1. General Introduction

Ocean is the biggest home to a wide diversity of organisms. It ranges from microscopic bacteria, algae and fungi to the biggest animal in the world. More than 250,000 marine species have been identified in the world. The largest ecosystem of the ocean covers 71 percent of the Earth’s surface and contains 97 percent of the planets water. This ecosystem is predominantly supported by primary production of phytoplankton in the photic zone, where it sustains the pelagic food web (Steele, 1974; MacIntyre et al., 1996; Underwood and Kromkamp, 1999). A small part of the organic carbon produced in the photic zone is exported and ultimately reaches the sediments, where it fuels the benthic community. Benthic habitats are important for a variety of reasons. East and near shore benthic ecosystem support a plethora of marine life by providing spawning nursery refuge and foraging grounds for both fin and shellfishes. Benthos helps in deposition, breakdown incorporation and turnover of organic matter in the seabed helping recycle nutrients to the overlying water column (Benoy and Kalff, 1999; Middelburg et al., 2000; Gacia et al., 2002; Volkman et al., 2008; Hardison et al., 2011). Phylum Annelida, class polychaeta contains the bristle-bearing segmented wowsms as called as Polychaetes. The polychaetes being the most dominant groups in benthic faunal communities contribute about 75% of the total macrobenthic community and their diet include microbial viz., bacteria, microalgae, protists and fungi.

Benthic organisms constitute an estuarine component both in east and marine coastal environment the term benthos refers to the benthic community that lives on or in the bottom of any body of water (Lee et al., 2010). They are divided into two groups namely infauna and epifauna. Infaunal organisms live in the sediments and their numbers and kinds are reliable and sensible indicators of benthic habitat. Epifauna lives on the sediment surface and are usually associated with surface structures such as shells, vegetation and animals colonies. Availability of benthic communities is depends on the type of sediment (Snelgrove et al.,
1997; Henman et al., 2001). For the convenience, benthos is classified into macro, meio and micro based on size. The different structure of polychaetes are available that is namely; lugworms, bristleworms, clam worms, fire worms, sea mice, palolo worms, feather duster worms, etc. Attain a sex characters of some polychaetes are separate. The few specialized body segments are shed into the coelom has produced gametes to leave the body through the nephridia. A ciliated free-swimming trochophore larval is developed after external fertilization. Bisymmetry, metamerism, segmentation, schizocel, closed vascular system, chain nervous system, spiral and determinant cleavage there was a common feature of polychaetes. Polychaetes worms are a morphologically and ecologically diverse group. Polychaetes display a variety of life history strategies and the factors that control reproduction, larval biology and adult distributions are as varied as these strategies. Adults can be free living or epibiotic, parasitic or mutualistic, wandering, swimming, sedentary or sessile. Seasonal reproduction is common in temperate polychaetes. In India, more than 10,000 species belonging to 70 families have been identified. Polychaetes having a prostomium in a front side and peristomium can make up in the head side for some times. Pygidium is present end of the part. A new growth/ new segments are formed in the anterior part. Look like the annelids are most attractive and many of the species are very beautiful. Benthos in general and polychaetes in particular play an important role in the ecology of marine communities in view of their structure and functions. Mainly affects on physical and chemical parameters of water and sediments and maintain sediment-water-column interface (Varadharajan et al., 2010; Kundu et al., 2010; Thilagavathi et al., 2013). A relatively small number of species are also of commercial important this series mostly from their use as live bait in support of the sea angling industry, which in some developed economies is the single largest participatory leisure activity. Polychaetes have become increasingly important as a resource in relation to the development of world aquaculture for crustacean since it has been
found that polychaetes can provide nutritionally balanced polyunsaturated fatty acids (PUFA) which are essential for egg maturation in cultured prawns (Fischer and Schmitz, 1981; Gambi et al., 1994).

Annelid groups of polychaetes are common members of estuarine ecosystems. For polychaetes worms, community composition and reproductive ability can be highly dependent on salinity and temperate gradients. Within temperate estuaries salinity temperature tends to cover along the estuarine gradient from river to mouth and interactions between these two physical factors may strongly influence species distributions through their impacts on benthic and pelagic stages. Salinity alone however has been investigated in more detail than temperature in estuarine polychaetes (Chu and Levin, 1989; Fong, 1991). Low salinities cause mortality, lower fecundity and prevent reproduction to varying degrees. Macrobenthos species are of special interest because most of them are sessile or have a limited mobility and are thus directly depend on environmental conditions, and they show marked responses to environmental changes depending on their species specific sensitivity and tolerance level. Polychaetes organisms are an important food source for higher tropic levels in most estuaries and near shore marine environment. In addition to this polychaetes in marine sediments play an important role in ecosystem process such as nutrient cycling, pollutant metabolism, and dispersion and in secondary production analyses of macrobenthos are essential in marine environment monitoring programmes (Pearson and Rosenberg, 1978; Elmgren et al., 1983; Snelgrove, 1998; Karoncke et al., 1998; Ajmal Khan et al., 2004; Beuchel et al., 2006; Varadharajan et al., 2010; Kundu et al., 2010; Thilagavathi et al., 2013). Macro benthos is useful in indicating the degree of contamination in this area. Their presence or absence indicates environmental conditions for sometime previous to benthic animals live in or on the seabed and since the seabed is mainly covered by sediments, this means that most
of the live in or on the sediment. The hydrological parameters play an important role in the
diversity of benthos populations.

Surfaces in a natural aqueous environment are readily colonized by bacteria. The
attachment of bacteria occurs at solid–liquid interface, and the process is controlled by the
in situ characteristics of the surface. After attachment, bacteria produce numerous
extracellular macromolecules that form a matrix on the substratum. These extracellular
molecules serve many important functions for the biofilm bacterial communities, such as
buffering against microenvironmental changes, localization of extracellular enzymes,
sequestering nutrients, attachment and movement on surfaces, and protection against toxins,
grazing and digestion (Decho, 1990).

The effects of biofilms on recruiting marine invertebrate larvae might involve cues
either released into the surrounding water or present on the surface of the substratum
(surface-bound). Steinberg et al. (2001) argued that inducers of marine invertebrate
settlement are more likely to be water-soluble primary metabolites. However, for many
marine invertebrates, including most members of fouling communities that encrust piers and
anchored ships, the cues are found to be large, insoluble, surface-bound molecules associated
with biofilms (Hadfield and Strathmann, 1996).

Characterization of antimicrobial peptides has received great attention in the recent
past due to their applications as food preservatives without any toxic effects on host and
therapeutic agents. At present, preservation of food is a serious concern for almost all
countries across the world. Since lactic acid bacteria (LAB) produce an array of antimicrobial
substances they were used as natural bio-preservatives for special applications
(Holzapfel et al., 1995; Cotter et al., 2005 and Deegan et al., 2006). Other bacteria such as
Enterococcus, Streptococcus etc., were also reported to produce various bacteriocins and they
are also being considered for different applications. However, production of antimicrobial
peptides by *Bacillus* strains has been increasingly characterized in the recent past and many peptides produced by this group of bacteria found to be suitable for various applications (Abriouel *et al*., 2011).

Pathogenic bacteria permanently threaten the health of human and animal. Microorganisms are the major cause of food-related diseases and spoilage during production and storage of food and beverages (Koponen, 2004). Lactic acid bacteria play an important role in producing many antimicrobial substances that are active against other bacteria such as food spoilers and pathogenic bacteria. The most interesting antimicrobial substance is bacteriocin (Wittayacom, 2004). Bacteriocins are proteinaceous antibacterial compounds exhibit bactericidal activity against species closely related to the producer strain. These substances are produced by many species of bacteria and among them the lactic acid bacteria are important producers (Riley and Chavan, 2007). However, only very little information about bacterocin are available.

Until now, a number of isolates were discovered. As the strains were isolated, it became apparent that there is a need arose to differentiate the strain. Consequently, different methods were developed for classifying the strains and confirming their identification. The first classification studies were mostly based on morphological and biochemical characteristics. Since the phenotypic properties of strains are insufficient to separate different strains, new methods are required to show strains differences. Bacteriocins are a heterogeneous group of ribosomally synthesized antibacterial peptides that inhibit strains and species that are usually, but not always, closely related to producing bacteria (Tagg *et al*., 1976). Both Gram positive and Gram negative bacteria produce small, heat-stable bacteriocins. Bacteriocins produced by Gram positive bacteria are often membrane permeabilizing cationic peptides with fewer than 60 amino acid residues (Jack *et al*. 1995).
The bacteriocins produced by lactic acid bacteria offer several desirable properties that make them suitable for food preservation: (1) They are generally recognised as safe substances (2) They are not active and nontoxic on eukaryotic cells (3) Become inactivated by digestive proteases, having little influence on the gut microbiota (4) They are usually pH and heat-tolerant (5) They have a relatively broad antimicrobial spectrum, against many food-borne pathogenic and spoilage bacteria (6) They show a bactericidal mode of action, usually acting on the bacterial cytoplasmic membrane; No cross resistance with antibiotics, and (7) Their genetic determinants are usually plasmid-encoded, facilitating genetic manipulation (Galvez et al., 2007).

The genus *Lactobacillus* is quite diverse and consists of a number of different species with little commonality. They are gram-positive rods with a size range of 0.5-1.2 ×1-10 μm and non spore formers, producing lactic acid as a fermented end product (Carl-Abatt et al., 1999). The genus *Lactobacillus* includes over 25 species, and the first level of differentiation is based on the end-product composition. Some are homofermentative where as others are heterofermentative in nature. Lactic acid bacteria (LAB) are heterotrophic and generally have complex nutritional requirements as they lack many biosynthetic capabilities and most species have multiple requirements for amino acids and vitamins. Hence they are often associated with oral cavity of animals, intestines and decaying plants and animals.

Lactic acid bacteria (LAB) are very much useful in food industries. They grow in food and reduce the pH low enough to inhibit the growth of most of the other microorganisms including common human pathogens, thus increasing the shelf life of foods. They also improve the flavor and texture of foods. *Lactobacillus spp.* are important either as deliberate or accidental ingredients in many food products. They produce many metabolites, which are useful in food industries. Another important property of *Lactobacillus* is the production of
bacteriocin. A great deal of attention has been directed towards their potential role as probiotics (Ivanova et al., 2000).

*Lactobacillus acidophilus* is the most prevalent species in most of the naturally fermented foods. Like all the other species of *Lactobacillus*, *Lactobacillus acidophilus* also produces many useful metabolites. It has the ability to block receptor sites for gram negative bacteria and is used as a probiotic. It is an important player in antimicrobial defense and is effective against both extra and intracellular pathogens. *Lactobacillus acidophilus* is also capable of digesting semi-digestible fibers such as onion, garlic and yeasts. It may therefore help in digestive problems like gas and bloating. Recent research has shown that *Lactobacillus acidophilus* has the ability to break down bile acids and lower cholesterol (Johns et al., 2004).

Bacteriocins are proteinaceous compounds of bacterial origin that are lethal to bacteria other than the producing strain. Bacteriocin production is a selective advantage for the organism in a complex microbial niche. Incorporation of *Lactobacillus* spp. as a starter or the inclusion of a purified or semi purified bacteriocin preparation as an ingredient in a food product may provide a margin of safety in preventing the growth of pathogens. Generally, bacteriocins are given names according to the genus or species of the strain that produces it. For example, plantaricin is the bacteriocin produced by *Lactobacillus plantarum* (Joerger et al., 2000). In complex dynamic environment like marine ecosystem many species are expected to produce bacteriocins (or) similar substances. Bacteriocins are a diverse group of proteins with different molecular structures and modes of action. Therefore, classification of bacteriocin is difficult. Bacteriocins can be distinguished broadly, based on whether they originate from gram negative or gram positive bacteria. The bacteriocins of gram-positive bacteria, primarily those produced by lactic acid bacteria, have been divided into three major classes. This classification is mainly on the molecular mass of the bacteriocin, and it does not
distinguish them based on their mode of action (Meghrous et al., 1999). Bacteriocins are active even in very low concentrations against specific strains. This property has been widely used for the identification of types of strains within several bacterial species and this technique is termed as bacteriocin typing. The present study deals with a bacteriocin, produced by a polychaete associated bacterium.

Enzymes have played an important role in many aspects of life since the dawn of time. In fact, they are vitally important to the existence of life itself. Civilizations have used enzymes for thousands of years without understanding what they were or how they work. Over the past several generations, science has unlocked the mystery of enzymes and has applied this knowledge to make better use of these amazing substances in an ever-growing number of applications. Enzymes play crucial roles in producing the food we eat, the clothes we wear, even in producing fuel for our automobiles. Enzymes are also important in reducing both energy consumption and combating environmental pollution. Enzymes are proteins with highly specialized catalytic functions, produced by all living organisms. Enzymes are responsible for many essential biochemical reactions in microorganisms, plants, animals, and human beings. They breakdown proteins, fats, carbohydrates and fiber making it possible to benefit from the nutrients found in those foods while removing the toxins. Enzymes turn the food we eat into energy and unlock this energy in usable form by the body. Their presence and strength can be determined by improved blood and immune system functions.

Enzymes are essential for all metabolic processes. Although like all other proteins, enzymes are composed of amino acids, they differ in function in that they have the unique ability to facilitate biochemical reactions without undergoing any change. This catalytic capability is what makes enzymes unique. Enzymes not only work efficiently and rapidly, they are also biodegradable. Enzymes are highly efficient in increasing the reaction rate of biochemical processes that otherwise proceed very slowly, or in some cases, not at all.
Cellulases refer to a group of enzymes which act together to hydrolyze cellulose into soluble sugars. They are distributed throughout the biosphere such as plants, animals and microorganisms. However, cellulases from higher plants such as *Lantana camara* and *Cuscuta reflexa* are mostly involved in fruit ripening and senescence (Chatterjee and Sanwal, 1999). Few animals such as the blue mussel *Mytilus edulis* (Bingze *et al.*, 2000), the green mussel (Marshall, 1973), the edible snail *Helix pomatia* (Maeda *et al.*, 1996), termites and protozoa (König *et al.*, 2002) were reported as cellulase producers. Protozoa such as *Epidinium caudatum* and *Eudiplodinium ostracodinium*, Archaea such as *Sulfolobus solfataricus* (Moracci *et al.*, 2001) and *Pyrococcus furiosus* (Voorhorst *et al.*, 1999) are also been recorded as cellulase producers. However, microorganisms are considered to be the main source for cellulases with novel and high specific activities. Microbial cellulases are the most economic sources, as microorganisms can grow on inexpensive media such agriculture residues and food industries by-products.

In the most familiar case of cellulase activity, the enzyme complex breaks down cellulose to beta-glucose. This type of cellulase is produced mainly by symbiotic bacteria in the ruminating chambers of herbivores. Aside from ruminants, most animals (including humans) do not produce cellulase in their bodies, and are therefore unable to use most of the energy contained in plant material. Enzymes which hydrolyze hemicellulose are usually referred to as hemicellulase and are usually classified under cellulase in general. Cellulosic materials, its derivatives and polymers with glycosidic linkages are substrates of cellulolytic enzymes. Cellulose is the most abundant organic biopolymer on earth with an estimated annual production of 180 billion tons in nature (Amor *et al.*, 1995; Delemer, 1999). However, its usage depends upon its hydrolysis to available saccharides. Cellulose can be converted into glucose by either chemical, physical treatments or enzymatic hydrolysis. Acid or high temperature degradation is unsatisfactory, because the resulting sugars are partly
decomposed. Cellulosic materials, its derivatives and polymers with glycosidic linkages are substrates of cellulolytic enzymes (He et al., 2000). Biological degradation of cellulose by cellulases is preferred for industrial purposes due to the high yields of desired hydrolytic products with minimal by-products (Parry et al., 2001). Effective utilization of cellulosic material through bioprocesses will be an important key to overcome the shortage of foods, feed and fuels, which the world may face in the near future, because of the explosive increase in human population (Ohmiya et al., 1997).

The development of economically feasible technologies for cellulose production and for the enzymatic hydrolysis of cellulosic materials will enable us to utilize the large quantities of biomass such as the residues of both food industries and agriculture. Instead, cellulosic materials are often burned to clean the fields after harvest each year, and subsequently produce air pollution (Miyamoto, 1997). Most cellulases studied have similar pH optimization, solubility and amino acid composition. Thermal stability and exact substrate specificity may vary. However, it should be remembered that cellulase preparations generally contain other enzymatic activities besides cellulase and these may also affect the properties of the preparation. The cellulase preparations are effective between pH 3 and pH 7. The optimum pH and temperature generally lays between pH 4 and pH 6 and 40°C and 55°C respectively.

Cellulase is inhibited by its reaction products eg. Glucose and cellobiose. Cellulases are inhibited by silver, chromium, lead, mercury, and zinc salts at about 1 to 3ppm concentration (Reese, 1963). Inhibition by heavy salts is due to non specific salt formation (Verma et al., 1963). Mercury inhibits cellulases completely, whereas, manganese, silver, copper and zinc ions are only slightly inhibitory. Cellulases have a wide range of applications. The main potential applications are in food, animal feed, textile, fuel and chemical industries. Other areas of application include the paper and pulp industry, waste
management, medical/pharmaceutical industry, protoplast production, genetic engineering and pollution treatment (Beguin and Albert, 1993).

In food industry, cellulases are being used (i) in the extraction of fruit juices and oil from seeds, (ii) in the clarification of fruit juices, (iii) to increase the soaking efficiency and homogeneous water absorption of cereals, (iv) in the removal of external soybean coat in production of fermented soybean foods such as soysauce and miso (v) in the isolation of proteins from soybean and coconut (vi) for the efficient isolation of starch from corn and sweet potato (vii) for the gelatinization of seaweeds to increase digestibility (viii) for the extraction of agar from seaweeds and (ix) to digest ball-milled lignocellulose which can be used as food additive (Beguin and Anbert, 1993). Cellulases can also be used for (a) improving the nutritive quality of fermented foods (b) improving the rehydrability of dried vegetables and soup mixtures (c) the production of cello-oligosaccharides, glucose and other soluble sugars from cellulosic wastes and (d) for the removal of cell wall which will facilitate the release of flavors, enzymes, polysaccharides and proteins (Mandels, 1985).

The application of cellulase enzyme preparations in food production also include the breakdown of the cellulose in citrus products, increase of the aromatic character of fruit juices through the hydrolysis of flavor glucosidic precursors, decrease the bitemess of citrus juices through the hydrolysis of prunin (Riou et al., 1998). Removal of fiber from edible oil press cakes, increase in starch recovery from potatoes, refinement of flour, extraction of proteins from leaves and grasses, tenderizing fruits and vegetables prior to cooking, extraction of essential oils and flavoring material from plant degradation of vegetable tissues and the extraction of green tea components, modifying food materials such as vegetables, soybeans and rice to increase the yield of the nutrients. New products were obtained by treatment of cellulosic materials with cellulases to produce carbohydrates, which can be used as food or for alcohol fermentation, or for industrial chemicals and beverages. Cellulose
microfibril fragments can be used as noncaloric food additives. Hyperabsorbent cellulose fibers from fragmented cellulose microfibrils are used in biomedical and household absorbent material. The cellulases are used to hydrolyse α-1,3 and α-1,4 glucan which are present in low grade barley and help in the filtration of beer and to increase the aroma in wines. The recombinant yeast producing α-1, 3 and α-1, 4-glucanases have already been used in brewery industry (Beguin et al., 1993).

Cellulases are used (a) as a supplement in feed for ruminents and monogastric animal and (b) in pretreating lignocellulosic material, dehulling of cereal grains, treating silage to improve the digestability of ruminents and monogastric animals (Mandles, 1985). Another interesting application is that the cellulase genes can be cloned to produce transgenic animals which would secrete the required cellulases into the gastrointestinal tract of the animal and help in the digestion of roughage efficiently (Beguin et al., 1993). They are widely applied in textile processing to improve fabric appearance by reducing fuzz, piling, and enhancing the softness, luster and color brightening of cotton fabrics (Ohmiya et al., 1997). Cellulases are also widely used as digestion aids and as detergents (Ito, 1997; Ozaki et al., 1995 and Murata et al., 1991, 1993). Cellulases are also used in the treatment of paper industry (Yinbo et al., 1996).

There are excellent illustrations available on how different industry structure and market considerations can affect the uptake of enzyme technology. Conventional stone washing uses abrasive pumice stones in a tumbling machine to abrade and remove particles of indigo dyestuff from the surfaces of denim yarns and fabric. Cellulase enzymes can also cut through cotton fibres and achieve similar effect without the damaging abrasion of the stones on both garment and machine; moreover, there is no need for the time-consuming and expensive removal of stone particles from the garments after processing. Machine capacity can be improved by 30-50% due to reduce processing times, product variability is reduced
and there is also less sludge deposited in the effluent. In addition, either cellulases or mixture of glucanases have been used for the production of plant and fungal protoplasts, in producing hybrid strains as well as in other genetic engineering experiments. Thus, cellulases have a wide range of potential applications, but considerable future research effort is necessary to exploit the commercial potential of cellulases to the fullest extent. Hence the present study on bioprospecting of polychaete associated bacterium covering bacteriocin and cellulase production.
1.2. REVIEW OF LITERATURE

Distribution and Identification of Polychaetes

Macrobenthos in marine sediment plays an important role in ecosystem processes such as nutrient cycling, pollutant metabolism, dispersion and in secondary production. Sanders (1968) made a detailed study on diversity of benthos in tropical east and shallow region of east coast of India. Bavanarayana (1975) studied the benthic organisms of Kakinada bay. Ajmalkhan et al. 1975 studied the bottom fauna of variation zone of Vellar estuary. Community composition (Bilyard and Carey, 1979; Bromberg et al., 2000; Elias et al., 2001; Maggiore and Keppel, 2007) is related closely to sediment characteristics in intertidal and subtidal soft sediment habitats. Fernando et al. (1984), and Fernando (1987) investigated the benthic fauna in relation to environmental parameters in, Vellar estuary. Polychaetes species richness and diversity also been studied (Gambi and Giangrade, 1986), Ansari et al. 1986 studied the macrobenthic commuites of coastal waters and their study revealed that macrofauna community was dominated by polychaetes in the presence of high sediment organic content from Mandovi and Zauri estuaries of Goa, West coast of India. Varshney et al. (1988) studied the biomass and population density of macro fauna and in relation to environmental conditions from the West Coast of India. Ansari et al., 1994 studied the Macrobenthic assemblages in the soft sediments of Marmugao harbour, Goa. Density of macro fauna species was studied by Scaps et al., 1998 and Gutierrez et al., 2000. Sunilkumar (1996) examined the vertical distribution and abundance of sediments dwelling macro invertebrates in an estuary mangrove biotope Southwest coast of India. Padmavathi and Sathyanarayana (1999) investigated the distribution of nutrients and major elements in the estuarine and adjoining coastal waters of Godavari and Bay of Bengal. Subramanian and Mahadevan (1999) made seasonal and diurnal variation in the hydrological characteristics of
coastal water of Madras. Vijayalakshmi (1999) studied the biomonitoring and histopathological impact of Tributyltin (TBT) pollution in the coastal areas of Parangipettai and Cuddalore. A review on the biodiversity of soil dwelling organisms in Indian mangroves was carried out by Kumar (2000). Brown et al., 2000 have studied to assess the influence of sediment contaminants and natural environmental factors on macrobenthic community from the estuaries of the northern Gulf of Mexico. Sagasti et al., 2000 characterized the abundance and species composition of sessile and mobile epifaunal assemblages in the York River, of the Chesapeake Bay in United States. Lercari et al., 2002 have documented the variation of sandy beach macrobenthic in intertidal environment of Uruguay waters which revealed that the diversity components were significantly decreased towards the source of disturbance. The marine zone of Vellar estuary was explored for temporal changes in community structure of benthos using advanced statistical tools was studied by Murugesan et al., 2002. Sunilkumar (2002) had extended the study on biomass distribution, horizontal zonation, relative dominance and vertical distribution of Polychaetes in littoral sediment of Cochin estuarine mangrove. Belan (2003) investigated the benthic abundance patterns and species composition in polluted, Amursky Bay, Japan. Rouabah and Scaps (2003) studied the comparison of the life cycles of two populations of the polychaetes Perinereis culifera from the Bay of Algiers in Mediterranean Sea which revealed no difference in the life of the populations. Harkantra and Rodrigues (2004) studied the species diversity, biomass and population density of soft bottom macrofaunal in relation to water quality in the estuarine system of Goa. Rajaram et al., 2005 studied on hydrological parameters from Uppanar estuary from Cuddalore. Jayaprakash et al., 2005 made a baseline study of Physico-chemical parameters and trace metals in Ennore creek.

Bick and Arlt (2005) investigated the intertidal and subtidal macro and meio fauna of a glacier Fjords on Spitsbergen. Narayanaswamy et al., 2005 investigated on the ecology of
bathyal polychaetes fauna at an Arctic–Atlantic boundary from the Faroe-Shetland Channel, North-east Atlantic. Gobin and Warwick (2006) investigated the macrobenthic assemblages associated with mangrove mudflats in the Dagua river estuary. Mendez (2007) have examined the relationship between the deep water polychaetes and estuary fauna in the southeastern Gulf of California, where canonical correspondence analysis indicated that depth, oxygen and temperature of water were the major variables influencing the faunal distribution. Pavithran et al., 2007 have investigated the diversity of macrobenthos in the central Indian Ocean Basin and reported 27 species with a dominance of polychaetes and the density of macro fauna was low since it was sustained by low primary productivity. Maggiore and Keppel (2007) have studied the molluscs and polychaetae distribution in the mud flats along the Dese estuary of Italy and evaluated the taxonomic biodiversity and zonation pattern of soft macrobenthos in an area of lagoon. Salgado et al., 2007 studied the distribution patterns of the macro zoobenthos assemblage in the salt marshes of Tejo estuary in Portugal. Tomiyama et al., 2008 investigated the spatial intertidal distribution of bivalves and polychaetes in relation to environmental conditions in the natori river estuary in Japan which revealed that substrata (silty, clay content) and salinity were the important variables influencing the faunal assemblages. Tomiyama et al., 2008 reported that the spatial intertidal distribution of bivalves and polychaetes in relation to environmental conditions in the Natori estuary, Japan. Pradhan et al., 2009 studied physico-chemical characteristics of coastal waters in Devi estuary Orissa. Quadros et al., 2009 investigated on the impact of changing ecological conditions on polychaeta assemblages along the extremely polluted Thane creek on the west-coast of India. Varadharajan et al., 2010 have studied the seasonal abundance of macro benthic composition the Palk Bay regions the dominated only for polychaetes than there others, east coast of India. Kundu et al., 2010 have studied the diversity and seasonal variations of macro-benthic fauna and associated environmental factors influencing the
benthic community in the inshore waters of Vellar estuary in Parangipettai. Macro faunal species diversity, biomass and population density were recorded and in relation to environmental influences of Mandovi and Zuari estuaries of India by Sivadas et al., 2011. Balasubramanian et al., 2012 have studied the biochemical changes of polychaetes Laeonereis ankylosea with environmental changes in Gadilam River, east Coast of India. Mark and Blanco (2013) investigated on the macro faunal community and found mostly polychaetes were dominant. Totally 751 polychaetes specimens and 133 species were identified in the Mid-Atlantic Ridge from the North Atlantic Ocean. Stark et al., 2014 studied anthropogenic disturbance and biodiversity from heavily contaminated to uncontaminated in different regions in Antarctica. Thilagavathi et al., 2014 investigated the macrobenthic communities in different mangrove ecosystems which identified that one hundred fifty six species were recorded including 102 polychaetes, 10 bivalves, 11 gastropods, 24 amphipods, 6 isopods and 3 cumacea. Miebaka and Erema (2014) studied the abundance, distribution and diversity of macro benthic assemblages in Azuabie creek, upper Bonny estuary of the Niger Delta. Warwick (2014) investigated the species size distributions, functional traits and intermediate size between meiobenthos and macro–benthos, interestingly, the size spectrum from subtidal sand in Algoa Bay, South Africa.

**Bacterial forms associated with polychaetes**

ZoBell and Allen (1935) first proposed that bacteria on marine surfaces provide settlement cues for some macroorganisms. They noted that when clean substrata were submerged in the ocean, typically several days passed before macroorganisms started attaching to them, a period during which the dominant organisms found on the substrata were bacteria. Zobell and Allen concluded that the ‘delay’ in attachment of macroorganisms reflected the time required to build up sufficient bacterial films on the surfaces to induce settlement of plants and animals. Bartlett et al., 1988 have obtained that typical *P. atlantica*,
the mucoid type (M), produces large quantities of EPS, which is considered to be a primary adhesive agent for cell-to-surface attachment. A variant of *P. atlantica*, the crenated type (C), yields less than 5% of the EPS produced by M-type cells. No differences in lipopolysaccharides or membrane proteins have been detected between the M- and C-types. Bartlett and Silverman (1989) have isolated genetic analysis showed that the insertion of a 1.2 kb DNA sequence in the *eps* locus results in EPS– (C-type), whereas the excision of this sequence reverses the phenotype to EPS+ (M-type).

Eanthon and Prieur (1990) have isolated fifty two heterotrophic bacteria from the deep-sea hydrothermal vent polychaete, *Alvinella porphiriana*, which were tested for susceptibility to cadmium, zinc, copper, arsenate and silver ions by an agar dilution technique. Metal resistance was exhibited by 96% of the total isolates. The frequencies of resistance to heavy metals were: copper, 88%; zinc and arsenic, 58%; cadmium, 38%. However, strains demonstrated a high susceptibility to silver. 71% of the strains tested were multiresistant to metals. Decho (1990) observed that bacterial biofilms modify both physical and chemical characteristics of substrata, and may enhance initial attachment by larvae and serve as food for juvenile and adult marine invertebrates. Szewczyk *et al.*, 1991 have identified among such molecules are the extracellular polysaccharides (EPS) secreted by most bacteria. EPS have been proposed as important factors in inducing larval settlement of various marine invertebrates. Grossmann and Reichardt (1991) reported on intertidal flats of the North Frisian Wadden Sea, in which total abundance and biomass of bacteria were examined at 6 sites in the particle transport system of Arenicola marina burrows. Both bacterial abundance and biomass showed maxima on the input side, with distinctive peaks in the polychaete's foregut (oesophagus), and declined on the egestive side (hindgut and fecal casts) by 70%. Cell sizes did not differ significantly among sampling sites. In feeding experiments using fluorochromelabelled (killed) bacteria and indigestible fluorescent
particles as a reference standard, disappearance rates of total bacterial biomass were 80 to 90%. Gut extracts showed lysozyme activity. Akagawa-Matsushita et al., 1992 has attempted to test the importance of EPS to the settlement of larval *Hydroides elegans*, and they employed the bacterium *Pseudoalteromonas atlantica*, a common colonist of coastal marine substrata in both Atlantic and Pacific Oceans. Hadfield et al., 1994 studied settlement of the larvae of *Hydroides eleganssand* required found that it a minimum microbial density, and the percentage of larval settlement correlated with bacterial density in natural biofilms which composed of multiple bacterial species, filamentous algae, diatoms and fungi. Vincent et al., 1994 collected heterotrophic and mesophilic marine bacterium HYD-1545 was isolated on a metal-amended medium from the dorsal integument of the hydrothermal vent polychaete *Alvinella pompejana*. This strain, which can be assigned to the genus *Alteromonas* on the basis of its G+C content and phenotypical features, produced large amounts of an acidic polysaccharide in batch cultures.

The polysaccharide was excreted during the stationary phase of growth and contained glucose, galactose, glucuronic acid, galacturonic acid, and 4,6-O-(1-carboxyethylidene)-galactose as major components. This polysaccharide was a polyelectrolyte, and the viscosity of its solutions depended on the ionic strength. The decrease in viscosity with increasing NaCl concentrations and the effect of Ca21 in decreasing the viscosity at low Ca2+ concentrations support a model in which the polysaccharide carries anionic groups. However, an unusual behavior was observed at higher concentrations and could be related to intermolecular interactions involving Ca2+ ions. Hofmann et al., 1996 investigated many benthic marine invertebrates and found that they had complex life cycles that consisted of one or more planktonic larval stages and an adult stage. The transition between the two phases includes settlement of the larva and its irreversible metamorphosis into the juvenile form they
observed that transport and subsequent recruitment of larvae are essential for dispersing, colonizing new habitats and maintaining populations.

Walters et al., 1997 analyzed the exact location where a larva of *H. elegans* permanently settled and found that it may not be affected by local hydrodynamics, neither conspecific individuals nor other physical characteristics of the substratum overcome the requirement for a well-developed biofilm in stimulating the settlement process. Francoise Lucas and Georges Bertru (1997) investigated on the bacteriolytic activity in the polychaete *Nereis diversicolor* was which which found in extracts of faeces and sediment, and in three sections of the digestive tract: foregut, midgut and hindgut. Using agarose-gel tests, they found a lysozyme-like activity was able to lyse *Micrococcus lysodeikticus* cells and stable to heat at acidic pH. The digestive extracts have also appreciable lytic action on plant leachate bacteria. In whole extract (tissues and contents), the lytic activity was higher in the hindgut, whereas in the contents the lytic activity was higher in the midgut.

The presence of bacteria or the food intake seemed to stimulate bacteriolysis activity since very low lysis of leachate bacteria was found in the digestive tract of starved animals. Craig Cary et al., 1997 studied the *Alvinella pompejana* a polychaetous annelid that inhabits high-temperature environments associated with active deep-sea hydrothermal vents along the East Pacific Rise. A unique and diverse epibiotic microflora with a prominent filamentous morphotype were found associated with the worm’s dorsal integument, the 13B and 5A phylotypes were identified and localized on *A. pompejana* by in situ hybridization, demonstrating that these two phylotypes were, in fact, the prominent filamentous bacteria on the dorsal integument of *A. pompejana*. These findings indicated that the filamentous bacterial symbionts of *A. pompejana* were epsilon *Proteobacteria* which do not had an obligate requirement for *A. pompejana*. Carpizo-Ituarte and Hadfield (1998) demonstrated that in a well-developed biofilm, larvae contacted the surface repeatedly with its apical tuft
and then attached to the surface with a sticky mucous thread secreted from its tail region. Immediately, it secreted a proteinaceous primary tube, within which metamorphosis proceeded, including resorption of the prototrochal cilia, elongation of its body and differentiation of the collar region. Soon after, the metamorphosing worm begun to secrete the calcareous secondary tube and develop branchial tentacles. The irreversible process of metamorphosis was completed within 12 hrs of contact with a biofilm. Unabia and Hadfield (1999) have observed the planktotrophic larvae of *H. elegans* which become competent to settle and metamorphose in approximately 5 days, but do so only in the presence of a bacterial biofilm. Matthew *et al.*, 1999 isolated bacteria that colonized the dorsal integument of the polychaete annelid *Alvinella pomerjana*, which inhabited the high-temperature environments of active deep-sea hydrothermal vents along the East Pacific Rise.

They opined that the composition of this bacterial community was characterized in previous studies by using a 16S rRNA gene clone library and *in situ* hybridization with oligonucleotide probes. They found that phylogenetic analysis separated the clone families into groups that probably represented two genera of previously uncharacterized sulfate-reducing bacteria. The presence of dissimilatory bisulfite reductase genes was found to be consistent with recent temperature and chemical measurements that documented a lack of dissolved oxygen in dwelling tubes of the worm. The diversity of dissimilatory bisulfite reductase genes in the bacterial community on the back of the worm suggested a prominent role for anaerobic sulfate-reducing bacteria in the ecology of *A. pomerjana*.

Lau and Qian (2001) studied two bacterial strains viz., *Roseobacter* sp. and an unidentified species in the α-subclass *Proteobacteria* group, specifically induced settlement in larva of *H. elegans*; and the response was reduced in the presence of streptomycin. Hadfield and Paul (2001) found that most species settlement of larvae was not random but correlates with biological, chemical and physical factors in the environment. Larvae that are
competent to metamorphose typically were able to remain pelagic in the water column until they encounter proper environmental cues, which may indicated suitable habitats. However exceptions were found like small lecithotrophic larvae that were simply exhausted their energy reserves during extended swimming periods. Negri et al., 2001 reported selective settlement on biofilms has been shown for larvae of many marine invertebrates, including the scleractinian corals Acropora willisae and A. millepora. Cambon-Bonavita et al. (2002) obtained to describe an aerobic, mesophilic and heterotrophic marine bacterium, designated HYD657, able to produce an exopolysaccharide (EPS). It was isolated from a East Pacific Rise deep-sea hydrothermal vent polychaete. Karine alain et al., 2002 examined of microbial diversity associated with hydrothermal vent polychaetes of the family Alvinellidae, molecular analysis of the bacterial assemblage associated with mucous secretions of the Northeastern Pacific vent polychaete Paralvinella palmiformis. Using a molecular 16S rDNA-based phylogenetic approach, clone libraries were constructed from two samples collected from active sulfide edifices in two hydrothermal vent fields. The remaining sequences were related to the taxonomic groups Fusobacteria, Green non-sulfur bacteria, Firmicutes, Q- and N-Proteobacteria. They claimed that was the first report of the presence of Verrucomicrobia, Fusobacteria and green non-sulfur bacteria on hydrothermal edifices.

The potential functions of the detected bacteria were discussed in terms of productivity, recycling of organic matter and detoxification within the P. palmiformis microhabitat. Barbara et al. (2003) reported the colonization of deep-sea polychaete Alvinella pompejana as tubes on the sides of black smoke chimneys along the East Pacific Rise. A diverse, yet phylogenetically constrained episymbiotic community was obligately associated with its dorsal surface. The results indicate that the ATP citrate lyase gene was not only a consistent presence in these episymbiont communities but it was also expressed.
Phylogenetically distinct forms of ATP citrate lyase were also found associated with and expressed by bacteria extracted from the tubes of *A. pompejana* have also demonstrated the persistent presence and expression of this gene in the episymbiont community, both the episymbiont and the surrounding free-living communities displayed a chemolithoautotrophic form of growth and therefore contribute fixed carbon to other organisms in the vent community.

George *et al.* (2004) found that the marine infaunal burrows and tubes greatly enhanced solute transport between sediments and the overlying water column and were seemed to be the sites of elevated microbial activity. The microbial communities in tubes of the marine infaunal polychaete *Diopatria cuprea* collected from two different sediment habitats were examined. The bacterial communities in the tubes from a sandy sediment differed from those in the tubes from a muddy sediment. Han-Seung Joo *et al.* (2004) studied the produced an extracellular alkaline protease from alkalophilic *Bacillus* sp. 103, was isolated from the hemolymph of an unique Korean polychaete (*Periserrula leucophryna*) living in the tidal mud flats of Kwangwha Island in the Korean West Sea. Maximum enzyme activity was achieved when the bacterium was grown in 2.0% soybean meal, 1% casein, 1% wheat flour, 0.5% K$_2$HPO$_4$, 0.5% sodium citrate, 0.01% MgSO$_4$ and 0.4% sodium carbonate at 37 °C for 48 h incubation period with an agitation of 250 rpm. The enzyme had an optimum pH of around 10 and maintained its stability over a broad pH range between 5.5 and 12. The optimum temperature was around 45–50 °C, and stability was exhibited up to 50 °C. The alkaline protease showed extreme stability towards SDS and oxidizing agents such as hydrogen peroxide and sodium perborate, which showed activity retention of more than 90% even on treatment with 1% SDS or hydrogen peroxide for 72 hrs of incubation. Petit *et al.* (2006) investigated low frequency ultrasound was used to depolymerize a high-molecular-weight exopolysaccharide produced by a deep-sea hydrothermal bacterium *Alteromonas*.
macleodii. The influence of several parameters was examined including the duration of ultrasonic irradiation, exopolysaccharide concentration, reaction temperature and volume of the sonicated solution. With the aim of optimizing the depolymerization, the native exopolysaccharide was simultaneously treated with hydrogen peroxide and ultrasound, and that study identified the sonication conditions that produce low-molecular-weight derivatives from the native exopolysaccharide (>106 Da) with good reproducibility. Eniko Kadar et al. (2006) screened tissue partitioning of micro-essential metals in the vent bivalve Bathymodiolus azoricus and associated organisms (endosymbiont bacteria and a parasite polychaete) from geochemically distinct vents of the mid-atlantic ridge.

Craig et al. (2008) demonstrated the gut contents of two polychaete deposit feeders, Nereis succinea and Amphitrite ornata, exhibited a significantly higher ratio of bacteria resistant to both cationic and anionic surfactants. In contrast, bacteria in the gut fluids of a holothuroid, Leptosynapta tenuis, showed surfactant susceptibility similar to that of bacteria from sediments. Analyses of 16S rRNA gene sequences revealed that the majority of surfactant-resistant isolates were previously undescribed species of the genus Vibrio or were of a group most closely related to Spongiobacter spp. They also tested a subset of resistant bacteria for the production of biosurfactants. The majority produced biosurfactants, as demonstrated via the oil-spreading method, but in all cases, production was relatively weak under the culture conditions employed.

Li et al. (2009) analyzed the phylogenetic diversity and axial distribution of microorganisms in three sections of the gastrointestinal tracts of the polychaete Neanthes glandicincta was evaluated using both most probable number method and cloning analyses of 16S rRNA genes in this study. Quantification of the density of microorganisms in the gut showed that aerobic microorganisms decreased from anterior to posterior, while anaerobic ones showed a reverse trend. The total numbers of microorganisms decreased significantly
(p<0.05, analysis of variance) but more rapidly from the anterior to the middle segment. The results revealed a difference in microbial community structure along the gut of *N. glandicincta*. The various phylogenetic diversity and axial distribution of microbes along the gut indicated an environmental gradient from anterior to posterior sections affecting the structure of the microbial community. Shankar *et al.* (2010) assessed the antibacterial activity of bacteria associated with polychaetes. Polychaetes were collected from the coastal environment and the bacterial communities associated with the surfaces were isolated using traditional culture method. Four biofilm bacteria (*Galionella* sp., *Alteromonas* sp., *S.aureus*, *Klebsiella* sp.) were isolated from the marine water were used as target organism for screening. Three surface associated bacteria were isolated from the surface.

The EPS of the three surface associated bacteria were isolated and tested for their antimicrobial activity. The results showed higher activity against *Alteromonas* sp. The bioactive compounds were separated by thin layer chromatography. The results showed higher activity against *Alteromonas* sp. Based on the study, concluded that bacteria associated with Polychaetes would serve as a potential source for the isolation of bioactive compounds. Matthew *et al.* (2011) reported that the tolerant species of polychaete worms can survive in polluted environments using various resistance mechanisms. One aspect of resistance not often studied in polychaetes is their association with symbiotic bacteria, some of which have resistance to metals and may help the organism to survive. They used “next generation” 454 sequencing of bacterial 16S rRNA sequences associated with polychaetes from a copper- and zinc-polluted harbor and from a reference site to determine bacterial community structure. They found changes in the bacteria at the polluted site, including increases in the abundance of bacteria from the order Alteromonadales. They opined that changes in the bacteria
associated with polychaetes may be relatively easy to detect and could be a useful indicator of metal pollution.

**Bacteriocin Production**

Nazime Mercan and Cumhur (2004) have focused a total of 19 strains of *Bacillus sphaericus* are compared both in vegetative and sporulated stages according to their filtrate protein profiles obtained by Native-PAGE and SDS-PAGE. When the strains were compared in the sporulated stage, filtrate protein profiles obtained by Native-PAGE differentiated the strains according to their phage and serogroups. On the other hand, the typing according to filtrate protein profiles was correlated with serotyping and phage typing. The discrimination of *B. Sphaericus* strains by Native-PAGE is more useful. Hashium *et al.* (2010) isolated lactic acid bacteria from 50 samples of different sources (fermented foods 30 samples, chicken intestine (10 samples) and human intestine and vagina (10 samples). Three hundred isolates were isolated from Baghdad, Iraq (220 isolates from human intestine and vagina and 80 isolates from fermented foods) and the others from Bangkok (Thailand). These isolates were screened for bacteriocin production using the agar well diffusion method and *Shigella dysenteriae* DMST 15110 was used as an indicator strain. Seventy two isolates gave clear inhibition zones against the growth of indicator strain. One isolate, F14 (from Thailand fermented fish) that produced largest inhibition zone against the indicator strain was chosen for further study, and identified as *Weissella citaria*. Mohankumar and Murugalatha (2011) studied the antimicrobial activity of *Lactobacillus* producing bacteriocin isolated from raw milk of cattle’s like cow, buffalo and goat and characterized the bacteriocin. Bacteriocin producing organisms were screened by Agar spot assay test. The antibacterial protein bacteriocin was characterized based on the sensitivity to heat, different pH values, acid neutralization test, sensitivity to chloroform, NaCl and incubation period. Lactobacilli from
raw milk samples that inhibited certain pathogenic organisms by producing bacteriocin may be beneficial for a probiotic culture to be triumphant in colonizing and to contend with pathogens. Ozdemir and Biyik (2012) observed a novel bacteriocin Toebicin 218 which was isolated from *Geobacillus toebii* HBB-218, a soil inhabiting Gram positive bacterium. The cell free culture supematants of *G. toebii* HBB-218 showed antibacterial activity against many Gram positive bacteria including thermophilic strains. Purification of the bacteriocin was achieved after ammonium sulphate precipitation, gel filtration and ion exchange chromatography. Tricine-SDS-PAGE yielded a single protein band observed with a molecular mass of 5.5 kDa.

The antibacterial compound was heat stable and sensitive to proteolytic enzymes. Bacteriocin production started at the early logarithmic phase and maximum production was observed at the end of the stationary phase. Iyapparaj *et al.* (2013) worked on the optimization and partial purification of bacteriocin produced by a goat milk isolate *Lactobacillus* sp. MSU3IR against the shrimp bacterial pathogens. Mahrous *et al.* (2013) investigated on isolates of *Lactobacillus* spp. which had potential for producing bacteriocins to suppress the growth of *Escherichia coli* ATCC 25922 and *Bacillus subtilis* NCIB3610, and optimized the process of bacteriocin production. Piyush Baindra *et al.* (2013) produced two antimicrobial peptides from a rhizosphere soil sample and identified strain as *Bacillus subtilis* based on both phenotypic and 16S rRNA gene sequence phylogenetic analysis. It grew optimally up to 14% NaCl and produced antimicrobial peptide within 24 hrs of growth. The peptides were purified using a combination of chemical extraction and chromatographic techniques. The MALDI-TOF analysis of HPLC purified fractions revealed that the strain SK.DU.4 secreted a bacteriocin-like peptide with molecular mass of 5323.9 Da and a surface-active lipopeptide (m/z 1056 Da). The peptide mass fingerprinting of low-molecular-weight
bacteriocin exhibited significant similarity with stretches of secreted lipoprotein of *Methylothermobacterium album* BG8 and displayed 70% sequence coverage.

**Cellulases Enzyme Production**


Saravanan and Jayaraaj (2004) carried out the comparative studies on degradation of cellulose in coirpith using the fungus *Pleurotus sajorcaju* and *Trichoderma viride*. Baig *et al.* (2004) reported the saccharification of banana agro waste by cellulolytic enzymes. Heidorne *et al.* (2005) have studied the production of cellulases and hemicellulases by *Ceriporiopsis subvermispora* cultured on wood chips of *Eucalyptus grandis* and *Pinus taeda*. Kathiresan and Manivannan (2006) have reported the cellulase production from *Penicillium fellutanum* isolated from coastal mangrove rhizosphere soil. Ariffin *et al.* (2006) reported the

Emtiazi *et al.* (2007) reported the cellulase activities in nitrogen fixing *Paenibacillus* isolated from soil in N-free media. Ibrahim and Ahmed (2007) have isolated and identified a cellulase producing thermophilic bacteria and an optimum cultural conditions were studied. Lee *et al.* (2007) have studied the purification and characterization of cellulase produced by *Bacillus amyloliquefaciens* DL-3 utilizing rice hull. Nishida *et al.* (2007) identified the primary structure of a cellulase from the Japanese sea urchin *Strongylocentrotus nudus*. Hatami *et al.* (2008) conducted a study on investigation of cellulolytic bacteria and their
1.3 OBJECTIVES OF THE PRESENT STUDY

The prime objective of the present study was to collect information on polychaetes available in the Uppanar estuary and use their associated microbes for bioprospecting. To achieve this, the following objectives were kept for the study.

✓ To study the density and diversity of polychaetes
✓ To study the bacterial density of polychaetes and their seasonal variation
✓ To bio-prospect the associated bacteria in the following aspects
  o Bacteriocin production
  o Cellulase enzyme production