Method:

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 1 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 fish pathogens were obtained from diseased fish collected from Chandrapur Dist. and identified using standard procedure. Thus, a total 40 bacterial culture were isolated from skin,gills,intestine operculum, tail region, liver etc. of the fish. Of these 6 common bacterial pathogens were identified are belonging to the genera viz., Klebsiella sp., Pseudomonas sp., E. Coli, Staphylococcus sp., Protius valgaris and Vibrio fluvialis. The isolated fungal cultures were identified at center for higher learning and research in Micrbiology, S.P. College Chandrapur (MS). A total 20 fungal culture were identified, of these 2 common pathogens were identified are belonging to the genera Fusarium sp. and Aspergillus sp. 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 370c with intermittent shaking and the titre inoculum was maintained around 108cfu/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 oC. 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:

Organisms and growth conditions Total 6 different species of bacteria were isolated from Channa morulias fish from Wainganga river which includes, Klebsiella sp., Pseudomonas sp., E. Coli, Staphylococcus sp., Protius valgaris and Vibrio fluvialis. Two common fungus pathogens were identified are belonging to the genera Fusarium sp. and Aspergillus sp.was predominantly present in the Wainganga river water. 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 aeruginosa and Vibrio fluvialis, Protius valgaris and Klebsiella (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 amended with rose bengal (a wide spectrum antibiotic) for 5 days.The inhibitory activities of isolates of Bacillus thuringiensis and Streptomyces antibioticus against. Staphylococcus sp., Streptococcus sp., Bacillus sp., Lactobacilli sp. (Gram +ve),

E. coli , Pseudomonas sp, and Vibrio fluvialis and Klebsiella (Gram -ve), fungi Fusarium sp. and Aspergillus sp. are shown in Table 1. In the case of E. coli maximum growth was inhibited by Streptomyces antibioticus followed by B.t. (B-2),(B-14) and (H-16). On the other hand, in the case of Pseudomonas sp. maximum growth was inhibited by B.t.(B-14).The inhibition efficacy of B.t.(B-14) was found to be superior followed by B.t. (B-2) and (B-16). 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-14). In the case of fungal pathogen, Fusarium sp. and Aspergillus sp. only B.t.(B-16) was found to be effective. Overall, B.t. (B-14) a broad spectrum maximum inhibitory action against all the tested bacterial pathogens but not effective on fungus species . Followed by B.t. (B-2) was inhibitory only to E. coli and Pseudomonas sp. The present investigation has shown 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 morulous 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 (B-16) derived exotoxin does not have any toxic or harmful effect on fish.

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

Antagonistic organism		**Inhibition zone –mm (average of 3 replication)**						
	E. coli	*Proteus Vulgaris sp.*	*Pseudomonas Sp.*	*Klebsiella sp.*	*Staphylococcus sp.*	*Vibrio fluvialis*	*Fusarium sp.*	*Aspergillus sp.*
B.t .(B-2)	41	ND	24	ND	ND	ND	ND	ND
B.t .(B-14)	24	26	44	38	24	18	ND	ND
B.t (B-16)	22	24	28	44	22	17	32	28
S.antibioticus	32	ND	ND	22	ND	ND	ND	ND

ND: Not Detected

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. 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. 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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