INTRODUCTION

Aquatic ecosystems are well utilized for aquaculture in recent decades. The use of unutilized resources and modern technology hiked aquaculture production. However, the number of outbreaks of bacterial diseases in cultured fish has also increased and it causes decline in aquaculture production. Fish health is closely associated with characteristics of aquatic environment and the microbes present. The intimate contact with water which, eases the movement of chemicals into and through the mucus coated skin, and other external layers becomes a disadvantage to the fish when nefarious chemicals, pollutants, and contaminants enter the aquatic environment (Adams, 1990, Wester *et al.*, 1994). These chemicals can have adverse effects on the fish's physiological pathways including the important mechanisms that help to protect the fish against diseases *viz* the nonspecific and specific immune responses. Without complete immune protection and barriers against viral, bacterial, fungal and protozoan pathogens that are ubiquitous in the environment, the animal is disadvantaged and become susceptible to disease-causing agents. In addition, during their whole life, farmed fishes are inevitably subjected to various stresses like handling, transportation, crowding, infections, exposure to pollutants, and physiological changes that may lead to immunosuppression and consequent infections. Even though vaccines are available in developed countries against a few diseases, aquaculture still experiences high loss of fish stocks due to outbreak of diseases. Part of this is because even efficient vaccines lose their effect one year after vaccination, and new pathogens are constantly gaining territory (Robertsen *et al.,* 1994). Further, it seems unlikely that cultured fish can be vaccinated against all potential diseases. Hence, the significance of a suitable client in preserving the health of living organisms is widely recognized (Lygren *et al.,*1998).

In recent years, the increasing consumer concern about the residues of antibiotics, hormones, growth promotors, and the danger of development of antibiotic resistant strains has led to the use of **immunostimulants** in aquaculture. By definition, immunostimulants are substances that can enhance the nonspecific defense mechanisms as well as specific immune response if the treatment is followed by infection or vaccination (Anderson, 1992a). Many natural and synthetic substances have been reported that potentiate the fish immune system and increase disease resistance (Klontz and Anderson, 1970, Amend and Flender, 1976, Munn and Trust, 1983, Siegel *et al.*, 1983,Blazer and Wolke,1984, Oliver *et al.*, 1985, Cossarini, 1985, Azuma and Jolles, 1987, Grayson *et al.*, 1987, Kitao *et al.*, 1987, Yano *et al.*, 1993; Anderson *et al.*, 1989, Kijita *et al.*, 1990, Siwiki *et al.*, 1990, Anderson, 1992a , Logambal,1996, Rajkumar,1996 , Jayameena, 1997, Hemapriya, 1997, Venkatalakshmi and Michael ,2000, Logambal *et al,* 2000, and Sudhakaran *et al.*, 2006). The search for new immunostimulants continues as an attempt to improve intensive fish farming. According to Rodriguez (2004) such new products should posses two characteristics.

1. Provide general stimulation and be
2. Economically affordable.

These two characters are well fulfilled by whole microorganisms.

1. First, they are rich sources of immunostimulant substances such as β -glucans, chitin, vitamins, genetic material etc. At the same time, they act as a source of nutrients and micronutrients that affect the general fish physiology.
2. They are cheap sources of immunostimulants. New strains can be generated by genetically manipulating strains with a high content of specific substances.

This concept of using beneficial whole microorganisms is termed as **"probiotics"** and is gaining importance, as an eco-friendly disease management tool. In addition to disease resistance, they also improve water quality and growth of farmed fish. Probiotics are defined as microbial dietary adjuvants that beneficially affect the host physiology by modulating mucosal and systemic immunity as well as improving nutritional and microbial balance in the intestinal tract (Fuller, 1989). Beneficial bacteria in the best cases could be used to substitute the use of antibiotics as preventive agents of disease (Nikoskalainen *et al.*, 2001) and as growth promoters (Byun *et al.*, 1997). Immunomodulation by probiotics have been well documented in mammals including man (Gill 1998, Kato *et al* 1984, Kato *et al* 1999, Ogawa *et al* 2001, Malin *et al* 1996). The immunomodulatory role of microbes as far as known, still needs elaborate investigations in fish. Recently there are a few studies that explore the immunomodulatory role of probiotics in fish. The non specific immunostmulation and colonizing efficiency in gut, skin, mucous of *Lactobacillus rhamnosus* were studied in rainbow trout (Nikoskalainen, *et al.*, 2003) and in turbot (Villamil *et al* 2002). The probiotic yeast cells *Saccharomyces cerevisiae* was recorded for its immunostimulatory activities in rainbow trout (Ortuno *et al* 2002) and in gilt head seabream (Rodriguez *et al* 2004). A mutant fungal probiotic *Mucor circinelloides* was demonstrated to be more effective in immunostimulation than the wild strains in sea bream (Rodriguez *et al*, 2004).

Immunologists are working for a long period in the field of immunomodulators, including immunosuppressors and stimulators, on the immune protection in fish, and their putative points of action

(Anderson, 1996). Many of the assays for detecting the changes in the protective mechanisms of fish due to immunomodulation are derived from those used in fish disease diagnostics and immunization programs. Study of specific immune response may include measuring the levels of humoral antibody with tests such as ELISA (Arkoosh and Kaattari, 1987), complement fixation test (Ingram, 1990) and microtiter agglutination (Roberson, 1990). For these assays, an immunization regimen is required to induce the specific antibody and specific cellular responses. Teleost fishes were shown to exhibit humoral immune response to a variety of antigens (Sailendri and Muthukaruppan, 1975; Sailendri,1973, Michael and Pricilla, 1994; Rajavarthini and Michael, 1996; Sudan and Michael,1995; Mohan, 1997; Ali, 1988; Jayaraman *et al.*, 1979) and has been used for studying a wide variety of immunomodulators (Anderson, 1996; Rajkumar, 1996; Logambal, 1996; Venkatalaksmi and Michael, 2000; Binu Ramesh *et al.*, 2006; Prabhakar *et al.*, 2006).

The assays for testing changes in the non specific defense system may require more background training and insight of the technician because they often require more detailed microscope work or sophisticated equipments to record the chemical changes.(Anderson, 1996). There are many immunological assays for analysis of non specific defense mechanisms like NBT Assay (Nitroblue Tetrazolium Assay) (Anderson *et al.*, 1992b), phagocytic assay (Sovenyi and Kusuda, 1987), lysozyme assays (Ellis, 1992) chemiluminescent response, pinocytosis assay chemotaxis assay (Mathews *et al.*, 1992). These assays of macrophage function have been tested on several species of fish and the results indicate that such methods can be applied as bioindicators of fish health (Warinner *et al.*, 1998; Weeks and Warinner, 1984; Weeks *et al.*, 1986; 1987, Venkatalakshmi and Michael, 2000; and Logambal *et al.*, 2003).

An initial step in many immunological studies is to collect and examine the leukocytes (Rowley, 1990). According to Anderson, (1996) though the hematological assays are generally less sensitive as bioindicators of immunomodulation, they are easy to do and technical training and laboratory requirements are minimal.

Knittel (1981) suggested that the ultimate test for modulation of the immune system by a substance is to show increased resistance to disease by comparing the test animals to control fish. The use of probiotics to displace pathogens by competitive processes or by release of growth inhibitors is now gaining acceptance in the animal industry as a better, cheaper and more effective remedy than administering antibiotics to promote health of animals (Westerdahl *et al.*, 1991). The increasing concern about the danger of development of antibiotic resistant strains and residues of antibiotics have lead to the use of biological or probiotic feed additives in the animal feeds (Verschuere *et al.*, 2000a). For most of the applications, it is recommended to use probiotic strains that are able to survive the gastro intestinal passage and have the capacity to adhere to the host intestinal tissues in order to prolong their health effects

(Fuller, 1986,1989; Havenaar *et al.*, 1992; Ouwehand *et al.*, 1999). While most of the probiotics are completely excreted a few days after ingestion (Joborn, 1998; Marteau and Vesa, 1998; Robertson *et al.*, 2000; Nikoskalainen *et al.*, 2003), only a transient colonization (Nikoskalainen *et al.*, 2003; Joborm, 1998 and Gatesoupe,1999) or *in vitro* adhesion in the gut (Villamil *et al.*,, 2002) was shown to the best of our knowledge. Of course the fungal probiotics *Debaryomyces hansenii* and *Rhodotorula glutinis* were shown to inhabit the gut of *Onchorynchus mykiss* juveniles for 30 days and 65 days respectively (Andlid *et al.*, 1995). The bacterial probiotics, which are well studied and widely used in aquaculture, belong to the genus Lactobacillus has good records of so many probiotic characters. However, no observations are available for prolonged gut colonization of Lactobacillus.

Probiotics are described as live microbial feed supplements (Fuller, 1989) or as water additives (Moriarity, 1998). Gatesoupe (1999) insisted that microbial cells that are administered in such a way to enter the gastro intestinal tract and to be kept alive with the aim of improving health, alone can be designated as probiotics. Verschuere *et al.*, 2002a argued that even if a probiotic is not able to colonize the gut, it should be able to adhere to the aquatic animal associated tissues like mucous or skin. If a probiotic fails to colonize, a regular administration of the same is required to obtain its effects (Marteau and Vesa, 1998). To overcome this problem, in the present study, an attempt is made to utilize all the benefits of probiotics without intestinal colonization using immobilization technology. Recent researches on immobilized cell (IC) technology applied to probiotic cultures have emphasized the importance of, and, interest in this new technology. In dairy industry, IC technology has been shown to offer many advantages for biomass and metabolite production compared to free cell systems such as high cell density and very high volumetric productivity (Doleyres *et al.*, 2004a), reuse of biocatalysts, high process stability (physical and biological) over long fermentation periods (Lamboley *et al.*, 1997) and retention of plasmid bearing cells (D-Angio *et al.*, 1994; Huang *et al.*, 1996). Improved resistance to contamination, uncoupling of biomass and metabolite production, stimulation of production and secretion of secondary metabolites and physical and chemical protection of cells are also recorded (Doleyres *et al.*, 2004b; Doleyres and Lacroix, 2004 and Lacroix *et al.*, 2004). However these benefits have not yet been exploited for the aquaculture industry.

A simple indicator for assessing the pressure of any chemical or biological agent on fish is growth. Growth and food utilization have significant relationship with fish health and aquaculture production. The various dietary components like Protein (Akiyama *et al.*, 1981) Lipid (Bromely, 1980) Fatty acids (Paul *et al.*, 1996) Carbhohydrates (Bergot, 1979; Beamish *et al.*, 1986) as well as quality of diet (Ebanasar and Jayaprakas, 1995b) have been found to contribute significantly to growth and biochemical composition of fishes. In addition to this, feed additives like vitamins, (Naik *et al.*, 1999; Murthy and Naik, 2000, 2002; Bhanja *et al.*, 2001; Muruganandham *et al.*, 2003, 2004a and b), hormones

(Nirmala and Pandian, 1983; Arul, 1986; Jayaprakas and Sindhu 1994 & Jayaprakash and Sambhu, 1994) and growth promoters (Unnikrishnan, 1995; Sambhu and Jayaprakas, 2001 & Abraham *et al*, 2001) and herbal products (Eswari, 2004) also have significant role on growth and food utilization of fishes. An understanding of the influence of any water additives or feed supplements to be used in aquaculture, on growth and food utilization of fish is essential for obtaining high production potential.

Aeromonas hydrophila, is a causative agent of one of the major fish bacterial diseases in India. A wide antigenic variety of motile *Aeromonas* markedly limits the development of vaccines (Newmann, 1993, Stevenson, 1998). However vaccines to protect farmed fish against *A. hydrophila* are not commercially available to date (Anbarasu *et al.*, 1998). Pretreatment with glucan before vaccination increased the antibody production (Selvaraj *et al.*, 2005). Similarly Venkatalakshmi and Michael (2001) and Logambal *et al.* (2000) showed the potential of *Ocimum sanctum* leaf extracts in stimulating the immune response of vaccinated tilapia against *A. hydrophila.* Five candidate probiotics isolated from clown fish was shown to compete with *A hydrophila* and *V alginolyticus* reducing pathogen attachment (Vine *et al.*, 2004). Probiotics, along with herbal treatment significantly boost recovery from *A hydrophila* infection (Harikrishnan and Balasundaram, 2005). Residual problems and development of resistant genes arising with the use of antibiotics, threaten the environment. In fact, *Aeromonas hydrophila* is reported to be resistant to almost all antibiotics. In this scenario, probiotics seems to be the better alternative.

Tilapia is often used as a good experimental model and is extensively used in genetic and physiological studies in relation to pollution, stress, or growth promotors (Baskaran *et al,* 1989; Pratap *et al,* 1989; Pandibaskaran and Palanichami, 1990; Varadharajan and Subramanian,1991; Jones Nelson and Sunilkumar, 1996; Ramakrishnan *et al.,* 1991; Ebanasar and Kavitha,2003). Tilapia is selected as the experimental model because of its economical importance worldwide and also for the following reasons (Popma and Masseri, 1999).

1. Tilapia eat a wide range of natural food organisms; tolerate poor water quality and easily spawned.
2. Worldwide harvest of farmed tilapia has now surpassed 800,000 metric tons and of the most widely farmed fresh water fish in the world, tilapia are second only to carps.
3. Tilapia is a good fish for warm water aquaculture.
4. Consumers like tilapia for their firm flesh and mild flavor.
5. It is a regular and prolific breeder. So all sizes and maturity stages are available through out the year.

In the view of the above considerations, the present treatise proposes to study the effect of probiotic bacteria on the health, growth, food utilization and immunomodulation of *Oreochromis mossambicus.*

The probiotic bacteria selected for the present study is *Lactobacillus sporogenes,* which is a human probiotic. A human probiotic is purposely selected because fish are for human consumption (Nikoskalainen, 2001 b).

The objectives for the present study was to investigate the effect of administration of the bacterial probiotic *Lactobacillus sporogenes* as water additive, feed supplement or immobilized cells in the adult tilapia *Oreochromis mossambicus* on:

- specific antibody response to the fish pathogen *Aeromonas hydrophila*
- non specific immune response, i.e. number of activated neutrophils
- total peripheral blood leukocytes
- differential count of blood leukocytes
- host disease resistance and efficiency of probiotic
- *Lactobacillus sporogenes* to
- colonization of fish gut
- survival in tank water.

In addition to this, growth and food utilization efficiency of tilapia fingerlings are also assayed.