GENERAL INTRODUCTION

The worldwide production of cultured fish increases every year due to the research and developmental advances in the aquaculture technologies. In general, various species of marine and fresh water fish have been cultured in enclosed spaces such as ponds or net cages with the intention of increasing the productivity per unit space. However, overcrowding tends to produce poor physiological environment for fish with increased susceptibility to infections.

It is also known that the diseases of aquatic organisms differ in several important ways from diseases of terrestrial animals. The culturable aquatic organisms come in direct contact with the pathogenic microbes, and they are continuously surrounded by the same medium in which such microbes grow, multiply and get transported. Thus a disease condition in one organism will inevitably result in a direct challenge by the pathogen to all the other fish or shrimp in the same pond or farm. The factors, which influence the ecology of the water masses, will affect the growth and survival of the disease-producing microbes, and reduce the ability of the cultured species to resist infection. The success of commercial aquaculture is therefore dependant on good water quality and on the success in preventing disease outbreaks.

The culturable species of fish are prone to a variety of infectious agents including, the viruses, bacteria, fungi and protozoa. In India, very little attention is paid to study the viral diseases, whereas a number of reports are available about the rest of the microbial agents. Of these, the bacterial pathogens at times initiates acute infections and cause total mortality. Predominant bacterial pathogens belong to the Gram-negative group. As more and more culture or rearing and hatchery attempts are carried out, new diseases also start appearing. Considering this, it becomes imperative to isolate, characterize and record (catalogue) the bacterial pathogens as well as the host responses towards the invaded pathogen.
Apart from improved nutrition and minimizing stress conditions, the effective health management programme emphasizes a systematic approach to routine health monitoring to detect early stress, use of sensitive detecting techniques and preventive therapy (Subasinghe and Shariff, 1994).

Among the different early disease diagnostic methods, the hematological investigations were studied extensively. Of these, measurement of hemoglobin, hematocrit, erythrocyte and leucocyte counts were considered as important ones in assessing the health of fish as well as monitoring the qualitative and morphological phenomena occurring in fish blood subsequent to exposure to pathogen; sub-lethal concentrations of pollutants as well as vaccine or bacterin administration. Therefore, investigations on fish hematology and detection of changes in the blood cells form an important prophylactic component in the case of acute infections (Rehulka, 1996). The hematological changes are to be documented taking into account the variations with regard to age, development, starvation, temperature and other multivariable physiological and pathological conditions. In addition, changes in the level of serum or plasma protein are important parameters in discussing specific disease conditions as well as progression of the disease in general. The information available on the normal blood parameter of *Etroplus maculatus* is scanty. There is no published account also regarding the changes in the blood due to the disease process.

In aquaculture, medication of fish cannot be restricted to the diseased individuals, which is possible with terrestrial animals. Although various chemotherapeutics have been used to treat the microbial infections for the past 20 years, such usage represents a treatment of a complete aquatic ecosystem, which results in the selection of resistant microbial strains. It also alters the commensal association between the host organisms and the microbes, which in the healthy aquatic community, constitutes a protective barrier against infection. Application of chemotherapeutants will give a short relief only and may even render fish and shrimp susceptible to secondary infections. The incidence of drug-resistance bacteria has become a major problem in fish culture (Raa *et al.*, 1992).

Much earlier research information highlights the influence of environmental pollutants, particularly on chemotherapeutics and organic pollutants and their influence on immune system of animals. It is inferred that both the humoral and cellular immune functions
and, consequently health and resistance to infections were adversely affected (Studnicka et al., 2000).

Considering these aspects, the disease control or disease management in aquaculture should focus on preventive measures related to water quality, technology and husbandry, and be combined with efforts to eliminate factors, which precipitate the disease conditions. Besides, the level of resistance to infection in the cultured organisms should be increased to reduce the risk of disease. It is possible to achieve through vaccines, immunostimulants and selective breeding.

In many of the developed countries, specific immunization programme has become a proven and accepted tool in integrated and comprehensive aspect of aquatic animal health management since its inception about 50 years ago. The prophylactic use of vaccines can significantly reduce mortality and other disease related losses, lower the unit cost of production, reduce the dependence for drugs (chemicals) and improve the general overall quality of the product (Busch, 1994). Already vaccines are commercially available for bacterial infections such as vibriosis, redmouth disease and furunculosis and for viral infections such as IPN (Leong-Jo-Ann, 1993). Vaccination may be hence one of the most effective methods of controlling fish disease, although it is specific to a particular pathogen.

Unlike vaccines or bacterins, which trigger the production of specific antibodies towards one particular pathogen, the immunostimulants enhance the non-specific defense mechanisms in animals (Fenichel and Chirigos, 1984). The active principles of immunostimulatory cell wall preparations are various muramylpeptide fragments, lipopolysaccharides (LPS), lipopeptides, acyl-oligopeptides and specific bacterial peptides. Administration of various immunostimulants in several fish species has been successful in enhancing non-specific immune responses as well as in providing some protection against infection (Anderson, 1992 and Duncan and Klesius, 1996). The immunostimulants represents an alternative as well as a supplement to vaccines in the protection of farmed fish. As there is no memory component and the response is likely to be of short duration, use of immunostimulants is considered as an effective means of increasing the immuno-competency and disease resistance.
Immunostimulants can be administered before, with or after vaccines to amplify the specific immune response in general elevation of humoral antibody levels and the number of antibody-secreting cells (ASC) (Burrels and Williams, 1998; Siwicki et al., 1998; Sakai, 1999). Enhanced resistance to infections was reported when the immunostimulants were administered along with vaccine (Nikl et al., 1991). Field trials and commercial experiences in intensive rearing conditions have shown that the inclusion of immunostimulants in the feed can reduce or even prevent disease outbreaks (Anon, 1997). This protection is particularly important for fish that are raised in or released to environments where the nature of pathogens is unknown and immunizations by specific vaccines may be futile (Sahoo and Mukerjee, 1999). Thus, the use of immunostimulants may be an effective means of increasing the immunocompetency and disease resistance of fish, thereby minimize the need to medicate or vaccinate fish during epizootics of certain pathogens. However, the importance of immunostimulants as prophylactic agents in cultured fish has received little attention (Park and Jeong, 1995).

Considering the bacterial disease and the need for developing effective eco-friendly managerial strategies, the thesis was initiated with the following objectives:

1. To isolate and characterize the pathogenic bacteria from infected Etroplus maculatus.
2. To evaluate the lethal time and lethal value of the pathogenic bacterial isolate.
3. To study the enzymatic activities of the exocellular products produced by the bacteria and characterize the exocellular protein responsible for the infection and necrosis.
4. To detect the tissue level changes after infection using the histological methods.
5. To quantify the hematological alterations in E. maculatus produced by the bacterial cells as well as the exocellular products of the bacteria.
7. To develop bacterin and to evaluate the Percent Relative Protection offered by the bacterin using challenge with the same pathogenic bacterial isolate.
8. To evaluate the combination of vaccine and immunostimulants on the protection against the specific disease conditions in E. maculatus.