CHAPTER 1

1. GENERAL INTRODUCTION

Probiotics are live microorganisms thought to be healthy for the host organism. Probiotic terms derived from Greek words Pro (favour) and bios (life). So in purely literary terms probiotics can be defined as organism or substance that favour our life (Saurabh et al., 2005). The word “Probiotic” was introduced by Parker, 1974. According to his original definition, probiotics are organisms and substances which contribute to intestinal microbial balance. Probiotics as ‘a live microbial feed supplement that beneficially affects the host animal by improving its intestinal balance. “Probiotics” generally includes Bacteria, Cyanobacteria, Microalgae, Fungi, etc. (Fuller, 1992).

1.1. Probiotics

Probiotics are defined as live microbial feed supplements that improve the health of man and terrestrial livestock by its valuable secondary products. This microbial cell that is administered in to our gastrointestinal tract and alive with the aim of improving health without any negative effects. Similarly, in nature some of the microbes present in the gill and digestive tract of the fish can provide the security from disease causing pathogenic microbes. Past few years, the research in the field of probiotics for aquatic animals is increasing with a demand for environment friendly aquaculture (Fuller, 1992). The gastro-intestinal micro biota of fish and shellfish are peculiarity dependent on the external
environment, due to the water flow passing through the digestive tract. Most bacterial cells are transient in the gut, with continuous intrusion of microbes coming from water and food (Newbold, 1995).

1.2. Probiotics Natural Environment

Most attempts to propose probiotics have been undertaken by isolating and selecting microbes from aquatic environment such as *Pseudomonas*, Lactic Acid Bacteria, *Bacillus* and yeasts (Gatesoupe, 1999). Probiotics generally include *Loctobacillus*, *Bacillus*, *Pseudomonas*, yeasts and algae (Rahana, 2003).

The use of probiotics in the culture of aquatic organisms is increasing with demand for more environment friendly aquaculture practices (Gatesoupe, 1999). Today aquaculture aims at increasing production, which leads to a shift in culture practice from extensive and intensive culture. During past two decades aquaculture industry has seen tremendous growth especially for economically important fresh water fishes, shrimp, marine fish and bivalves. The increasing amount of the world fish is being supplied while catches of wild fish have stagnated at approximately 90 million metric tons amount of formed fish has increased from 10 million metric tons in 1984 to more than 20 million metric tons in 1996 (Lone et al., 1999). These intensive culture practices with high stocking density also pose threat of various diseases out breaks due to degradation of water quality and soil parameters. The poor water quality after results in disease out breaks resulting in huge economic loses (Laxmi et al., 2003).
1.3. Probiotics and their Uses

Intake of probiotics could improve intestinal microbial balance, leading to enhanced absorption of food, increased activity of digestive enzyme and reduced pathogenic problems in the gastrointestinal tract (Balcazar et al., 2006; Vine et al., 2006). The nutrition of the aquatic organisms is essential for their profitable aquaculture, and the formulation of effective feed depends on our knowledge of the nutritional biochemistry and physiology of the cultured species (Lemos et al., 2000). A successful probiotic is expected to have a few specific properties in order to certify a beneficial effect. In order to have a beneficial effect in the form of growth promotion via digestive enzymes, the strains should also have the capacity to colonize the fish by adhesion (Olsson et al., 1992) and to produce digestive enzymes.

Bacteria live in every corner of the aquatic environment. The fish egg is the first stage of a fish life cycle that could be exposed to bacteria. Therefore, a relatively dense, non-pathogenic, and diverse adherent microbiota present on the eggs would probably be an effectiveness barrier against the formation of a colony by pathogens on fish eggs. Fish probiotics are defined as harmless bacterial strains to prevent or control fish diseases caused by pathogenic bacteria as well as boosting growth performance in farmed fish (Gatesoupe, 2000). Fish probiotic microorganisms will of course have to be non-pathogenic and non-toxic in order to avoid undesirable side-effects when administered to fish.
1.4. Microbial Feed Additive

The use of microbial feed additives (probiotics or direct fed microbials (DFM)) and enzymes in animal diets is not new. In 1924, Eckles and Williams have published a report on the use of yeast as a supplementary feed for lactating cows, while in 1947, Mllgaard reported improvements in health and skeletal formation in pigs with impaired mineral absorption supplemented with lactic acid Bacillus. However, it is only in the last two decades that a clear consensus has started to develop on how addition of such additives to the diet might stimulate productivity in farm animals.

Enzymes are used more as a feed treatment than as a supplement. Their purpose, principally, is to degrade indigestible or anti nutritional factors (such as protease inhibitors) within the feed, to improve digestibility of poor-quality feed or to remove inhibitors of digestion. Improvements to health are brought about by the removal of these anti nutrients and by rendering poorly digestible carbohydrates into a form that is digested in the ileum, resulting in a reduction in fermentable substrate entering the large intestine. Although the enzyme may be added to the feed rather than applied as a pretreatment, the principle is the same. The enzymes act on components of the feed, not on the digestive processes of the animal or on its microflora. Non starch polysaccharides (NSP), such as xylans and glucans in poultry feeds, can cause poor digestibility, resulting in sticky litter and hock burn in the birds. These problems have been successfully treated with xylanases and glucanases in the feed (Pettersson and Aman, 1989).
Bacillus sp. have been widely used as potential probiotics (Ziaei-Nejad et al., 2004), since they secrete a variety of antimicrobial compounds and exo enzymes (Moriarty, 1997). Rengpipat et al. (2000) reported that use of Bacillus sp. (strain S11) provides pathogenical protection by activating both cellular and immune defenses. However, only a few studies have been published on the nutritional effects of the Bacillus, particularly their effects on digestive enzyme activities of fishes.

The Bacillus sp produces siderospheres, growth hormones and enzymes and solubilize the phosphorus that enhanced the growth of paddy. The selection of probiotics for usually based on their antagonistic towards the pathogens. Bacillus sp. that strain was antagonistic to 63% of the isolate from the fish intestine (Gatesoupe 1999). Bacillus produces bacteriocins, siderophores, lysozymes, protease, hydrogen peroxides that are inhibit the pathogenic microbes (Laxmi et al., 2003). In aquaculture practice water quality deteriorates mainly due to accumulation of metabolic waste. Bacillus sp degrade organic matter facilities nutrients recycling competes with other pathogenic bacteria (Moriarty, 1997). Bacillus sp. are reported to more efficiently improve water quality for e.g., Bacillus sp also reduced the quantity of ammonia, nitrite in the water (Laxmi et al., 2003).

1.5. Significance of Probiotics

Probiotics are of considerable significance in fermentation industries for production of Enzymes and fermented foods of some probiotic Bacillus possess amylase enzyme activity this enzyme has more effective than common Bacillus
flora (Ara et al., 1993). Probiotic *Bacillus* sp are important constituents of the intestinal microflora of fish and animals. These organisms are the predominant group of bacteria in the normal intestinal flora of healthy fishes where they constitute more than 95% of the total population (Yildirim et al., 1999). Recently it has been reported that many beneficial effects on fish health. High numbers of probiotic *Bacillus* sp are reported to form a barrier pathogens by prohibiting colonization or by controlling the intestinal pH through the release of acetic and lactic acids, Enzymes and stimulate the immune response of the host, for these reasons they have been incorporated into various commercial fish foods.

*Bacillus* is currently being used for oral bacterio therapy and bacterioprophylaxis of gastrointestinal disorders (mostly as a direct result of antibiotic treatment), many of which lead to diarrhoea. Ingestion of significant quantities of *B.cereus* thought to restore the normal microbial flora following extensive antibiotic use or illness. Probiotic preparations of *B.cereus* are sold commercially in most European countries, although little is understood about how these bacteria exert their therapeutic benefit. *B.cereus* is a gram-positive, nonpathogenic, spore-forming organism normally found in the soil, and the robustness of spores is thought to enable passage across the gastric barrier, where a proportion of spores germinate in the small intestine and populate, albeit briefly, the intestinal tract (Mazza, 1994). In addition, the clinical effects of *B.cereus* as an immune stimulatory agent in a variety of diseases, as an *in vitro* and *in vivo* stimulant of secretary immunoglobulin A and as an *in vitro* mitogenic agent have been documented. Other topical examples of such probiotic bacteria are the gram-positive Lactobacilli and Lactococci, which are sold commercially
for both human and veterinary use. *B. cereus* is a medically important gram-positive microorganism can serve as a probiotic agent (Yuksekdağ *et al.*, 2004).

*Bacillus* groups have been reported to produce antimicrobial compounds, which show a great variety as to their inhibition spectrum. Antimicrobial substance produced are smaller peptides and do not contribute to the formation of resistance to pathogens. Feed conservation rate is decreased due to the fact that many of the probiotic microorganisms produce enzymes like amylase, protease and lipase, whether in gut or in environment. The pathogens inhibited by probiotic microorganisms the process is called competitive inhibition (Fuller, 1989). *Bacillus* sp were isolated from primary clarifier of dairy effluent, this strain has the ability to ferment lactose as well sporulate under stress environment (Sreekumar and Soundarajan, 2010). The quality of successful probiotic organisms is determined by its extracellular secretions such as enzymes, by products and antibiotics these secretions are highly temperature sensitive.

The *Bacillus* showed good protein profile at 35°C which is an optimum temperature for a better probiotic activity. The strain also showed stress related adaptation for a thermal stress of 40°C by inducing 30 kDa protein, this fact would suggest that *Bacillus cereus* is able to increase the synthesis of a number of thermal responsive proteins to cope with increasing temperatures. At the same time, it was observed that the repression of many proteins occurred at 40°C compared with the profile at 35°C, indicating that not only induction but also repression controls were set up at 40°C. Therefore it is proved that *Bacillus*
*cereus* is capable to survive under the internal gut temperatures and express its probiotic activity (Sreekumar and Soundarajan, 2010).

Fluorescent Pseudomonads have been used as biocontrol agents in several rhizosphere studies (Sullivan and Gara, 1992), where their inhibitory activity has been attributed to a number of factors, such as the production of antibiotics (Lemos *et al*., 1985), or iron-chelating siderophores (Kloepper *et al*., 1980). Pseudomonads constitute a large part of the microflora of the gills, skin and intestinal tracts of live fish (Buyer, 1994; Shanahan *et al*., 1992) and are only rarely reported as pathogens of fish. Aquatic Pseudomonads are often antagonistic against other microorganisms, including fish pathogenic bacteria and fish-pathogenic fungi. One study demonstrated that bathing Atlantic salmon presmolts in a strain of *Pseudomonas fluorescens* reduced subsequent mortality from stress induced furunculosis. When tested in vitro, iron limitation has been found to facilitate the antibacterial activity of fluorescent pseudomonads (Gram, 1999).

Thus, inhibition may be due to the production of siderophores, which deprive the fish pathogen of iron. Production of siderophores is a virulence factor in many microorganisms, such as members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Vibrio anguillarum* (Wooldridge and Williams, 1993).

The four digestive enzymes amylase, protease, cellulase and lipase play an important role in fish nutrition. Without these enzymes, fish cannot survive due to unutilization of nutrients. Probiotic microflora can able to produce
digestive enzymes. Starch is a polysaccharide which composed of glucose monomers. Starch is digested by amylase enzyme which breakdown the starch into glucose (Parker, 1974). This glucose is easily utilized fish for their growth. Lipid is an essential nutrient for fish and it is a complex molecule composed of glycerol and fatty acids. The lipolytic enzyme split the lipid into glycerol and fatty acids. The fish utilize the essential fatty acids for their metabolism. Proteins are complex molecules composed of number by peptide linkages. Proteolytic enzymes breakdown the protein into amino acids. These amino acids are easily utilized by fish (Fuller, 1989). So there is a symbiotic relationship between fish and bacteria. Digestive enzymes are extracellular enzymes, which secrete outside to the bacterial cell and found in the intestine of the fish. In intestine, the digestive enzyme can able to convert the complex nutrients into small units. This digestive enzyme producing non-pathogenic normal microbiota is known as fish probiotics. The intestinal tract of fish larvae is much simpler in organization and shorter than that of the adults (Trust and Sparrow, 1974). The degree of survival and well being of carp larvae dictate the success and failure of the fish culture programme.

Due to undeveloped (without normal flora) intestinal tract and non-availability of proper larval diet, in general the rate of survival is very low. Supplemental nutrition in the form of artificial compounded feed in appropriate quality and quantity is one of the important factors and development of larval diet should be given priority. The importance of a quality larval diet has been increasing day by day with the advancement of multiple spawning of carps.
Several attempts have been made to develop diets to replace live food in the rearing of fish larvae (Vine et al., 2004b).

1.6. Need for the Study

Ingredients for the feed preparation are fish meal, rice bran, cod liver oil, ground nut oil cake and vitamin mineral mixture. These are natural products, which contain starch, cellulose, lipid and protein. In our study, the feed ingredients are fermented by extracellular enzyme producing microflora isolated from fish gut. This probiotic feed used to spawning of fish larvae and observed better survival and growth of fish larvae (Ghosh et al., 2002a).

In present investigation isolation of some selected microbes from the intestine of fish and analysis of its probiotic activity. Production of the probiotic amylase enzyme from the isolate and optimization of the enzyme production were carried out. The role of selected microbes in the water quality management of fish pond water and also to ascertain the efficacy of selected microbes in the fish growth parameters. The present study will through more highlight on the probiotics and their significance in the growth of fishes.