MICROBIAL DISEASES OF FISHES AND THEIR BIOLOGICAL CONTROL USING BACILLUS THURINGIENSIS WITH SPECIAL REFERENCES TO CHANNA MARULIAS AND CLARIAS BATRACHUS: A REVIEW

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ABSTRACT

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 two fresh water fishes Clarias batrachus and Channa marulias. 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. The Bacillus thuringiensis represents a group of organisms that occur naturally and can be added to an ecosystem to achieve insect pest control. The present paper reviews the potential of Bt for the control of microbial fish diseases.

Keywords: Bacillus thuringiensis (Bt), Biological control, Channa marulias, Clarias batrachus.

INTRODUCTION

Biological Control of Insects Using Bt

Bt is a facultative anaerobic, gram-positive bacterium that forms characteristic protein inclusions adjacent to the endospore. Bt subspecies can synthesize more than one parasporal inclusion. Bt produces parasporal crystalline inclusions, which are toxic for certain invertebrates, especially species of insect larvae belonging to the insect orders *Coleoptera*, *Diptera* and *Lepidoptera*. The parasporal inclusions are formed by different insecticidal crystal proteins (ICP). The crystals have various shapes (bipyramidal , cuboidal, flat rhomboid, spherical or composite with two crystal types), depending on their ICP composition. A partial correlation between crystal morphology, ICP composition, and bioactivity against target insects has been established.

The genes that encode the ICPs are mostly on plasmids. Each ICP is the product of a single gene. Most plasmids with ICP genes are readily transferred by conjugation between Bt strains and may be transferred to related species of bacteria. The phenotypic classification has now been complemented by molecular biological characterization, based on the sequence of the crystal (*cry* and *cyt*) genes rather than target organism specificity. Different domains of the ICP are responsible for host susceptibility (receptor recognition) and toxicity (pore formation).

The Bt subspecies represents a group of organisms that occur naturally and can be added to an ecosystem to achieve insect control (Andrews et al., 1987; Stahly et al., 1991). In this monograph, a natural habitat is considered to be one where Bt can be isolated when there has been no previous history of application of the organism to that ecosystem, whereas a treated habitat is one where Bt can be isolated after a previous history of application of the organism for insect control. Insecticides formulated with Bt are being manufactured and used worldwide. These commercial Bt products may be applied as an insecticide to foliage, soil, water environments and food storage facilities. After application of Bt to an ecosystem, the

organism may persist as a component of the natural microflora.

Bt, like other members of the genus Bacillus, has the ability to form endospores that are resistant to inactivation by heat and desiccation and that persist in the environment under adverse conditions (Stahly et al., 1991). When considering the degradation of Bt in the environment, it is important to distinguish between changes in the numbers of viable spores and changes in biocidal activity. The survival and activity in the environment has been reviewed by Hansen et al. (1996).

Effects on aquatic vertebrates

The World Health Organization (WHO, 1982) reviewed laboratory and field studies, performed by that time, that examined the impact of Bt on frogs (*Hyla regilla, Rana temporaria*), goldfish (*Carassius auratus*), mosquito fish (*Gambusia affinis*), newts (*Taricha torosa, Triturus vulgaris*), rainwater killifish (*Lucania parva*) and toads (*Bufo* species). No adverse effects were reported. Under static renewal conditions, Boeri (1991) exposed rainbow trout (*Oncorhynchus mykiss*) to high concentrations (100 mg/litre) of a commercial Bta formulation for 96 h and observed no adverse effects (Table 11). Under static renewal conditions, Surprenant (1989) exposed rainbow trout (*Oncorhynchus mykiss*) to high concentrations (100 mg/litre) of a commercial Btte formulation for 96 h and observed no adverse effects.

During 30- or 32-day static renewal tests, bluegill sunfish (*Lepomis macrochirus*), sheepshead minnow (*Cyprinodon variegatus*) and rainbow trout (*Oncorhynchus mykiss*) were exposed to commercial Bti, Btk or Btte formulations at aqueous and dietary concentrations from 100 to 500 times the e x p e c t e d e n v i r o n m e n t a l c o n c e n t r a t i o n s (Christensen, 1990a,b,c,d,e,f,g,h). The results of these studies indicated that exposure to very high concentrations of Bti, Btk and Btte did not adversely affect the survival of these fish, nor did it produce lesions. In the Btk study, the rainbow trout had a 20% mortality during the last 4 days of the study (Christensen,1990b). This effect was attributed to the excessive competition for food that resulted from poor visibility due to the turbidity and the presence of suspended solids encountered in the water. In Canada, Buckner et al. (1974) assessed the impact of Btk on brook trout (*Salvelinus fontinalis* Mitchell), common white suckers (*Catostomus commersoni* Lacepede) and smallmouth bass (*Micropterus dolomieui* Lacepede) during a field trial for spruce budworm control. The fish populations were assessed visually in underwater surveys before and after the spray programme. No effect on their populations was seen. Two analyses of surveys of the impact of the larvicidal campaign in the Onchocerciasis Control Programme of West Africa, which compared fish populations during the programme with the normal yearly fluctuation, observed little or no effects on the non-target populations. However, few details were provided (Yameogo et al., 1988; Levêque et al., 1988; Calamari et al., 1998).

Effects of Bt on non-target organisms

Studies on mammals, particularly those on laboratory animals, have evaluated possible infectivity and toxicity of various Bt preparations, which include the ICPs, vegetative cells and spores. The ICPs, spores and vegetative cells of the Bt subspecies, which were administered by different routes, were mostly non-pathogenic and non-toxic to the various animal species tested. The vegetative cells and/or spores of Bt were demonstrated to persist for weeks without causing adverse effects. Bt has not been observed to adversely affect birds, fish or many other non-target aquatic vertebrates tested in a large number of laboratory and field studies. Relatively few species of aquatic invertebrates are susceptible to Bt under either laboratory or field conditions. Bt does not adversely affect earthworms.

MATERIAL AND METHOD

The Study Organisms

Channa marulias

Channa marulius 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.

Clarias batrachus

Clarias batrachus is a catfish (Froese, Rainer, and Daniel Pauly, eds.2006). It is highly nourishing and esteemed as food. It is mostly used in laboratories for experiment purposes but

also used as a food. The flesh has high nutritive value and its flesh is said to have wound healing effect and recuperative attributes. It is highly suitable for intensive culture due to its air-breathing habit.

The following methodology could be applicable for study of microbial fish diseases;

Sampling for Disease Diagnosis

The ideal specimens for disease investigation or health monitoring are live fish. Samples can be taken from these either on site or transported live to the appropriate laboratory. Transport of live fish Place the fish, representative of the problem, in a plastic bag filled to approximately a third with water and two thirds oxygen. Seal the bag with cable ties or equivalent and place in another bag and seal again. Then place the bag on ice or cool-packs in an insulated box e.g. polystyrene, place more ice on top and seal the container. The maximum transport time depends on water temperature, and the ratio between biomass, water volume and oxygen. As a rough guide the transport time should not exceed 12 hours and the biomass should not exceed one third of the water volume. Transport time is significantly reduced if oxygen is not used.

Live fish weighing more than approximately 300g should not be sent by normal goods transport (air, rail or road), but should either be sampled on site or sent via specialized forms of transport.

Transport of fresh material Unopened fish, reproductive products, virology samples, fish heads (for Myxobolus) may be dispatched for laboratory investigation in the fresh state.

All samples must be chilled to as close to 0°C without freezing. Pack samples in ice and in an insulated container and dispatch. The maximum transport time is 24 hours.

Parasitological Sampling of Fish

a) Examination of skin: first stun the fish with a sharp blow to the head. Then take scrapings for microscopic examination using a scalpel and scrape from front to back of the fish or around the fins (Figure A). Place scraping on a clean glass slide with a drop of water from the holding facility and cover with a coverslip.
b) Examination of gills: following gross examination of the gills, clip a small portion of gill lamellae with sharp scissors and place on glass slide (alternatively scrap the gill lamellae with a scalpel), add a drop of the holding tank water and cover with a coverslip. Examine under low power with high contrast or phase.
c) Examination of other organs: any other organs suspected of having parasitic infection can have squash preparations made from small sub-samples of tissue and examined similarly using light microscopy.
d) Observation and results.

Histological Sampling of Fish

Histology encompasses the scientific area concerned with the structure of tissues and histopathology the relevant branch of pathology. Histopathology can therefore provide information on the processes and changes occurring in tissues and in many cases form the basis for disease diagnosis and prognosis. Accurate sampling of tissues for histology is a vital part in the diagnostic procedure and to follow are guidelines for onsite sampling. Before sampling any fish note any behavioural abnormalities or visible external lesions.

Bacteriological Sampling of Fish

1) Examination and direct inoculation of solid media

Before examining a fish internally, the external body surface, including gills, tail and fins should be examined for the presence of any lesions. Observations should always be recorded on paper. Samples from these sites can be taken by searing the surface with a hot scalpel blade followed by insertion of a sterile bacteriological loop or swab. Material from the loop/swab is then plated out onto suitable agar medium by the spread plate technique.

Once external examination or sampling has been carried out, the body surface is opened to expose the internal organs. Care must be taken not to puncture the gastrointestinal tract. In the absence of any visible internal lesions a sample of kidney is taken and inoculated onto suitable agar medium. The surface of the kidney (or other organ) should be seared with a hot scalpel blade before insertion of the sterile loop.

Agar plates containing the streaked out samples should be incubated and examined

daily for any evidence of growth. The majority of bacterial fish pathogens will grow on Tryptone Soya Agar (TSA) within seven days. Media such as Marine Agar or TSA plus 1.5% salt (NaCl) can be used for marine pathogens.

Kidney smears

Direct examination of Gram stained kidney smears may give an indication of bacterial septicemia and is especially useful in the examination of fish for Bacterial Kidney Disease (BKD). A small portion of kidney is emulsified in a loopful of sterile physiological saline on a microscope slide, allowed to air dry then fixed by passing the slide through a bunsen flame several times. Direct observation of the slide is carried out using the X40 and X100 (oil immersion) objectives after being stained using Gram's method.

Inoculation of an Agar Plate

Petri dishes containing solid medium (agar) are used to provide a large surface of media for the cultivation of micro-organisms. Inoculation of an agar plate is often carried out using the streak plate technique. This involves diluting the culture or other sample. Organisms present in the sample will be separated and after suitable incubation each organism present will give rise to a colony. Although this colony contains many millions of organisms they will have all originated from one, and therefore all organisms in one colony are identical.

By using this method organisms can be cultured in the laboratory and if a mixed culture is present it will become apparent on plating out. This is essential before starting identification procedures as methods are only valid when carried out on pure cultures, i.e. cultures containing one type of organism.

Blood Sampling

Fish blood may be sampled for disease monitoring or health status analyses in the following areas:

i) Hematology (examination of blood cells and blood cell indices), e.g. red blood cell count, haematocrit
ii) Blood biochemistry e.g. hormones, enzymes, etc.
iii) Plasma parameters e.g. plasma chlorides (for salt water challenge tests in salmon)
iv) Serology for pathogen antibodies i.e. salmon pancreas disease virus antibody screening
v) Virology and bacteriology: some micro-organisms can be screened for using frank blood.

Virological Sampling

For virological sampling the fish organs and transport medium/conditions will be determined by the virus/es suspected. The laboratory where samples will be submitted should be contacted prior to sampling.

FISH DISEASES

A) VIRAL DISEASES

Viral Haemorrhagic Septicaemia

Viral Haemorrhagic Septicaemia (VHS) is an infectious disease caused by coldwater rhabdovirus which is of clinical and economic importance in rainbow trout farming in Europe. VHS is notifiable in Ireland.

Pathogen-Enveloped RNA virus belonging to the family Rhabdoviridae, genus Novirhabdovirus.

Infectious haematopoietic necrosis (IHN)

Infectious haematopoietic necrosis (IHN) is an infectious disease, caused by a rhabdovirus,

B) ***BACTERIAL DISEASES***

Mycobacteriosis

Mycobacteriosis in fish is a chronic progressive disease caused by certain bacterial species within the genus *Mycobacterium*. The species in fish are non-tuberculous mycobacteria (NTM) and do not ***cause major disease in healthy humans.***

Coldwater disease

Bacterial coldwater disease (CWD) is a serious septicaemic infection of fishes.

Pathogen- *Flavobacterium psychrophilum*

Bacterial Kidney Disease (BKD)

A serious disease of fresh and seawater, that results in an acute to chronic systemic granulomatous disease.

Pathogen-*Renibacterium salmoninarum*

Enteric Redmouth Disease (ERM)

Bacterial septicemic condition

Pathogen-*Yersinia ruckeri*

Furunculosis
Furunculosis is a fatal epizootic disease.
Pathogen-*Aeromonas salmonicida*

Bacterial Gill Disease
Bacterial gill disease is an important disease in farmed freshwater fishes
Pathogen-*Flavobacterium branchiophila*.

Vibriosis
Vibriosis is the term most commonly used to describe infections associated with *Vibrio anguillarum*,

Francisellosis
Pathogen-*Fransicella philomiragia*

Red mark syndrome (RMS) or Cold water strawberry disease
Red mark syndrome (RMS) is an infectious dermatitis of fishes which does not cause mortality but presents as dramatic haemorrhagic marks on the skin.
Pathogen- Not fully established although *Flavobacterium psychrophilum* and rickettsia-like organisms have been associated with the condition.

C) FUNGAL DISEASES
Saprolegniosis
Saprolegnosis is the term most commonly used to describe infections in fish and fish eggs.
Pathogen- *Saprolegnia parasitica*-
Epizootic ulcerative syndrome (EUS)
Epizootic ulcerative syndrome (EUS) is considered to be an infection with the Oomycetes
Pathogen- *Aphanomyces invadens* has an aseptate fungal-like mycelia structure and this oomycete has two typical zoospore forms.

D) PARASITES
Amoebic and protozoan infections
Costiasis
Costiasis is the term used for infection of fish by costia or *Ichthyobodo necator*, a protozoan parasite of skin and gills.
Pathogen-Ichthyobodo necator

Chilodonella and Trichodina spp.
Trichodina spp. and *Chilodonella* spp. are ciliated protozoans that can give rise to mortalities in young fish due to skin and/or gill damage.

Hexamita sp.
Hexamita sp.are flagellated protozoans often observed in the digestive tract of fish.

Amoebic gill disease
Amoebic gill disease (AGD) of farmed is a significant disease which results in respiratory distress and mortality.
Pathogen- *Neoparamoeba* sp. (originally described as *N. pemaquidensis*, but now *N. perurans*).

Ich or white spot
Ichthyophthirius multifiliis is a pathogenic protozoan ciliate which infects freshwater fishes. Affected fish are lethargic, dark in colour, may flash or rub themselves exhibit respiratory distress and then mortality.

RESULT AND DISCUSSION

From the above study following results were observed;

Bt may be safely used for the control of insect pests of agricultural crops and forests. Bt is safe for use in aquatic environments, including drinking-water reservoirs, for the control of mosquito, blackfly and nuisance insect larvae. Bt products should contain the ICPs and be free from other microorganisms and biologically active metabolites. New Bt products based on either new Bt strains and/or new ICPs require appropriate assessment. Food and Agriculture Organization of the United Nations (FAO) and WHO should develop standard specifications for Bt preparations as is done for chemical pesticides. Good industrial large-scale practice (GILSP) standards should be employed for the production of Bt products. The occurrence of resistant insect populations underscores the need for research on the relationships between *cry*-toxins and the ecology of Bt. More research on the fate of Bt spores and ICPs in the environment is needed. This should cover the natural occurrence of Bt in foods and its relationship to exposure to Bt from its pesticide use. Research into dose–response analysis and the consequent acceptable daily intake levels of Bt in the diet and beverages is a high priority.

Bt has been reported to survive in fresh water and in seawater for more than 70 and 40 days, respectively, at 20^0C (Menon & De Mestral, 1985). A higher percentage of Bt was found to survive for extended periods in lake water than in tap and distilled water, presumably due to the presence of more nutrients in lake water. Bt has not been isolated from any drinking-water supplies. Spores of Bt remained viable for shorter periods when suspended in moving water than when in static bottles, indicating that static laboratory trials may overestimate the longevity of these spores in the environment (Yousten et al., 1992).

CONCLUSION

Fisheries & aquaculture is gaining additional emphasis due to our concern in sustainability, greener solutions, conservation & food security. Detail studies microbial diseases in a fish species very much relevant in order to put forward conservation protocols and to propose newer & improved culture practices. Establishment of *Clarias batrachus* and *Channa morulias* in several continents & its popularity as a freshwater culturable fish species among consumers made the species suitable for meticulous reviews with respect to various parameters. Accordingly the demand for above mentioned fishes throughout the world is increasing & their several beneficial aspects remain as a hit among the Asians in particular. Besides in order to protect the genetic resources of these species from unwanted hybridization, which the species is very much vulnerable, the fish geneticists & the government bodies should work together. Habitat protection & sustainable consumption of this excellent fish species is the call of the day. Intensive aquaculture in the rural water bodies of Chandrapur and Gadchiroli with very little infrastructure development may bring-about socioeconomic development in many parts these tribal districts.

Owing to their specific mode of action, Bt products are unlikely to pose any hazard to humans or other vertebrates or to the great majority of non target invertebrates provided that they are free from non-Bt microorganisms and biologically active products other than the ICPs. Bt products may be safely used for the control of insect pests of agricultural and horticultural crops as well as forests. They are also safe for use in aquatic environments including drinking-water reservoirs for the control of mosquito, black fly and nuisance insect larvae. However, it should be noted that vegetative Bt has the potential for the production of toxins, the significance of which as a cause of human disease is not known.

An extensive literature exists on the consequences of exposure of non target organisms to Bt, including reports of several long-term field studies. The data have been reviewed periodically (e.g., WHO, 1982; Lacey & Mulla, 1990; Melin & Cozzi, 1990; Molloy, 1992; Otvos & Vanderveen, 1993). The range of non-target species that have been found to be susceptible to direct toxic action of Bt has remained small. A list of non-target species found to be insensitive to Bt was issued by Keller & Langenbruch (1993). In more than 30 years of commercial use, noserious, direct effects on non target organisms have been reported as arising from Bt based Microbial Pest Control Agents (MPCAs). Several studies which identified effects of Bt on predators or parasitoids of susceptible insect species are listed by Navon (1993), but the effects have been small. Mortality in bees has been observed after exposure to vegetatively growing Bt but the effect does not seem to be related to spores or ICPs.

Dietary exposure of the general population in some Asian countries, Bt has been added to domestic containers of drinking water for mosquito control. From these high Bt exposures in drinking water, no adverse effects in humans have been reported. In Africa, some rivers have been dosed with Bt at weekly intervals for blackfly control. No adverse effects in the human populations that drink the river water have been reported. Bt has been reported to survive for 1 to 2 months in fresh water and in seawater. However, viable Bt cultures have not been isolated from drinking water supplies (Menon & De Mestral, 1985).

This review 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 reviews it is found that Bt could be used as a potent and safe biological control agent against the microbial diseases of fishes like *Clarias batrachus* and *Channa morulias.*

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- *Bacillus thuringiensis :- (Bt)*
- Insecticidal crystal proteins :- (ICP)
- Crystal genes:- (*cry* and *cyt*)
- World Health Organization:- (WHO)
- Tryptone Soya Agar :- (TSA)
- Bacterial Kidney Disease :- (BKD)
- Viral Haemorrhagic Septicaemia :- (VHS)
- Infectious haematopoietic necrosis :- (IHN)
- Non-tuberculous mycobacteria :- (NTM)
- Coldwater disease :- (CWD)
- *Enteric Redmouth Disease :- (ERM)*
- *Red mark syndrome :- (RMS)*
- *Epizootic ulcerative syndrome :- (EUS)*
- Amoebic gill disease :- (AGD)
- Food and Agriculture Organization of the United Nations:- (FAO)
- Good industrial large-scale practice:- (GILSP)
- Microbial Pest Control Agents :- (MPCAs)
- Genetically modified organ isms :- (GMOs).