Introduction
1. Introduction:

Natural products have had a crucial role in identifying novel chemical entities with useful drug properties (Newman et al, 2000). Nearly 75% of the populations in under-developed countries depend on plant based medicines. According to WHO, this dependence is due to either economic or cultural reasons. Of late, there is growing awareness of the social benefit of effective drug therapy to treat various existing and emerging human diseases. It is expected that treatment will improve and also accessible to the world’s population. This expectation is predicated on a continued and determined effort by scientists, researchers and industries to discover new and improved drug therapies.

The ocean covers more than 70% of the earth’s surface and contains about 300,000 described species of plants and animals. The tropical marine habitats are rich sources of biological and chemical diversity. In the marine environment, the bioactive compounds, drugs, marine natural products are produced as secondary or non-primary metabolites. Natural products chemists considered them as sources of new and unusual organic substances. Synthetic chemists targeted these structures for the development of new analogues. Pharmacologists considered this for treatment of human/agricultural diseases and pests. Hence marine organisms are targeted for developing new medicines.
Among the phyla found in the oceans, the best sources of pharmacologically active compounds are present in bacteria, fungi, certain groups of algae and sponges. Some marine organisms such as dinoflagellates, echinoderms and some fishes are well known for their ability to produce potent toxins, but these are usually too toxic for medicinal use.

1.1 Marine microbes

Microorganisms are not only the causative agents of infection, but they also produce substances that can cure infections. After the discovery of Penicillin, about 50,000 natural products are discovered from microorganisms. More than 10,000 products are biologically active and more than 8,000 are antibiotics. For generations, it is known that seawater has bactericidal properties. This could be attributed to the production of antibiotics by planktonic algae and bacteria. Rosenfeld and Zobell in 1947 showed that bacteria produce antimicrobial products. Marine microorganisms encompass a complex and diverse assemblage of microscopic life forms and occur throughout the oceans. They are recorded from environments of extreme pressure, salinity and temperature. They developed unique metabolic and physiological capabilities that not only ensure in extreme habitats, but also produce novel metabolites which are not generally encountered from terrestrial microbes.

According to Gerwick et al, (2001) the greatest untapped source of novel bio compounds is marine microorganisms. Even with new techniques, it is noted that less than 1% of the
total marine microbial species diversity can be cultured with the common methods. This means chemicals produced by the rest of 99% of the microorganisms in the ocean have not yet been studied for potential commercial applications. Thus, these organisms constitute an enormous untapped resource and opportunity for discovery of new bio products with applications in medicine, industry and agriculture. In research and education, special emphasis has to be given to develop solutions for the identification, culture and analysis of uncultured marine microorganisms.

1.1.1 Bacteria

Highly brominated pyrrole antibiotic (Burkholder et al, 1966) was the first documented bioactive metabolite isolated from bacteria. The genera Alteromonas was found to be the producer (formerly: Pseudomonas bromoutilis) and was isolated from the sea grass (Thalassia sp.). The compound was highly active against Gram positive, but not against Gram negative bacteria. Antibiotic producing purple pigmented Chromobacterium (Alteromonas sp.) was isolated from North Pacific in 1977 and since then several antimicrobial compounds such as pyrrole, tetra bromo pyrrole and phenolics were isolated. The bioactive metabolite producing bacteria evolved the ability to concentrate the bromide ion from seawater and through oxidative pathways; this is incorporated into organic compounds. This results in metabolite bromination with enhanced bioactivities. There are differences in activity due to habitat and some of the compounds derived from marine microorganisms are listed in Table 1.
Table 1: Marine derived compounds

<table>
<thead>
<tr>
<th>S. No</th>
<th>Marine source</th>
<th>Drug</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sponge</td>
<td>Discodermolide</td>
<td><em>Discodermia dissoluta</em></td>
</tr>
<tr>
<td>2</td>
<td>Sponge</td>
<td>Isohomo-halichondrin B</td>
<td><em>Lissodendroryx sp.</em></td>
</tr>
<tr>
<td>3</td>
<td>Sponge</td>
<td>Bengamide</td>
<td><em>Jaspis sp.</em></td>
</tr>
<tr>
<td>4</td>
<td>Sponge</td>
<td>Hemiasterlins A &amp; B</td>
<td><em>Cymbastell sp.</em></td>
</tr>
<tr>
<td>5</td>
<td>Sponge</td>
<td>Girolline</td>
<td><em>Pseudaxinyssa cantharella</em></td>
</tr>
<tr>
<td>6</td>
<td>Bryozoan</td>
<td>Bryostatin 1</td>
<td><em>Bugula nentina</em></td>
</tr>
<tr>
<td>7</td>
<td>Sea hare</td>
<td>Dolastatin 10</td>
<td><em>Dolabella auricularia</em></td>
</tr>
<tr>
<td>8</td>
<td>Tunicate</td>
<td>Ecteinascidin 743</td>
<td><em>Ecteinascidia turbinata</em></td>
</tr>
<tr>
<td>9</td>
<td>Tunicate</td>
<td>Aplidine</td>
<td><em>Aplidium albicans</em></td>
</tr>
<tr>
<td>10</td>
<td>Tunicate</td>
<td>Isogranulatimide</td>
<td><em>Didemnum granulatum</em></td>
</tr>
<tr>
<td>11</td>
<td>Gastropod</td>
<td>Kahalalide F</td>
<td><em>Elysia rubefescens</em></td>
</tr>
<tr>
<td>12</td>
<td>Actinomycete</td>
<td>Thiocoraline</td>
<td><em>Micromonospora marina</em></td>
</tr>
</tbody>
</table>

(Source: Rajiv Gandhi Chair special publication, CUSAT, 2008)

Besides antibacterial and antifungal activity, most of the marine derived compounds showed anticancer activity which has high need in the medical world.
1.2 Anticancer activity

Despite recent advances in treatment modalities, cancer remains a major source of morbidity and mortality throughout the world and runs in the top three causes of death worldwide especially in the developed countries (WHO, 2009). Moreover, the incidence of many cancers, including cancers of the skin, prostate, breast, and kidney, continues to increase (Edwards et al., 2005).

“Cancer” is, in fact, a general term that refers to over 100 distinct diseases affecting many different tissues and cell types. However, all forms of cancer are characterized by abnormal cell growth resulting from a relatively small number of inherited or environmentally-induced genetic mutations (Renan, 1993). Hanahan and Weinberg (2000) have argued that in order for a cell to become cancerous, it must acquire six unique traits as a result of altered cell physiology. These defining traits of cancer cells are:

1. the ability to generate their own growth signals or respond to weak growth signals that are ignored by healthy cells;
2. insensitivity to antiproliferative signals;
3. resistance to cellular suicide mechanisms that normally cause aberrant cells to die by apoptosis;
4. the capacity for limitless replication;
(5) the ability to stimulate new blood vessel development in order to allow for tumour growth; and

(6) the capacity to invade tissues, at first locally, and later to spread or metastasize throughout the body.

Although localized cancers can often be successfully treated by surgery and/or radiation therapy, chemotherapy remains the usual treatment of choice for advanced or metastatic disease (Espinosa et al., 2003). Almost 60% of anticancer drugs are of natural origin, such as plants (i.e., vincristine, irinotecan, camptothecines) and microorganisms (i.e., doxorubicin, dactinomicines, mitomycin and bleomycin) (Grever, 2001). However, the use of conventional chemotherapeutic agents that typically target rapidly dividing cancer cells is often associated with deleterious side-effects caused by inadvertent drug-induced damage to healthy cells and tissues (Cassidy and Misset, 2002; Kalyanaraman et al., 2002). For these reasons, research and development of new classes of anticancer agents which exhibit efficient and selective toxicity to tumor cells is attracting increased attention. The development of a new class of anticancer drugs that lack the toxicity of conventional chemotherapeutic agents and are unaffected by common mechanisms of chemoresistance would be a major advance in cancer treatment.

In order to evaluate the biomedical potential of any plant or animal, both the chemical ecology of the organism and its evolutionary history must be considered. If it is assumed that secondary metabolites evolved from primary metabolites in a random manner, any
newly produced secondary metabolite that offered an evolutionary advantage to the producing organism would contribute to the survival of the new strain. The specific evolutionary pressures that led to chemically rich organisms need not be defined, but longer the period of evolution, the more time the surviving organism has had to perfect its chemical arsenal. Chemical defense mechanisms cannot be directly equated with potential biomedical activity, but it is remarkable how well the two correlate with reality. This could be understood by the fact that the targets of the chemical defenses, primary metabolites such as enzymes and receptors are highly conserved when compared with secondary metabolites. These evolutionary changes will occur only in the organisms residing in the extreme habitats so that they have to adjust their metabolism to survive. One of the extreme environments in the ocean is Sea Surface Microlayer (SML).

1.3 Sea Surface Microlayer (SML)

It is well known that the ocean provides several types of unique and potential environments such as extreme pressure, salinity, temperature and their combinations. One such environment is Sea Surface Microlayer (SML) which has not been extensively studied in India. This can be termed as the skin of the ocean and this air-sea interface denotes the upper 150-1000µm of the sea water. SML is a unique habitat characterized by high surface tension, high solar radiation, variable temperature, salinity and turbulence which are enriched with dissolved organic matter such as amino acids, carbohydrates,
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At the air – sea interface, the SML forms a physical boundary between the ocean and the atmosphere. Roughly considered as the uppermost 1mm of the ocean, the sea-surface microlayer is physico-chemically distinct compared with underlying water and is characteristically enriched with biogenic organic compounds such as lipids, proteins and polysaccharides (Liss and Duce, 2005). The presence of organic films as well as the surface tension forces of the interface itself provides an area of physical stability where compounds, particulate materials and organisms can concentrate (Hardy, 1982).

More recently it has been suggested that the SML is in the order of 50mm in thickness (Zhang et al, 1998). In comparison to the water column, the SML can be characterized as a physically more stable but climatically variable environment. Physical stability results from strong surface tension forces. Normal oceanic waves and ripples cause periodic changes in the thickness of the microlayer, but generally leave the microlayer intact. Breaking waves will disrupt the microlayer temporarily, but white caps cover only 3–4% of the ocean surface at any one time (MacIntyre, 1974).
1.3.1 Importance of SML

Surface films occur on all water bodies, marine, estuarine and freshwater, sometimes as visible slicks, and are rapidly reformed after mixing by wind or waves (Cunliffe and Murrell, 2009). The structure of the sea-surface microlayer film, is of great importance in the exchange of chemicals between the oceans and the atmosphere. During summer blooms of filamentous cyanobacteria, the net oxygen flux is significantly higher between the atmosphere and the surface microlayer compared with the ocean and the surface microlayer, which highlights the importance of autotrophic and heterotrophic organisms in this environment (Ploug, 2008). Also, bacterial activity within the microlayer can mediate the air–sea flux of other gases, such as methane (Upstill-Goddard et al., 2003).

SML is enriched with transparent exopolymer particles (TEPs) (Wurl and Holmes, 2008). TEPs are generally formed in surface waters due to the coagulation of biogenic polysaccharides (Fig. 1) particularly those produced by phytoplankton, and are some of the most ubiquitous gel particles in the marine environment. They are critical in the formation of marine aggregates, acting as the binding matrix or glue that holds the aggregate together (Verdugo et al., 2004).
The concentration of the enrichment factor depends on the assumptions of the thickness of the SML. It is beyond doubt that the rich organic SML support a distinct microbial community that fundamentally differ from the underlying euphotic zone. As this forms the interface between atmosphere and ocean plays an important role in heat momentum and mass exchange including gas fluxes. Hence, this understudied layer may be very important in issues related to global environmental change (GESAMP 59, 1995).

SML is a very dynamic system, due to the interplay of meteorological forcing on the sea surface and diverse import and export processes into and away from the interface (Fig. 2). Generally, material in the SML originates from the underlying bulk water, e.g. by upwelling, convection, diffusion or bubble transport as well as from wet or dry atmospheric deposition, e.g. by dust input or pollination events (Sodergen, 1987).
Material will leave the SML by evaporation and aerosol formation into the atmosphere as well as by dissolution or sedimentation in the underlying water. Sedimentation results from e.g. a collapse of the SML due to increased surface pressure and convergent forces (Wheeler, 1975).

**Fig. 2: Transformation processes influencing material concentration and properties of SML**

Wind is one of the main factors constantly acting upon the sea surface and induces aerosol formation as well as wave breaking. Breaking waves disrupt the SML and induce a transport of material from the SML into the ULW. Furthermore, breaking waves as well as photodegradation of dissolved organic matter cause the formation of bubbles in the ULW (Wangersky, 1976). Bubbles, which ascend through the water column, collect
surface, active compounds, e.g. produced by phytoplankton, as well as particulate material and cause transport back into the SML (Blanchard, 1974). Bursting bubbles will release part of this material into the atmosphere (Bezdek and Carlucci, 1972). Therefore, after disruption events, bubbles may speed up the reestablishment of the SML which has been described to be very fast (Hardy, 1982). In turn, high concentrations of surface active compounds modify physical processes in the air-water interface, e.g. capillary wave dampening and bursting bubbles phenomena (Garrett, 1972). This is especially pronounced in visible surface films (slicks) which are formed during high concentrations of surface-active compounds and thus by a strong reduction of surface tension under low turbulence conditions and are frequently observed in marine systems (Romano, 1996).

Langmuir circulation, visible through bands with altered reflectance characteristics on the sea surface, acts as a local concentrating mechanism (Wangersky, 1976) and additionally causes small scale variations in SML properties.

1.3.2 Sampling of SML

The optimal basis for studying the air-water interface is the investigation of an undisturbed SML, i.e. one in which the native surface to volume ratio is maintained (Hermansson & Dahlbaeck, 1983). However, since most chemical and biological characterizations depend on obtaining larger water volumes, sampling of the SML and therefore a change in its surface to volume ration is unavoidable. Current sampling devices obtain SML layers of different thickness depending on their operating mode. The
thinner a collected SML sample is, the more selective the respective sampling device is thought to be (Hu¨hnerfuss, 1981a & b). Therefore, sampling of the SML must take the following into account (Garrett & Duce, 1980 and Hu¨hnerfuss, 1981a & b):

a) As little dilution with bulk water as possible,

b) No bias due to sampler selectivity

c) Large sample volume in reasonable sampling time and

d) Good handling.

Additionally, wind speed, water temperature and wave state at the time of collection, have been reported to influence the thickness of SML samples (Carlson, 1982b and Falkowska, 1999).

A common sampling technique is the employment of hydrophilic and hydrophobic sheets and membranes, which float on the sea surface and collect very thin layers (1-30µm) of the SML (Crow et al, 1975). Over the years a variety of instruments have been used, including: the Prism dip (Baier, 1972), Screen sampler (Garrett, 1965), Rotating ceramic drum (Harvey, 1966), Stainless steel tray (Hatcher and Parker, 1974) and Glass plate sampler (Harvey and Burzell, 1972). The efficacy of these methods has been evaluated in the laboratory (Hatcher and Parker, 1974, Van Vleet and Williams, 1980) as well as under field conditions (Carlson, 1982a). For the isolation of microorganisms from SML different sampling technique should be followed as the above said method extricate more water along with SML. Hence, to study the microbiology of SML, Teflon plate method
(Hu¨hnerfuss, 1981a & b) and membrane method (Franklin et al, 2005 and Cunliffe et al, 2009) has been widely followed by researchers. In the present study, an improved methodology to sample SML using membrane is also studied to avoid the possible contaminations in membrane method. For this, a device has been designed to place membrane in it and sample SML. As teflon and glass plates are hydrophobic surface, the membrane used in this study is hydrophilic.

Membranes and sheets sample high concentrations of chemical and biological SML components (Crow et al, 1975 and Kjelleberg et al, 1979), but it is still a matter of debate, whether these sampling devices are selective, especially concerning bacterial parameters (Agogue et al, 2004 and Franklin et al, 2005). Taken together, these reports show that until today no sampling device is known to fulfill the several requirements for SML studies. Generally, samples from the SML are composed to control samples from the underlying water to better understand quantitative and qualitative changes within and between the compartments.

Another sampler called Mesh screen sampler is also occasionally used in the study of bacterioneuston. But, the previous comparison of membrane-collected and mesh screen-collected samples from an estuarine surface microlayer showed that samples collected using a mesh screen underrepresent the bacterioneuston because samples also contain subsurface water, which “dilutes” the bacterioneuston sample (Cunliffe et al, 2009a & b).
Hence, the mesh screen sampler is not considered as an accurate sampler in the study of bacterial diversity of SML.

1.3.3 Microorganisms of SML

Microorganisms in the surface microlayer are subjected to a combination of both favorable and detrimental factors. Favorable factors are high concentrations of inorganic and organic nutrients, while detrimental factors are intense solar radiation in the ultraviolet and visible spectra, high concentrations of heavy metals and organic pollutants, temperature fluctuations and salinity changes. Due to the diverse mechanisms which transport and maintain material in the SML as well as its consistent enrichment of organic substances, it has long been suggested that bacteria are highly concentrated in the SML. Even though bacteria are abundant in SML, other microorganisms like fungi can also be found in SML. In the northern coast of Taiwan, near the Keelung City, China an ascomycetes yeast strain SM-22 was isolated from the SML (Chang et al, 2013). This strain showed a cell surface hydrophobicity higher than 90%, moderate UV A/B resistance, and it degraded 68% of the total petroleum hydrocarbon content of an artificial seawater medium containing 1 % (v v⁻¹) diesel oil within 15 days. Strain SM-22 does not produce ascospores on common sporulation media and it can therefore be considered an anamorph of the genus *Yarrowia*. Hence, the author proposed the name *Yarrowia keelungensis* for this novel species.
The surface microlayer has often been considered as an extreme environment for microorganisms that may contain unusual species and taxa (Maki, 2002). This fact led to the thinking that a specific near-surface complex assemblage of bacteria, the bacterioneuston, would have a wide distribution in the world ocean (Tsyban, 1971).

A high correlation of bacterial numbers between the SML and underlying water supports the idea that abundances in the SML are strongly influenced by the bulk phase (Joux et al., 2006, Santos et al., 2009). Stability of the bacterioneuston was thought to be mainly dependent upon the surface tension rather than buoyancy (Sieburth, 1983) and strong hydrophobicity has been reported for neustonic bacteria (Dahlbaek et al., 1981). Thus, the SML was suggested to provide conditions comparable to solid substrate (Kjelleberg, 1985). Bacteria might additionally adhere to the SML either firmly, e.g. mediated by extracellular polymeric substances (EPS), or reversible (Marshall, 1985). Similar mechanisms also cause attachment of bacteria to particles and higher numbers of particle attached bacteria have been reported in the SML compared to the underlying water (Aller et al., 2005).

1.3.4 Bacterioneuston

Only little depletion of bacterial numbers in the SML compared to the underlying water was reported (Hermansson et al., 1987, Carlucci et al., 1991 and Munster et al., 1998). Variations in microlayer enrichment may be caused by processes such as bubble
scavenging, adsorption and diffusion. It is reported that bubble transport contributed strongly to bacterial accumulation in the SML (Bezdek and Carlucci, 1972). Generally, import from the underlying water was suggested to be the major source of enrichment of bacterial cells in coastal and open ocean SML samples (Kuznetsova et al, 2004). In addition, different reports suggest that microbial concentration exceeds that of underlying waters by orders of magnitude (MacIntyre, 1974 and Hardy, 1982), but despite their abundance and expected widespread distribution, bacterial communities thriving at the surface microlayer are poorly characterized (Norkrans, 1980).

Compared with the water only a few centimeters below the sea surface, the microlayer has been demonstrated to contain an abundance of bacteria (Harvey (1966), Morita and Burton (1970), Sieburth, (1971), Tsyban (1971), Sieburth et al (1976), Münster et al (1998)). For instance, some estimates commonly state that bacteria in the SML are $10^3$ – $10^5$ times more abundant than the bacterioplankton of the water column (Bezdek and Calucci, 1972).

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Isolation and cultivation techniques remain essential to understand the physiology and ecology of marine bacteria. Previous studies on bacteria from the surface microlayer have showed strains belonging to Proteobacteria and Actinobacteria (Table 2), including mostly misidentified genera such as *Pseudomonas, Chromobacterium, Aeromonas* and *Micrococcus* (Tsyban (1971), Sieburth (1971) and Fehon and Oliver (1979)). Evidence of unusual neustonic bacterial genera inhabiting the SML is therefore not available so far. This may be due to the well-known limitations for bacterial identification in the past. The application of DNA-based techniques (Pace et al., 1986) leading to the rapid identification of isolated strains makes it easier to survey the culturable bacteria inhabiting the surface microlayer.
### Table 2: Bacterioneuston recorded using different samplers in various waters

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sampler</th>
<th>Sample collected</th>
<th>Theoretical thickness (µm)</th>
<th>Sampling time (seconds)</th>
<th>Microbes isolated</th>
<th>Study area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glass plate method</td>
<td>Seawater and particles (Harvey &amp; Burzell, 1972; Cunliffe et al., 2009)</td>
<td>20 – 150 µm</td>
<td>45</td>
<td>Gammaproteobacteria, Alphaproteobacteria, Cytophaga, Flavobacterium, Bacteroides, Actinobacteria, Firmicutes (Agogue et al., 2005). Archae (Cunliffe et al., 2009a &amp; b)</td>
<td>The Bay of Banyuls-sur-Mer, Olympic Harbour in Barcelona, Kaneohe Bay, Ke’ehi Lagoon and an open ocean site</td>
</tr>
<tr>
<td>Method</td>
<td>Medium</td>
<td>Size Range</td>
<td>Bacterial Groups</td>
<td>Location</td>
<td></td>
<td></td>
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<td>-----------------</td>
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<td>------------</td>
<td>---------------------------------------</td>
<td>----------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Teflon plate</td>
<td>Sea water and particles</td>
<td>20 – 150 µm</td>
<td>Gammaproteobacteria, Alphaproteobacteria, Cytophaga, Flavobacterium, Bacteroides, Actinobacteria</td>
<td>(Hu’hnerfuss, 1981a &amp; b) The Bay of Banyuls-sur-Mer (France), off Olympic Firmicutes (Agogue et al, 2005), Collimonas sp. (Hakvag et al, 2009) Barcelona (Spain) and coast of Trøndelag, Norway.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Membrane Microbiology</td>
<td>35 – 42 µm</td>
<td>Gammaproteobacteria, Alphaproteobacteria, Cytophaga, Flavobacterium, Bacteroides, Actinobacteria</td>
<td>The Bay of Banyuls-sur-Mer (France), off Olympic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Introduction**

<table>
<thead>
<tr>
<th>Firmicutes (Agogue et al, 2005),</th>
<th>Harbour in Barcelona</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces sp (Hakvag et al, 2008),</td>
<td>Marine</td>
</tr>
<tr>
<td>Betaproteobacteria (Lanoil et al, 2001),</td>
<td>Biological</td>
</tr>
<tr>
<td>Synechococcus sp. (Cunliffe et al, 2009)</td>
<td>Field</td>
</tr>
<tr>
<td></td>
<td>Station, Espeland (Norway)</td>
</tr>
</tbody>
</table>

4 Modified membrane Microbiology 20 µm 10

Firmicutes (Bacillus sp.) and Pseudomonas sp. Palk Bay, Mandapam, India

1.3.5 Bacterioneuston activity

Although there are a number of studies regarding the number and activity of the neuston, there is little known about what constitutes a ‘real’ bacterioneuston. Therefore, discussion of the bacterioneuston is limited in the sense that many characteristics may be important for survival of organisms within this layer have not been investigated so far. It has been suggested that the bacterioneuston exhibits an effect on gas exchange and
transport mechanisms between the water column and the atmosphere and *vice versa* (Conrad and Seiler, 1988). Bacterial cells, which developed into a thick surface film showed increased formation of extracellular polymeric substances (EPS) and polyhydroxybutyrate (PHB), which might additionally indicate high organic substrate availability (Sieburth, 1983). Respiring bacteria are usually enriched in the SML (Hermansson and Dahlbaeck, 1983, Maki and Remsen, 1989) and most of them were attached to particles (Harvey and Young, 1980). Community respiration on the SML correlated to the enrichment of total organic carbon, further indicating bacterial uptake of organic material in the SML (Obernosterer *et al.*, 2005). Furthermore, ATP concentrations increased with more stability of the SML and highest enrichment factors were reported in slicks (De Souza-Lima and Romano, 1983), indicating influences of the wind speed on patterns of bacterioneuston activity.

Consequently, bacterial growth in the SML seems to be limited and low bacterial growth efficiencies in the SML have been measured. It was assumed that material in the SML is too refractory for bacterial uptake (Reinthaler *et al.*, 2008). Meanwhile, the physiological status of bacterial cells in the SML may be impaired by diverse stress factors acting upon the bacterioneuston community, e.g., increased exposure to UV-radiation, toxic compounds, etc. (Maki, 1993). Yet these factors have not fully explained the patterns of bacterioneuston activity. Thus, the complexity of processes in the SML still leaves large
uncertainties about the relation of bacterioneuston communities and the physical and chemical environment of the SML.

In addition, as the bacterioneuston inhabiting an extremely dynamic environment, it is an ideal source of habitat to explore metabolites which are different to terrestrial as well as other marine organisms. The study of secondary metabolites from bacterioneuston is scarcely done and especially in India the study of bacterioneuston has not been studied so far. As the need for new potential drugs are sternly increasing day by day, the study of bioactive compounds from bacterioneuston, their characterization and activity will be fruitful in this current era.

1.4 Bioactive compounds from bacterioneuston

High densities of metabolically active bacteria will most likely yield a competitive environment, and properties such as production of antibiotics may give organisms an advantage. Although a number of antibiotic producers have been isolated from the marine environment, there are no reports on the systematic screening of the marine surface microlayer for antibiotic-producing organisms (Kelecom, 2002 and Radjasa et al, 2007).

In a study on antagonistic interactions among marine pelagic bacteria it has been found that more than a half of the isolates expressed antagonistic activity, and this trait was more common with particle-associated than with free-living bacteria (Long and Azam, 2001). Particles often tend to accumulate on the sea surface, and one could therefore
expect that bacteria living on the sea surface might produce more antimicrobial compounds than other marine (i.e. Pelagic) bacteria.

A new strain belonging to the genus *Collimonas* was isolated from the sea surface microlayer off the coast of Trøndelag, Norway (Hakvag *et al.*, 2009). The bacterium, designated *Collimonas CT*, produced an antibacterial compound active against *Micrococcus luteus*. Subsequent studies using LC-MS identified this antibacterial compound as violacein, known to be produced by several marine-derived bacteria.

In this project, over 1000 cultivable bacteria were isolated from the sea surface microlayer, in different locations in the Trondheim fjord and along the coast of Trøndelag, Norway. The diversity of the isolates from the sea surface microlayer have been investigated by studying cultivable bacteria from two sampling locations as ‘model-samples’. Two *Streptomycetes* isolates from the sea surface microlayer displayed activity against a vancomycin-resistant *Enterococcus sp*. Analysis of the bacterial extracts indicated that this was due to the production of a novel antibacterial compound. Production of candididin was found to be widely distributed among *Streptomyces* bacteria isolated from the Trondheim fjord, Norway. These studies prove that bacterioneuston is rich in bioactive compounds which have potential activity against pathogens.