Biodiversity is the life sustaining systems of biosphere with intrinsic values. Rajasegar and Sendhilkumar (2009) discussed, India being a mega biodiversity has a long coastline of 8500 kms bounded with estuaries, backwaters, sandy beaches, near shore environments, coral reefs, sea grass meadows, algal communities etc. Many small islands are seen with a vast potential of marine biodiversity. India with a wide continental shelf is rich in fishery resources which make it the fourth largest producer of fish in the world and also the second largest producer of inland fishing (Thillaimaharani, 2010). Marine fishery resources are living natural resource which is self renewable with dynamic habitat. In India, the natural resources are highly rich where annual harvestable fishery potential to the country is estimated to increase in millions of tones day by day (Stevens et al., 2000; Varadharajan et al., 2009; Varadharajan et al., 2012).

The report of CMFRI (2005) estimated marine fish landing in India is approximately 2.54 million tonnes per year, among the maritime states in India, of which Tamilnadu contributes 15% of the total marine fish landings. Fisheries play an important role in our national economy, providing employment for 5.96 million people (Lakshmanan et al., 2009). Fish export in India has increased from 3.7 million tons to 5.3 million tons during the period 1990 to 1999, currently in India marine fish landings contributes nearly 90%.
As the world’s population increases inexorably at a current rate of almost 2% per year, the importance of seafood as a source of animal protein foodstuff gained more and more attention in recent years. The demand always exceeds its supply in many parts of the world thus encouraging people to go for aquaculture, as the marine natural resources declining day by day due to over exploitation and pollution. Aquaculture has been a fast growing industry throughout the world as the demand for fish and sea food increases at a significant pace (York and Gossard, 2004).

The seafood in India are used in different forms such as fresh, frozen, canned, dried, cured and other fishery products in domestic and foreign markets. They have essential amino acids, fatty acids, protein, carbohydrates, vitamins and minerals. They harbor a number of biohazards as well as chemical contaminations such as biogenic amines, bio-toxins, pathogenic bacteria and viruses (Gram et al., 2000; Ashie et al., 1996). The hazard of seafood industry may be physical, chemical or biological in nature. Even though, sea foods are nutritive, they act as a vehicle for the pathogenic bacteria naturally occurring in the aquatic environment referred to as indigenous or derived from the postharvest contamination (Wallace et al., 1999; Gillespie et al., 2001). It is considered that the quality of seafood depends on the quality of water where the fishes are caught and the sanitary conditions of the landing centre.
Seafood plays a significant role in causing food borne diseases, although it is important element of Mediterranean diets. Sea foods are susceptible to a wide variety of potentially pathogenic bacteria (Schmidt et al., 2000). Seafood forms a major vehicle for transmission of several bacterial diseases (Kumar et al., 2005). Contamination occurred as pathogenic microorganisms form a part of the normal flora of fish. In developing countries like India, the level of contamination in fishery products by various food borne pathogens and other chemical pollutants are very high due to lack of proper infrastructure facilities.

The fish contributes about 60% the world supply of proteins and that 60% of the developing world derives more than 30% of their animal protein from fish (FAO, 1994; Adedeji et al., 2011). At present, more than 76% of fish production is utilized globally for human consumption with the remaining going for miscellaneous purpose. Darlington and Stone (2001) assumed that fresh fish accounts for 45% of total consumption, followed by molluscs and crustaceans at 30%, canned seafood products at 13%, and frozen fish at 11.5%. Seafood obtained from wild and farm fishes have always been the important source of protein in human diet (Yagoub, 2009).

Andrew (2001) considered fish as a good and cheapest source of protein for maintenance of healthy body and vitamins B12, B6, fluorine, iodine and other elements which are needed for the development of strong teeth, prevention of goitre in man. Nutritive value in fish as protein supplement is
known as well since time immemorial (Shingadia et al., 2011) has a high biological value in nature (Anthonio and Akinwumi, 1991; Fagbenro and Arowosoge, 1998). Marine fishes are liable to variable number of environmental stressors bounded with chemical, natural and biological invaders. Marine fishes are largely surveyed using selective and invasive methods, which are mostly limited to commercial species, and restricted to particular areas with favourable conditions. It is considered that food has a long association with the transmission of disease.

The marine realm which covers the earth’s surface provides 70% as the largest inhabitable space for living organisms, particularly microbes among the three major habitats (Surajitdas et al., 2006). It is estimated that about half the biomass on earth is microbes (Whitman et al., 1998). Microorganisms occur nearly everywhere in nature and occupy an important place in the life of human. Marine microbial diversity in an environment always maintains the ecological balance through various microbiological processes (Sindermann, 1990). The human activities had a great impact in coastal areas in the last two decades with the effects of industrialization, intensive agriculture and coastal engineering gave serious threat to marine life (His et al., 1999) that led to environmental pollution. Rivers carry their pollutants to estuaries and finally to coastal areas either in dissolved, colloidal or particulate form, where harmful substances enter the food chain and become concentrated in fish and edible organisms via bioaccumulation process (His et al., 1999). Pollution being the
negative feedback of the environment affects the living organisms in the aquatic environment.

Fishes has always been a primary source of food for coastal populations, and one of the most internationally traded seafood. Fish and shellfish are highly perishable and they are prone to wide variations in quality due to differences in species, environmental habits, feeding habits and function as carriers of microbes and other health hazards (Yagoub, 2009). Fishes constitute an important part of the diet in many countries (Leisner et al., 2001) thus, 80% of animal protein in our diet comes mainly from fish alone (Rubbi et al., 1978).

The aquaculture industry of the world has been facing serious problem due to microbial diseases. At certain times, microorganisms manifest their presence in several ways, such as food spoilage, food borne illness, can transform food properties in beneficial way like food fermentation. Boyd (1984) made known fishes are conditioned by their environment and hence it is obvious yet fish also gets polluted of the growing and harvesting environment of fish is polluted chemically or microbiologically. Herbert et al. (1997) suggests fishes possess neutral to slightly acidic pH and high moisture content which favours the growth of a wide range of microbes, intrinsic and extrinsic factors also determines the initial bacterial contamination. Fishes passively carry human pathogens in their bodies which cause health hazards to fish
handlers and consumers (Buras et al., 1985). Microorganisms that occurred due to contamination reflect the environmental pollution (Adeyemo, 2003).

Fishery products are of great importance for human nutrition worldwide and provide clear health benefits, act as a source of food borne pathogens. Pathogenic bacteria naturally occurring in aquatic environments are referred as indigenous or derived from polluted waters and from post capture contamination (Wallace et al., 1999; Huss et al., 2000a, 2000b, 2003; WHO, 2000; Gillespie et al., 2001). There is only a significant amount of data on the microbiology of seafood produced or imported in different countries and only a small fraction of food borne disease outbreaks are reported. In most countries seafood accounts for up to 10% of all outbreaks (Huss et al., 2000b). A recent estimate suggests that the sea may support 2 million different bacterial species (Curtis et al., 2002) among the estimated number 0.01 to 0.1% of marine bacteria are culturable.

Ranjith Singh et al. (2011) quoted that bacteria are microbes that inhibit almost in all ecological niche. Microbes are mostly parasitic; some are autotrophs while some others are commensalisms. The aquatic system provides a good deal of facilities to the bacteria and other microbes. Biological agents, particularly bacteria and viruses are recognized as the etiological agents of infectious diseases in a wide range of marine animal species. The microbiological activity is by far the most important factor influencing fish
quality (Gram and Huss, 1996). Apart from affecting the major fauna of the aquatic systems such as fish, the pollutants also affect the commensals and parasitic microbes that have a close association with them.

Fishes are in direct contact with the environmental microflora and the opportunistic pathogens already present in the water invade the host under stress. Therefore, it is important to understand the microflora associated with fish culture environment. Aquatic organisms often harbour a great number of bacteria in their intestinal tract, gills and body surfaces which acquire from water, sediment and or food. Fishes receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria.

Being rich in nutrient, the environment of the digestive tract of fish confers a favourable culture environment for the microorganisms. Thus from several points of view, fish quality has become very important in the world. This is because consumers now are more aware of possible food hazards and malpractices which will affect the quality as a result of bad handling and processing. Therefore, consumers individually or collectively demand in respect of freshness, naturalness, microbial safety, free from pollutants and protection from damage.

Bacterial flora enters in high concentration into surface water through pollution of surface water through direct contamination by animal and human
waste, run off from farms, wildlife and sewage which are a growing environmental concern. Bacterial flora in fish and water environment are different in various geographical areas. Microbial adaptation through natural selection is a key process in the emergence of pathogens. New pathogens emerge because of changing ecology that connects a potential with a food chain, with increasing opportunities for contamination.

Bacteria are easy to isolate, culture, maintain and to improve their strains. Microbes are omnipresent and exist in a competitive environment. Heterotrophic bacteria commonly present in marine environments have received little attention, though special groups such as the agar digesters have been extensively investigated. Freshly caught fish microbial flora is largely a reflection of microbial quality of the waters from where they are harvested. Qualitatively, the bacterial flora of marine environments in different parts of the world recorded by different researchers showed some differences.

All living organisms including fish coexist with a wide range of pathogenic and non-pathogenic microorganisms which posses complex defence mechanisms which contribute for their survival. Marine bacterial diversity are used to assess the community structure and distribution pattern of microbes. Buckling et al. (2003) demonstrated that bacterial populations lose their potential to diversify, as they are adapting to a specific niche. Heterotrophic bacteria enclosed with a bulk of microbial populations are
responsible for biological transformation of organic matter and production of carbon dioxide (Sher and Sher, 1996; Surjita das et al., 2006) bacterial distribution depends on the water temperature, salinity, and other physiochemical parameters (Alavandi, 1990).

Bernard et al. (2000) discussed, from marine environments, colony forming units provide inadequate description on the relative abundance of bacteria, because traditional methods are unable to mimic the real environmental conditions under which natural population flourishes. International committee for microbiological food safety (ICMFS) has devised permissible counts for various pathogens in different food products.

Many bacterial species encountered in different fishes are potentially pathogenic, there is growing awareness on the influence of bacterial composition of fish, especially in the intestine, on the health and growth of the host. The spoilage of marine products is mainly due to bacteria which plays a major dominant role (Palaniappan, 1982; Ganesh et al., 2010).

Microbial growth and activity determines the shelf life and safety of various fresh and lightly preserved sea foods. Kaneko (1971) stated by monitoring the bacteria contents of fish organs, the quality of fish can be measured since these will affect the storage life and quality of the fishery products. Bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body (Douglas, 2007). Different types of association such
as ingestion, incubation, parasitism and contribution of exo-enzymes are reported to occur between gut microbes and it is known that bacterial communities cannot be treated as single functional entities but the individual populations require examinations (Harris, 1992).

Various aspects of reviews on microbial flora associated with fish intestine, gill, skin, egg, muscles and on relationships between intestinal microflora and the microflora of aquatic habitat studied by Ringo et al. (2008). From the studies of the intestinal microflora of warm blooded animals, that the bacterial population structure of the intestine influences the establishment of pathogenic micro-organisms in the intestinal tract was known (Van der Waaij, 1989; Singer and Nash, 2000; Huber et al., 2004).

From most of the fish digestive tracts bacterial species isolated have been reported to be aerobes or facultative anaerobes (Trust and Sparrow, 1974; Bairagi et al., 2002; Saha et al., 2006). The gastro intestinal microflora is generally thought to play an important role in the maintenance of gut homeostasis and is beneficial to health. These beneficial bacteria have gained attraction as probiotics. Various authors reported the average values of total bacterial counts on skin, gill, gut region of different fishes ranged between $10^3$-$10^5$, $10^2$-$10^7$, $10^4$–$10^8$ cfu/gm, cm$^2$ (Lima dos santos, 1982; Huang and Leung, 1993).
Conventional microbiological methods for identifying microorganisms in foods are reliable, however, they require several days to complete which may result in considerable loss of perishable foods. Hence, a rapid diagnostic method for surveillance of microorganisms is needed. Molecular methods such as PCR (Polymerase chain reaction) and other amplification procedures are coupled with nucleic acid probes carrying DNA or RNA base sequences are now widely used for identifying microbes. It was Kary mullis who first described PCR, a sensitive and rapid method of identifying infectious agents. By using 16S rDNA is a specific advantage that it permits the analysis of both cultivable and non-cultivable, anaerobic and aerobic bacteria and provides a rapid method to observe the changes in community structure in response to different environmental factors (Yang et al., 2001).

The PCR has gained potential to detect microbial species by amplification of gene sequences unique to that particular organism. PCR permits amplification of the region of the genome selected for analysis in a minimum period of approximately 1½ hours. Woese (1987); Stahl and Amman (1991); Amann et al. (1995) revealed analysis of 16S rRNA sequences is a simple, commonly used method for the identification of microorganisms, the 16S rRNA actually exhibit great variation in some regions. These differences in 16S rRNA sequence provide the basis for the design of nucleic acid probes of various specificities, ranging from probes targeting all living organisms to group-specific and species-specific probes.
Generally, rRNA represents about 80% of total nucleic acids in microbial cells, using the rRNAs as a target is the fact that these molecules are naturally amplified within the cell, thus, present in many hundreds of thousands of copies per cell. This natural amplification allows for direct detection of rRNA sequences without the need for intermediate amplification via PCR (Amann et al., 1995). It is noteworthy that rRNA sequences are well established as a molecular chronometer to infer phylogenetic relationships because they are present in all organisms and changes in the nucleotide sequences were deemed to occur in a clock wise manner (Woese, 1987 and Weisberg et al., 1991).

Many bacteria isolated from natural environments possess an important ecological quality, namely that of resistance to antibiotics, which can be gained in the course of selective processes (Nair et al., 1992; Silva and Hofer 1995). Goldstein (2007) cited bacterial resistance to antibiotics is an extensively investigated phenomenon of considerable medical importance and resistant bacteria are common in the natural environment (Kummerer, 2009a, b). Many microorganisms especially bacteria often produce antibacterial substances, that allow the ecological stability of ecosystem in marine medium (Fabregas et al., 1991). Metabolic by-products of complex biosynthetic pathways in microorganisms are antibiotics, which have been used by the farmers to prevent infection by pathogens as well as to treat the fish getting affected (Karunasagar et al., 1994).
Wise (2008) suggested that the rapid emergence of resistance to antibiotics amongst pathogens generates visions of the ‘potential post-antibiotic era threatening present and future medical advances’. Antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes in the past 60 years, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. First part of the problem is bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs and secondly another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture.

Antibacterial substances produced by antagonistic bacteria are extracted, characterized and used as antibacterial substances to control the growth of human pathogens associated with sea foods (Jayanth et al., 2002). Antibiotics are low molecular weight chemical substances produced by microorganisms, having growth-inhibitory effects on other microorganisms in high dilution. Antibiotic usage in the marine pathogens has shown resistance mechanisms to various antibiotics (Riquelme et al., 1996).

Emergence of resistant strains in fishes is also favoured by the antibiotics, used for the control of infections in fish farming. Isolation of antibiotic-resistant bacteria from fishes and other marine organisms has been
reported from several laboratories across the globe. Washed by rain and carried to the ponds and rivers, these antibiotics enter the body of fishes, which serve as a reservoir of resistant bacteria. Antibiotic resistant strains were less competent to survive in the environment and are slow-growers compared to their antibiotic-sensitive counter parts. It is believed that bacteria sense antibiotics as an environmental stress. Hence there might be some correlation between antibiotic-resistance and stress tolerance of bacteria. Antibiotic-resistance has been detected in many bacteria isolated from extreme environments.

High frequency of bacterial resistant to antibiotics is considered to be an important reservoir of resistance genes, important for their maintenance, mixing and mobilisation (Wright, 2007; Zhang et al., 2009; Taylor et al., 2011). Enhanced rate of horizontal gene transfer was observed by some investigators in some food-borne bacteria treated with sub-lethal levels of stress factors.

Gardener (1969) and Davis and Anandan (1970) reported comprising the fish gut microbiota of primitive people inhabiting areas not exposed to modern medical procedures. Apart from harboring antibiotic resistance genes in mobile genetic elements such as plasmids and transposons, many bacterial species possess an intrinsic mechanism for resistance to multiple structurally unrelated compounds. Resistance genes mostly reside on bacterial plasmids, in
some cases they are also found to be located on chromosomes and in a few cases organized in operons.

Nakahara *et al.* (1977) showed in a number of different bacterial genera showing plasmid mediated is also associated in some cases of antibiotic resistance. Not all antibiotics are active against all bacteria and bacterial species can have intrinsic resistance to one or more antibiotics. Intrinsic resistance refers to resistant microorganisms without any chromosomal mutation or acquisition of plasmid carrying resistance factors. R plasmids are considered as significant plasmids among various plasmids present in bacterial strains as they confer resistance to one or several antibiotics which possess a threat to chemotherapy. Treatment of cells with certain chemical agents such as acridine orange or ethidium bromide enhances the elimination of plasmids from host cells (Ansari and Khatoon, 1994).

Susceptibility to curing agent also varies among plasmids. Some plasmids are not curable by treatment of acridine orange or ethidium bromide (Amir and Khatoon, 1976). Loss of certain genetic markers after treatment of bacterial cell to plasmid curing agents also suggests for the plasmidial nature of the marker (Mesas *et al.*, 2004). It is known that bacteria can transfer resistance plasmids *insitu* to indigenous microflora. Interspecies and inter generic transfer of R plasmids has also been shown to occur (Mergeay and Gerits, 1978). The potential for plasmid transfer is especially significant in view of the fact that
many bacteria containing R plasmids, the possession of which is associated with antibiotic resistance, exhibit higher rates of survival in aquatic environments (Kelch and Lee, 1978).

For cloning purpose which have potential use in biotechnology such as the manufacture of biosensors and bioremediation processes, where the plasmid can be the source of resistance genes (Collard et al., 1994). The ability of a genetic marker for transferring from one bacterium to another through conjugation or transformation provides a good presumptive evidence for the involvement of plasmid, particularly if the frequency of transfer is high. Some of the plasmids have important characteristics such as drug resistance, metabolic functions, a restriction system or phage resistance and some have its own importance in industrial applications (Saarela et al., 2000; Cebici and Gurakan, 2003). Lavanya et al. (2011) discussed most of the natural isolates carried low molecular weight plasmid DNA, which may fetch interest on application to rDNA technology.

Phylogenetics, a field with a growing impact on a variety of life science areas, is experiencing an increasing but poorly met requirement for software supporting the advanced visualisation of phylogenetic trees. The development of powerful visualisation tools is a major challenge in bioinformatics. The major assumption for phylogenetics are molecular sequences used in
Phylogenetic construction are homologous and each position in a sequence evolved independently.

Phylogenetic inference methods are comparatively well developed, tools in the domain are characterised by a lack of effective visualisation techniques (Munzner et al., 2003). Phylogenetic analyses rely heavily on visual inspection, structural comparison, manipulation and exploration of phylogenetic trees, and thus present a number of visualisation challenges. Phylogenetic data is characteristically noisy and incomplete. As such phylogenetic inference is an optimisation problem, which may result in hundreds or thousands of near optimal or equally optimal trees (Amenta and Klinger, 2002).

Phylogenetic analysis refers to the act of inferring or estimating evolutionary relationships, which is usually depicted as branching, tree-like diagrams that represent an estimated pedigree of the inherited relationship among molecules, organisms or both. Phylogenetic analysis of a family of related nucleic acid or protein sequences is a determination of how the family might have been derived during evaluation. The evolutionary relationships among the sequences are depicted by placing the sequences as outer branches on a tree; the branching relationships on the inner part of the tree reflect the degree to which different sequences are related. To understand the historical relationships between genes, proteins and species in the aspect of evolutionary
biology, with the Phylogenetic tree playing a fundamental role in analysis and visualisation providing information about the origin and diversity of species.

The study of proteins and DNA that are homologous will give rise to a useful measure unaffected by environmental effects on phenotypical changes, thus help us in providing a measure for understanding evolutionary changes at molecular level (Vittal R. Srinivas, 2009). Most trees like cladograms or phylograms with equal edge lengths, despite branch lengths being an important indicator of evolutionary time. An evolutionary tree is two dimensional graphs showing evolutionary relationships among organisms or evolutionary relationships in genes from the separate organisms, thus dendrogram comparisons are under significant in development.

The separate sequences are referred to as taxa, defined as phylogenetically distinct units on the tree. It is important to recognize that each node in the tree represents a splitting of the evolutionary path of the gene into two different species that are isolated reproductively. Molecular phylogenies are very informative compared to those based on traditional or morphological characters, because they are wider in scope and data handling is consistent and objective (Ignacimuthu, 2009).
1.1 AIMS AND OBJECTIVES:

The aim and objectives of the present study are,

- To study the quantitative and qualitative analysis of bacterial flora associated with skin, gill and gut regions of the marine edible fishes.

- To characterize the isolated bacteria both morphologically, biochemically and by molecular methods.

- To analyze the antibiotic resistance in bacteria.

- To determine the plasmid curing in the selected samples.

- Plasmid isolation to study the molecular weight of the sample.

- To analyze the phylogenetic tree using bioinformatics tools.