Ecology of seagrass

Seagrasses are flowering plants (angiosperms), specially adopted to grow in marine environment. Seagrass is predominantly noticed in the inter-tidal region of tropical coastal seas. Due to their dominant spatial presence in many locations, they are more readily referred to as ‘habitats’, they are also sometimes referred to as ‘ecosystems’ in their own right (Larkum et al., 2006). They have been found to occur in all coastal areas of the world (although not continuously), except along the Antarctic shores (Hemminga and Duarte, 2000).

Seagrass meadows are, on an area basis, very productive ecosystem with an average standing stock seagrass dry weight (d.w.) of 460 g m\(^2\), and an average growth rate of 5 g d. w. m\(^2\) day\(^{-1}\) (Duarte and Chiseano, 1999). Seagrasses can be patchy in distribution but more often they form large swaths of vegetation, sometimes over 10,000 km\(^2\) in area (Hemminga and Duarte, 2000). With their extensive root–rhizome system and well-developed canopy, there beds provide many important ecosystem services (Duffy, 2006). Seagrasses, although a predominant and specialized group of marine flora, are poorly understood compared to other ecosystems.

In India, the maximum extent (3000 ha) of seagrasses occurs along the Gulf of Mannar and Palk Bay (Jagtap and Imandar, 1991). In India, about 15 species have been recorded, belonging to seven genera that account for 30.16% of the total seagrasses reported in the world. The coast of Tamil Nadu (southeast)
harbours all the 15 species. The east coast supports a greater number of species than the west coast.

**Overview of Seagrass ecosystem.**

Seagrasses are a unique group of angiosperms that have adapted to exist fully submersed in the sea. They profoundly influence the physical, chemical and biological environments in coastal waters, acting as ecological engineers (Wright and Jones, 2006). There are relatively a few species globally (60 species) and these are grouped in 10 genera and 5 families (Short and Coles, 2001). Generally they are divided into five temperate and five tropical genera (Green and Short, 2003).

The family Zosteraceae includes genera *Zostera* and *Phyllospadix*. Hydrocharitaceae notably includes *Enhalus, Thalassia* and *Halophila*. The family Potamogetonaceae includes *Cymodoceae, Halodule* and *Syringodium* and Posidoniaceae includes genus *Posidonia*. Additionally, a fifth family Ruppiaceae is sometimes accepted as a family of seagrass. They are common in brackishwater and the species *Ruppia* is a very important seagrass in parts of the Mediterranean region, particularly in the Black, Aral and Caspian Seas (Green and Short, 2003).

seagrass meadows is greater than in adjacent unvegetated areas and faunal densities are orders of magnitude higher inside the meadows (Hemminga and Duarte, 2000).

**Seagrass-associated bacteria**

Seagrass act as nutrient sinks, buffering or filtering nutrient and chemical inputs to the marine environment, that support a diverse assemblage of microorganisms ranging from mutualistic to parasitic species (Crump and Koch, 2008). Although prokaryotes are usually stigmatized as pathogenic, many bacteria have neutral and beneficial effects on their host plant (Lodewyckx et al., 2002). Bacterial biofilm associated with seagrass can promote plant growth by deterring insect and animal herbivory, and occupying an ecological niche similar to that of phytopathogens (Azevedo et al., 2000).

Negative impacts on seagrasses could arise from increased shading by thick biofilm and possibly also from pathogenic bacteria present in the biofilm. Pathogenic microbes on seagrasses can devastate populations of marine plants and animals. Aquatic angiosperms have several physiological traits such as oxygenation of rhizosphere (Sand-Jensen et al., 1985), and production of antimicrobial agents (Bushmann and Ailstock, 2006) that influence the composition of attached microbial communities and hence encourage the growth of mutualistic microbial populations (Kloepper et al., 1980; Mayak et al., 2004).
Overview of biofilm-forming microbe – bacteria

Historically microorganisms have primarily been characterized as planktonic, freely suspended cells and as occurring ubiquitously in nature. They are found in soil, marine and fresh water, sewage sludge and even in extreme environments such as hydrothermal vents (Hugenholtz et al., 1998). They are described on the basis of their growth characteristics in nutritionally rich culture media.

Van Leeuwenhoek rediscovered the microbiological phenomenon and explained how microorganisms attach to and grow universally on exposed surfaces. Studies revealed that surface-associated microorganisms exhibit a distinct phenotype with respect to gene transcription and growth rate. In the last decades, it has been commonly acknowledged that bacteria prefer an attached lifestyle if nutrient conditions are favourable and thus are mainly found on surfaces (Costerton et al., 1995; Stanley and Lazazzera, 2004). Since then much research has been done on bacterial biofilms, their role and function in healthcare, wastewater treatment, industries and ecology (Morris and Monier, 2003; Pasmore and Costerton, 2003; Parsek and Fuqua, 2004; Stanley and Lazazzera, 2004).

Biofilm is defined as the aggregation of microbes that occurs at solid–liquid interfaces enclosed in an extracellular polymeric substance (EPS) matrix and develops on all surfaces in aquatic environments (Venugopalan et al., 1998). In general, biofilms contain water, EPS (upto >90% of organic matter), cells, entrapped particles, precipitates, adsorbed ions, and polar and apolar organic molecules (Donlan, 2002).
The bacteria in biofilm live predominantly associated with surfaces as biofilm communities in natural and man-made environments (Stoodley et al., 2002). Biofilms found in nature are usually multispecies aggregations in which bacteria of different metabolic characteristics coexist and may act as symbionts (Burmolle et al., 2006).

The process of biofilm formation generally begins with the formation of a biochemical conditioning film on which bacteria and other microorganisms colonize (Costerton and Lappin-Scott, 1995). Bacterial colonization occurs via a two-step process beginning with reversible attachments of cells that are held by physical forces and can be easily removed by gentle washing. Non-reversible attachments of cells are due to mechanisms such as hydrogen bonding, ligand interaction and the production of extracellular polysaccarides (Biancetto et al., 2001; Jayaram and Seetharaman, 2003).

The microbes present in a biofilm have an increased resistance to dessication, grazing and antimicrobial agents compared to their planktonic counterparts (Fux et al., 2005; Jefferson, 2004; Mah and O’Toole, 2001; Matz and Kjelleberg, 2005; Sutherland, 2001). These surface-associated microbes, because of their ubiquity, diverse metabolic capabilities and high enzymatic activity, play a crucial role in biogeochemical cycling (Moss et al., 2006).

Direct observations show that biofilm-associated organisms account for a major part of ecosystem processes both numerically and metabolically (Costerton et al., 1995). They are also more resistant to grazing by flagellates due
to the thickness of the biofilm and the EPS matrix, which makes them less accessible (Jurgens and Matz, 2002).

Plants and their heterotrophic bacterial communities possibly strongly interact as biofilms especially in aquatic systems. Numerous studies have confirmed the relevance of symbiotic associations such as plant–microbe interactions for the survival of most terrestrial species (Montesinos et al., 2002). Most researchers have focused their interest in documenting and studying microbes associated with their hosts but little is known about those present in marine plants.

**Secondary metabolites in seagrasses**

Seagrasses are a rich source of secondary metabolites, particularly phenolic compounds (Mcmillan et al., 1980) that limit pathogenic bacterial and fungal colonization of plant surfaces, allowing only certain microbes to become established (Bushman and Ailstock, 2006; Harrison, 1982; Jensen et al., 1998; Newby et al., 2006). Antimicrobial defences of marine organisms are largely uncharacterized, although from a small number of studies it appears that chemical defences may improve host resistance (Kubanek et al., 2003). Phenolic compounds are well known as allelopathic agents in terrestrial plants (Swain, 1977) and similar ecological functions have been found in extracts of seagrasses that had antifouling activity (Jensen et al., 1998). Extensive chemical investigations of the extracts from marine organism have led to the discovery of a variety of secondary metabolites with antimicrobial activities against human pathogens (Pesando, 1990).
Seagrasses from India have been largely left out of education, research and management consideration. In recent years there has been a growing interest in biofilms, owing to their significance in environmental, industrial and medical areas. Application of the naturally occurring biofilm microbes associated with aquatic angiosperms may improve seagrass restoration (Cammarata, 2008).

Hence the present investigations were made with the following objectives:

1. To study the ecology of seagrasses occurring along the Kanyakumari coast and to record the radioecological nature in view of the nuclear power plant, Koodankulam.

2. To isolate and characterize the biofilm-forming bacteria on seagrass blades

3. To monitor the seasonal abundance of bacteria in the seagrass blades (phyllosphere) and to compare the epiphytic and endophytic bacterial load of the seagrasses along with the study of hydrological parameters.

4. To study the interaction between associated bacteria and to monitor various bioactivity present in the seagrasses.

5. To screen the phytochemicals present in the seagrasses and to study the seagrass larvicidal and insect repellent activity which provides valuable information on broader seagrass health, in addition to human health.
REVIEW OF LITERATURE

There are several studies from Indian waters characterizing the biochemical and molecular constituents to assess the development of biofilms in various hard surfaces (Bhosle et al., 1989, 1990, 2004; Venugobalan et al., 1998; Bhosle and Wagh, 1997; D’Souza and Bhosle, 2003). The bacteria that developed on hard surfaces were also studied by Devi (1995), Palanichamy et al. (2002) and Nancharaiah et al. (2004).

Marine biofilm and biofouling have also been subjected to substantial research effort throughout the world, and numerous published studies are available from marine and coastal waters of various geographical regions. Fuhrman et al. (1993) investigated the phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans.

Bacteria associated with seagrass

The study and assessment of epiphytic assemblage and the biofilm formation on plants are limited in the marine ecosystem especially on aquatic angiosperms or seagrasses. Terrestrial plant-associated biofilm and the epiphytic bacterial studies have been carried out extensively by various scientists. However, there has been relatively little examination of phyllosphere microbiology when compared to other bacterial habitats.

Beattie and Lindow (1999) investigated the bacterial colonization on terrestrial leaves and the study proved that bacteria can modify their environment on and within leaves to enhance their colonization on plants, by increasing local
nutrient concentration or by producing a layer of extracellular polysaccharides. Lindow and Leveau (2002) also reported about the phyllosphere microbiology (microbes on leaves) in terrestrial plant species. Koutsoudis et al. (2006) reported about quorum sensing that initiates biofilm formation and host colonization.

Ramey et al. (2004), Welsh (2000), Morris and Monier (2003) and Danhorn and Fuqua (2007) studied the biofilm formation on leaves by plant-associated bacteria, and their significance. Marco et al. (2005) have reported the colonization of *Pseudomonas syringae* on bean leaf surfaces which demonstrated a high level of epiphytic fitness on plants.

Monier and Lindow (2003, 2005) also reported the bacterial colonization on bean leaf surfaces. Idris et al. (2004) recorded the bacterial communities associated with flowering plants. More than 85 different species of microorganisms in 37 genera have been reported in the phyllospheres of rye, olive, sugar beet and wheat (Hirano and Upper, 2000; Legard et al., 1994; Thompson et al., 1993).

The biofilm bacteria and the epiphytic bacterial communities were also studied in aquatic plants by a few researchers. Lemos et al. (1985) made a survey of antibiotic-producing bacteria from five species of green and brown seaweeds and studied their antibiotic-producing capacities. Heterotrophic bacteria attached to seaweeds were also reported by Shiba and Taga (1980). The colonization and invasion of leaves by the epiphytic bacteria from the aquatic macrophyte *Ceratophyllum demersum* L. was also recorded by Underwood (1991).

Hempel et al. (2008) also reported a comparative analysis of the epiphytic bacterial community composition on two submerged macrophytes, *Chara aspera*
Willd. and *Myriophyllum spicatum* L., in two different brackishwater and freshwater habitats and found that bacterial communities were influenced by host plant and environmental factors.

Bacterial enrichment in seagrass meadows also led to the study of associated microbes by a few researchers. Worldwide, scientists reported the occurrence of epiphytes on seagrass beds. Pereg *et al.* (1994) isolated a population of bacteria from *Halophila stipulacea* (Forsk.) Aschers seagrass beds.

Wahbeh and Mahasneh (1984) also reported the difference in heterotrophic association of bacteria which were seen attached to leaves, rhizomes and roots of three seagrass species (*Halophila ovalis* (R.Brown) Hook. F., *H. stipulacea* (Forsk.) Aschers. and *Halodule uninervis* (Forsk.) Aschers.) in Jordan.


Hamisi *et al.* (2004) in coastal Tanzania reported the cyanobacterial occurrence and diversity in seagrass meadows. In the northwest Mediterranean Sea, Balata *et al.* (2007) recorded the pattern of spatial variability of *Posidonia oceanica*-associated epiphyte assemblage in both the leaves and rhizomes in three
different habitats. Results showed the absence of significant difference in 
association in plant parts as well as habitat.

Barnabas (1992) in South Africa reported the bacteria on and within leaf 
blade epidermal cells of *Thallasodendron ciliatum* (Forssk.). Cifuentes *et al.* (2000) studied the prokaryotic diversity in *Zostera noltii* (Hornem.) colonized marine sediments and rhizosphere samples. In the Gulf of Elat, 
Weidner *et al.* (1996) reported the diversity of uncultured microorganisms 
associated with *Halophila stipulacea* (Forsk.) Asch.

Drake and Dobbs (2003) recorded the effects of epiphyte load on *Thalassia 
testudinum* J. Blanks & D. Solander ex Koenig and *Zostera marina* (L.). The 
abundance of seagrass-associated bacteria and its productivity was studied by a 
few researchers. Blum *et al.* (1988) reported the abundance of bacteria and fungi in 
*Thalassia testudinum, Syringodium filliforme* Kuetz, and *Halodule wrightii* Asch. from Florida Bay.

In the Lee Stocking Island and in Exuma Island of Bahamas, Moriarty and 
Pollard, (2004) registered the diel variation in bacterial productivity in *Zostera 
capricornii* Asch. from Australia. The seasonal dynamics of bacterial biomass and 
productivity of the eelgrass *Zostera marina* L. was investigated by Tornblom and 
Soundergaard (1999). Moriarty *et al.* (1985) also recorded the microbial biomass 
and productivity in seagrass beds. Kirchman *et al.* (1984) found out the 
productivity of epiphytic bacteria associated with *Zostera marina* L. in the 
northwest Mediterranean region.

The invention and introduction of molecular methods to microbial ecology has increased our knowledge of bacterial communities. Fisher *et al.* (1998) recorded the molecular characterization of epiphytic bacterial communities on charophycean green algae *Desmidium grevillii*, *Hyalotheca dissiliens* and *Spondylosium pulchrum* and found that the majority are undescribed bacterial species.

Rao *et al.* (2006) recorded the colonization of *Pseudoalteromonas tunicata* and *Roseobacter gallaeciensis* and the competition of microbes on the marine algae *Ulva australis* that showed difference in colonization strategies. Chand *et al.* (1992) enumerated and characterized the bacterial colonies such as *Actinobacter*, *Cyanophaga*, *Flavobacterium*, *Pseudomonas* and *Vibrio* on a submerged aquatic plant, *Myriophyllum spicatum* L.

The characterization of bacteria from seagrass blades by molecular method was also attempted by a few researchers. The phylogenetic analysis of the bacterial community associated with leaves of the seagrass *Halophila stipulacea* identified
*Pseudomonas, Marinomonas, Oceanospirillum* and *Roseobacter* from the leaves (Weidner *et al.*, 2000).

Uku *et al.* (2007) characterized and compared the prokaryotic epiphytes associated with two East African seagrasses such as *Thalassia ciliatum* and *T. hemprichii*. The analysis revealed the presence of Cytophage-Flavobacteria-Bacteroides (CFB).

The attached bacterial population shared by four species of angiosperms (*Vallisneria americana* Michx (freshwater), *Potomogeton perfoliatus* L., *Stuckenia pectinata* (L.) Boerner (brackishwater) and *Zostera marina* (marine water)) was investigated by Crump and Koch (2008) which led to the identification of leaf-attached phylotypes that belongs to Bacteroides, Alphaproteobacteria, Betaproteobacteria that host potentially mutualistic populations.

**Interaction of bacteria with host plant**

More comprehensive reviews of phyllosphere microbiology also address another important feature of interesting association. The inclination for bacteria to colonize plant surfaces is a double-edged sword, they can prove either beneficial or potentially destructive (Dunner, 2002). Plants serve the microbes in two different ways: they give mechanical support or they may provide some nutrients for the microbes.

A comparative study was made on the adhesion of epiphytic bacteria and marine free-living saprophytic and pathogenic bacteria on seagrass leaves and abiotic surfaces to prove the bacteria–plant symbiotrophic relationship.
*Cytophaga* sp KMM 3552 and *Pseudomonas citrea* KMM 461 on *Zostera marina* seagrass blades showed increased number of viable cells, i.e., 3–7-fold after 60 h of incubation when compared to abiotic surfaces (Kurilenko *et al.*, 2007).

The interaction of microbes with seagrasses was also studied by a few researchers. In the coast of United States of America, Orth and Montfrans (1984) investigated the epiphyte–seagrass relationships with a role of micrograzing. Harlin (1975) studied the epiphyte–host relationship in seagrass communities in United States of America.

Furthermore, the epiphytes on leaves are also involved in processes such as carbon cycles, nitrogen cycles and nitrogen fixation, affecting the health of the individual plant (Lindow and Brandl, 2003; Yang *et al.*, 2001). Algam *et al.* (2005) investigated a method for introducing *Bacillus* for the growth promotion and suppression of wilt in tomatoes.

The nitrogen-fixing potential of associated epiphytes on seagrass has also been documented by various authors (Goering and Parker, 1972; Capone *et al.*, 1979; Moriarty and O’Donohue, 1993; Mcglathery *et al.*, 1998; Pereg *et al.*, 2002) and they have shown that epiphytes on seagrass leaves were responsible for nitrogen fixation and thus were important contributors to the nitrogen budget of seagrass communities.

Adithya *et al.* (2007) studied the diversity of assimilatory nitrate reductase genes from plankton and epiphytes associated with seagrass beds. Welsh (2000) reported a symbiotic association between seagrass and nitrogen-fixing bacteria based on mutual exchange of fixed carbon and nitrogen.
Donnelly and Herbert (1999) studied the bacterial interaction in the rhizosphere of seagrass communities in coastal lagoons.

The diverse collection of bacteria found on leaves sometimes includes a few pathogens that can incite diseases. Von Bodman et al. (2003) studied quorum sensing in plant pathogenic bacteria and the steps involved in biofilm formation and infection in *Pantoea stewartii* ssp. *stewartii*.

In Florida, seagrass–pathogen interaction was also studied by Latina et al. (2005). Montesinos et al. (2002) studied several implications for the management of plant diseases that are derived from the knowledge of plant–microbe interactions and the new biotechnological methods used for plant disease control.

Armstrong et al. (2001) investigated the symbiotic role of marine microbes on living surfaces and described an ecological role for epibiotic bacteria associated with the surface of the seaweed, *Codium* sp. that play a protective role releasing compounds into surrounding seawater and helping to prevent excessive fouling of the surface thus useful in bioprocess application.

**Allelochemical interaction**

In the past, many studies described allelochemical interactions between bacteria and phototrophic organisms. The bioactivity of plant extracts against colonizing bacteria and epiphytes were reported in terrestrial as well as in aquatic plants. The antimicrobial activity was well studied in marine plant especially algae.
From northeast Brazilian coast, Lima-Filho et al. (2002) studied the antibacterial activity of the extracts of six macroalgae. The screening of antimicrobial activities in red, green and brown algae from Spain was reported by Gonzalez-del-Vak et al. (2001).

Ballesteros et al. (1992) studied the biological activity of extracts from some Mediterranean macrophytes. Methanotrophic bacteria and their activity on submerged aquatic macrophytes were reported by Sorrell et al. (2002). A novel mechanism for rapid epiphytic control in marine macrophytes was studied by Littler and Littler (1999).

Even though the biofilm-associated microbes on seagrass are not well studied, the antifouling activity was well documented. Engel et al. (2002) analysed the chemical ecology of marine microbial defence and in 2006 reported the antimicrobial activity of plant extracts of Halodule beaudettei (C. den Hartog) C. den Hartog, Syringodium filiforme and Thalassia testudinum that inhibited Halophytophthora spinosa, Schizochytrium aggregatum and Pseudoalteromonas bacteriolytica from tropical Atlantic marine plants against marine pathogens.

In India Mayavu et al. (2009) studied the bioactive potential of seagrass species *Cymodocea serrulata* and *S. isoetifolium* against biofilm-forming bacteria from ship hull. The bacteria tested were *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus vulgaris*, *Escherichia coli*. Recently in India, Umamaheswari et al. (2009) reported a potential antibacterial activities of seagrasses *Halophila ovalis* and *Halodule pinifolia* against *Acinetobacter* spp., *Salmonella typhi*, *Micrococcus* spp., *Shigella sonii*, *Vibrio cholerae*, *Staphylococcus* spp., *Proteus vulgaris*, *P. mirabilis*, *Pseudomonas aeruginosa* and *Salmonella paratyphi*. B. from Vellar estuary in southeast coast of India.

Sreenath Kumar et al. (2008) studied the bioactivity of *Cymodocea serrulata*, *Halophila ovalis* and *Zostera capensis* against human pathogens such as *E. coli*, *Staphylococcus aureus*, *S. subtilis*, *Micrococcus luteus* and *S. typhimurium*. Ross et al. (2007) studied the antifungal defences of seagrasses from Indian River Lagoon, Florida. From the Mediterranean coast the antibacterial and antifungal activities of extracts from rhizomes of seagrass *Posidonia oceanica* was identified by Bernard and Pesando (1989).

Haroon, (2006) showed the effect of methanol extracts of some common and widely distributed macrophytes (leaves and stems) collected from Manzalah lake on the growth of toxigenic strain of *Aspergillus parasiticus* in a chemically defined media and proved the inhibitory effect of macrophytes on fungal growth.

A new antibiotic from seagrass *Thalassia testudinum* was found out by Jensen et al. (1998) that prevent a zoosporic fungi. Ross et al. (2007) studied the antifungal defence of seagrasses from the Indian River Lagoon, Florida.
Spencer and Ksander (1999) studied the phenolic acids and nutrient content for aquatic macrophytes.

Newby et al. (2006) reported an important component zosteric acid from Zostera marina which showed an effective antifouling activity that reduce the attachment of freshwater bacterial attachments on coatings, and they investigated the antifoulant compound zosteric acid that reduces the freshwater bacterial attachment on coatings.

**Phytochemicals in seagrass**

Few of the researchers reported the biological activity and the presence of active principle in the seagrasses. Zapata and Mcmillan (1979) reported the phenolic acids in seagrasses. Mcmillan et al. (1980) also described the phenolic compounds in the seagrasses. Bernard and Clement (1983) reported the antibiotic substances from seagrass Posidonia oceanica. Bushmann and Stephen (2006) also reported the antimicrobial compound in estuarine submerged aquatic plants.

Hamout’ene et al. (1995) studied and characterized xerobiotic metabolism from leaf sheaths of marine seagrass Posidonia oceanica. Swain (1977) studied that the secondary compounds as protective agents. Vergeer et al. (1995) investigated the wasting disease and the effect of abiotic fraction and infection with Labyrinthula zosterae on phenolic content of Zostera marina shoots. Harrison (1982) from Canada reported the control of microbial growth and amphipod grazing by the phenolic content from Zostera marina seagrass blades.
In Egypt from Bardawal Lake Howayda et al. (2007) found an antimicrobial compound from Cymodocea nodosa, Ruppia cirrhosa. Similar studies in Indo-Pacific marine plants were also reported by Puglisi et al. (2007). Bhosale et al. (2002) studied an antifouling potential of Cymodocea rotundata from India against species of Bacillus and Pseudomonas.

Kongkum and Jangaramruarg (2005) isolated indole-3-carboxaldehyde from seagrass Halodule pinifolia and crystals of s8 from Enhalus acoroides (L.F.) Royle, and Halophila ovalis from Kung Krubaen Bay, Chantaburi. Ioanna et al. (2008) screened a new metabolite from Cymodocea nodosa such as diarylheptanoids, meroterpenoid, brominated briarane diterpene that was active against methicillin-resistant strains of Staphylococcus aureus, Mycobacterium phlei, M. smegmatis and M. fortuitum.

Numerous reports on the mosquito larvicidal activity of terrestrial plants were recorded. Mittal and Subbarao (2003) analysed the prospects of using herbal products in mosquito control. Das et al. (2006) evaluated the mosquito larvicidal efficacy of plant extract. Suwannee et al. (2006) evaluated the larvicidal activity of medicinal plant extract. Shaalan et al. (2005) also studied about the use of botanical phytochemical with mosquitocidal potent. Sukumar et al. (1991) studied about the botanical derivatives in mosquito control.

Few reports on seagrass were also documented. Prabha Devi et al. (1998) reported the larvicidal activity of some marine macrophytes including seagrass Halophila ovalis and Syringodium isoetifolium against Artemia salina. The
mosquito larvicidal activity of seaweeds *Plocamium telfairiae* and *Laurencia nipponica* was reported by Watanabe *et al.* (1998, 1999)

**Ecology of seagrass**

Seagrasses have been described as coastal canaries, global biological sentinels of increasing anthropogenic influences in coastal ecosystems (Orth *et al.*, 2006). Fundamental monitoring programme should provide a powerful tool for coastal resource managers through improved tracking of seagrass population over time.

Throughout the world the seagrass ecological study focussed on various aspects. Guidetti (2001) reported the temporal dynamics and biomass partitioning in three Atlantic seagrass species *Posidonia oceanica, Cymodocea nodosa* and *Zostera marina*. In India, Kannan and Thangaradjou (2006) studied the biomass and productivity of seagrasses. Lipkin (1979) investigated the quantitative aspects of seagrass communities especially *Halophila stipulacea* in Sinai (northern Red Sea).


According to Jagtap *et al.* (2003), the major seagrass meadows in India exist along the southeast coast (Gulf of Mannar and Palk Bay) and in the lagoons of Island from Lakshadweep (Arabian Sea) and Andaman and Nicobar in Bay of
Bengal. The seagrass species diversity is reported to be high in the Gulf of Mannar and Palk Bay, while it is low in the Bay of Bengal (Parthasarathy et al., 1991).

Most of the earlier seagrass studies in the Gulf of Mannar have focused only on their quantitative, taxonomic and structural components. Few studies in India focussed on the distribution, associated flora and fauna. Thangaradjau et al. (2007) studied the distribution pattern of seagrass meadows in the Mandapam group of Islands. At Minicoy Lakshadweep Island, Ansari (1984) studied the benthic macro- and meio-fauna of seagrass *Thalassia hemprichii* (Ehrenb.) Asch. Ranjitham et al. (2008) also investigated the associated fauna of seagrass in Vellar estuary.

Studies on number of general parameters are needed for the encouragement or elimination of seagrass from specific location. The present study of seagrass-associated bacteria, its bioactive interaction and the ecology would be the base line data to know the changes in seagrass population in future.
AIM AND SCOPE OF STUDY

Even though considerable literature is available on biofilm-forming microbes on abiotic substratum, the biofilm formation of bacteria on biotic surface especially on plants, their interaction with host plants and the host bioactivity work was lacking from India especially in southmost Tamil Nadu. So this led to the initiation of the present study. The importance of seagrass in coastal ecosystems had drawn more attention these days, as it can provide food and shelter for microbes, algae, fish and other organisms and also keep the coastal ecological system healthy.

Seagrass ecosystems are sensitive to impacts linked with human activities and are undergoing substantial declines (Jagtap et al., 2003). Epiphytes play an integral role in the ecology of seagrass communities including food web dynamics (Fry and Parker, 1979) and nutrient cycling (Harlin, 1973; McRoy and Goering, 1974). In addition epiphytes are major contributors to the overall productivity of seagrass meadow (Moncreiff et al., 1992), and are considered an important factor influencing the distribution and abundance of seagrasses (Kuo and McMomb, 1989).

Microbiologists are also having increased attention during recent years towards seagrasses which constitute potential bioactive substances. There are very few reports concerning anti-fungal, anti-viral, anti-bacterial activity of crude extracts of marine plants including seagrasses (Bernard and Pesando, 1989; Garg, 1993; Prabha Devi et al., 1997). Considering the importance of seagrass
ecosystem and the biofilm-forming microbes, particularly bacteria, the thesis was carried out with the following objectives:

1. To study the distribution, morphology, biomass of the seagrass ecosystem and the radioactivity of seagrass of the southern coast of Kanyakumari.

2. To isolate and characterize the biofilm-forming bacteria from seagrasses blades of *Halodule pinifolia* and *Syringodium isoetifolium*.

3. To analyse the epiphytes and endophytes seasonal microbial load (bacteria), to study its changes based on hydrological conditions.

4. To study the surface interaction and bioactivity of seagrass against pathogens.

5. To screen the phytochemicals and to test the larvicidal and insect repellent activity.
DESCRIPTION OF THE STUDY AREA

The study site was chosen based on seagrass abundance and the investigation on biofilm-forming bacteria on seagrasses; the bioactivity and the assessment of microbial load on the seagrasses were conducted along the east coast of Kanyakumari. Kanyakumari district, that have a coastline of about 68 km, is situated on the southern extremity of the Indian peninsula (latitude between 8°4′ and 8°21′N and longitude between 77°26′ and 77°30′E).

The district has 62 km of coast on the western side and 6 km of coast on the eastern side. Since this district is situated at the extreme south of the Indian subcontinent the coastline is formed nearly by three seas. The southeastern boundary is the Gulf of Mannar (Bay of Bengal) while on the south and southwest the boundaries are Indian Ocean and Arabian Sea. But the main part of the coast faces the Arabian Sea. The coast receives two monsoons, the southwest and northeast monsoon. The northeast monsoon occurs between October and December while the southwest monsoon occurs between June and September.

The coastal ecosystem of Kanyakumari district is studded with 44 coastal fishing villages. Beaches in specific areas of the district are rich in mineral content and the sands look partly black. The state-owned Indian Rare Earths Ltd. mines the sands for zircon, ilmenite, monazite, thorium and other minerals. Kanyakumari is 20 km to the south of Nagercoil; the place is famous for its distinctly beautiful (reddish) sunrise and sunset. It is an important tourist centre of India. The coastal landscape of Kanyakumari is mainly composed of beach ridges of rocky and sandy
region. The selected site for this work was off Kanyakumari Catholic Church. The study site also reflected the seasonability influence of the southwest and northeast monsoon with variability seen in nutrient influxes, the floral composition as well as other physical characteristic of this site. The study site is in between Vattakottai on the eastern side and Cape Comorin on the western side. Fisherman housing structures were constructed in the marine-terrestrial zone. Since the study site was bounded by man-made constructed stone walls, seagrass are abundant in this area and in no other places such abundance was noticed.

**Enlarged view of the study area**
This work was intended to assess the biofilm community of *Halodule pinifolia* and *Syringodium isoetifolium*, focusing on five approaches. The first part of this work is aimed to study the ecology and radioecology of available seagrasses of Kanyakumari Coast. The second part was to isolate and characterize the biofilm-forming bacterial population of the two seagrasses *H. pinifolia* and *S. isoetifolium* which relies on standard microbiological techniques and molecular characterization of predominant bacterial strains. The third part is to study the seasonal load of microbes and the fourth part is to find out the interaction and the bioactivity of the seagrasses. The fifth part is to screen the phytochemicals of the seagrass blades and to study the larvicidal activity and insect repellent activity.

Three seasons were recognized in the present study *viz.* post-monsoon season from February to May, pre-monsoon season from June to September and norh east monsoon season from October to January. During the research period (2008 to 2010) several field trips were conducted in different seasons to survey the seagrasses of entire coast of Kanyakumari District. Based on richness a single site was chosen and thoroughly studied for its microbial load. The study sites have plenty of seagrasses *Halodule pinifolia* and *Syringodium isoetifolium* attached to flat rock.