Chapter I

Survey and Molecular Systematics of Marine Cyanobacteria and Diatoms
INTRODUCTION

As primary producers in the world’s oceans, they are keystone species in global nutrient cycling. About 20% of the total carbon and silica sequestered are fixed by less than a few hundred species in the marine plankton (Guillard and Kilham 1978; Goldman 1993; Hasle and Syversten 1996; Mann 1999). Knowledge of the geological age of the diatoms gives palaeoclimatologists a clue as to how long these organisms have been modifying the biosphere (Kooistra and Medlin 1996; Siever 1991). The cell wall structure is species specific, demonstrating that diatom silica morphogenesis is genetically encoded.

Cyanobacteria are prokaryotic oxygenic phototrophs found in almost every conceivable habitat on earth (Ferris et al. 1996; Ward et al. 1997; Nubel et al. 1999, 2000; Abed and Garcia-Pichel 2001; Garcia-Pichel and Pringault 2001). They exist in different morphologies including unicellular and filamentous forms (Castenholz 2001). While unicellular types exist as single cells, suspended or benthic, or aggregates, filamentous types may be thin or thick, single trichome or bundles either with or without a sheath. Cyanobacteria are able to perform different modes of metabolism with the capacity to switch from one mode to another (Stal, 1995).

All cyanobacteria carry out oxygenic photosynthesis but some cyanobacterial species can switch to the typical bacterial anoxygenic photosynthesis using sulfide as electron donor (Cohen et al. 1986). Under anoxic conditions and during the dark, cyanobacteria carry out fermentation (Stal 1997). Some cyanobacteria form heterocysts and have the ability to fix atmospheric nitrogen (Capone et al. 2005). Phylogenetic analysis of cyanobacteria based on 16S rDNA genes showed that they are a diverse, monophyletic phylum of organisms within the bacterial radiation. Research on cyanobacteria in the last decades focused largely on their ecology, morphology, physiology and 16S rDNA-based phylogeny but relatively little has been done on their potential uses in biotechnology. The overwhelming available knowledge on the diversity and physiology of cyanobacteria serves as an excellent base for exploring their applications in biotechnology (Thajuddin and Subramanian, 2005).
In the last few years, cyanobacteria have gained much attention as a rich source of bioactive compounds and have been considered as one of the most promising groups of organisms to produce them (Bhadury et al. 2004; Dahms et al. 2006). These cyanobacterial metabolites include antibacterial (Jaki et al. 2000 and MubarakAli et al., 2008), antifungal (Kajiyama et al. 1998), antiviral (Patterson et al. 1994), anticancer (Gerwick et al. 1994), antiplasmodial (Papendorf et al. 1998), algicide (Papke et al. 1997) and immunosuppressive agents. Screening of cyanobacteria for antibiotics has opened a new horizon for discovering new drugs. Some cyanobacteria have also been found to intracellularly accumulate polyhydroxyalkanoates (PHA), which are comparable in properties to polyethylene and polypropylene (Steinbuchel et al. 1997). These biodegradable plastics could replace oil-derived thermoplastics in some fields. Recent research on cyanobacteria has demonstrated that they form ideal consortia with chemotrophic bacteria and can effectively be used to cleanup oil contaminated sediments and wastewaters (Abed and Koster 2005).

Cyanobacterial taxonomy has been established based on morphological features, such as the shape and dimensions of the cells, presence of structurally differentiated cells, and whether the cells grow as solitary cells or in colonies (Paerl, 1988). Molecular methods have become an indispensable tool for characterization of cyanoprokaryotes and the assessment of evolutionary relations among them in recent decades. The direct sequencing of various genes is the most common method used in taxonomy of cyanobacteria. However, RFLP (Restriction Fragment Length Polymorphism) is also widely applied, especially for more detailed examination of the genetic variability of closely related taxa (Ernst et al., 1995) or to infer the extent of cyanobacterial diversity in nature. Also random amplified polymorphic DNA (RAPD) analysis is sometimes used in order to discriminate between genotypes of close relatives. Much less common are the allozyme or the whole-cell protein analysis (Neilan, 1995).

Diatoms are single celled photosynthesizing eukaryotic algae that produce intricately structured cell wall made of nanopatterned silica (SiO₂) and are found in
almost every aquatic environment including fresh and marine water, soil, in fact almost anywhere in moist. They are non motile, or capable of only limited movement along a substrate by secretion of mucilaginous material along a slit like groove or channel called a raphe, both benthic and planktonic forms exist. Diatoms are formally classified as belonging to the division \textit{Chrysophyta}, Class \textit{Bacillariophyceae}. The \textit{Chrysophyta} are algae which form endoplasmic cysts, store oil rather than starch, posses a bipartite cell wall and secrete silica at some stages of their life cycle. Diatoms are nearly all autotrophic, heterotrophic members are rare (Li and Volcani 1987).

Diatoms are commonly between 20-200 microns in diameter or length, although sometimes they can be up to 2 millimeter long first appeared more than 180 million years ago. First recorded occurrence of diatoms are from Jurassic however, these are uncertain and the earliest recorded well preserved diatoms are centric forms from Aptian-albian stages of the Cretaceous. At first diatoms are thought to be animals because what are now called raphid diatom move at a speed of upto $25\mu\text{ms}^{-1}$ when attached to surfaces. Chris Bowler (2001) explains that molecular phylogeny and morphological studies suggest that diatoms originated probably as the result of a eukaryote being invaded or engulfed by a photosynthetic eukaryote, most probably a red algae.

Diatoms are phylogenetic similarity to green algae and higher plants is derived from the primary endosymbiotic event, which is thought to have occurred at least 700 million years ago (Kowallik, 1992). Therefore, diatom cells have a range of features that make them highly divergent from the classical cellular structure of higher plants, including:

\begin{enumerate}
\item The use of the brown carotenoid pigment, fucoxanthin for light energy transfer within the light harvesting complexes of photosystems I and II
\item The presence of four membranes around their plastids. The inner two membranes are equivalent to the membranes that normally surround higher plant chloroplasts, whereas the second membrane (as counted from the outside)
is thought to be derived from the endosymbiont’s plasma membrane, and the outer membrane is continuous with the endoplasmatic reticulum of the host cell.

(c) The presence of a rigid cell wall composed largely of amorphous silica (i.e. glass). The exquisite lacework-like patterning of diatom cell walls is reproduced with high fidelity from generation to generation and is species specific. For these reasons, it has been used since the last century for taxonomic classification.

Current phylogenetic trees place diatoms close to Alveolata lineages and far from the green and red lineages of other photosynthetic eukaryotes (Baldauf et al., 2000). The expressed sequence tags (EST) program has revealed the presence of genes encoding enzymes involved in cAMP metabolism, as well as genes encoding the components of the animal extracellular matrix, such as fibronectins, elastins, and tenascins, none of which appear to be present in higher plants. As a consequence, one could almost consider diatoms as photosynthetic animals rather than unicellular plants.

The rigid part of the cell wall (frustule) of diatoms is composed of amorphous silica (Pickett-Heaps et al., 1990; Parkinson and Gordon 1999; Vrieling et al., 2000), located in the girdle bands and two valves: the epitheca consisting of an epivalve and epicingulum, and the hypotheca consisting of hypovalve and hypocingulum (Round et al., 1990). The timing of diatom valve formation has not been directly determined in an individual cell, however, some estimates were made based on measurements on whole synchronized cultures. For instance, 2D valve formation in Melosira varians Agardh takes approximately 8 minutes. Cell wall morphogenesis in Ditylum brightwelli (West) Grunow, and in Navicula pelliculosa (Kutz) Hilse occurs during respectively a 53 min and a 2-3 h period of the cell cycle (Sullivan and Volcani, 1981).

Commonly, in pennate diatoms the development of the new hypovalve starts from the axial area and grows towards the outer cellular edges (Pickett-Heaps et al., 1990). Subsequently, the frustule thickens in the third dimension and is completed by coverage with a casing followed by exocytosis of the valve and girdle bands, and
finally cell separation (Darley et al. 1976; Darley and Volcani 1971; Pickett-Heaps et al., 1990).

Application of the M13 PCR fingerprinting method was useful for almost all forms of cyanobacteria strain and species discrimination. PCR amplification techniques viz., repetitive DNA element PCR (REP-PCR), short randomly repeated repetitive PCR (STRR-PCR) and arbitrarily primed PCR (RAPD-PCR) were used for the taxonomic discrimination among the strains of the unicellular cyanobacterium, *Synechococcus elongatus* collected across the coastal regions of the Indian subcontinent. In comparison with the STRR and RAPD, the REP primer set generates fingerprints of lower complexity, but still the phenogram clearly differentiated the strains. In conclusion, described PCR fingerprinting methods can be considered as promising tools for the differentiation at the strain level of cyanobacteria from the same species (Muralitharan and Thajuddin, 2010). 151 unique cyanobacterial genes in 8 studied genomes and found a few examples of largely conserved gene order, which could prove useful for solving problems of cyanobacterial evolution on a larger scale (Martin et al., 2003).

Molecular approaches have been divided into two classes: PCR independent and PCR based approaches. Molecular assessment of cyanobacterial biodiversity has been studied by using markers like 16S rDNA, phycocyanin locus, *nif* gene, *rpo* gene, ITS region etc. A general overview of biodiversity assessment, molecular techniques and markers used for biodiversity assessment recommends combinatorial approach with different molecular markers. It is likely to improve the degree of resolution and provide as possible the broadest picture and in depth information about biodiversity documentation (Kumari et al., 2009).

Due to innate biotechnological potentials and most abundantly available in marine ecosystems, both cyanobacteria and diatoms has been selected in the present study.
REVIEW OF LITERATURE

Diatom Biology

Diatoms were first discovered by light microscopy and mistakenly addressed as unicellular animals due to their brownish color and their capability to move on substrates (Lind et al., 1997). According to the species concept, organisms mating with each other belong to the same species. Diatom taxonomy thus was based mainly either on the identification of ribosomal sequences (Medlin et al., 1996) or more classically on the morphology and the shape of the frustules, the extracellular silica cell walls. These frustules, which are formed by two valves that fit together like Petri dishes, often are highly ornamented and show species-specific structures.

The ability of diatoms to genetically define these structures makes them highly interesting for nanotechnological applications (Drum and Gordon, 2003). Because of their siliceous composition frustules were often well preserved in fossil deposits (Damste et al., 2004) and strong sedimentation of diatom shells in ancient oceans led to the deposition of siliceous earth or diatomite, a material of high importance for industrial uses (Harwood, 1999).

Diatoms play a significant ecological role; about half of the global annual net primary production in the oceans is due to phytoplankton which is dominated by diatoms (Falkowski et al., 1998). Diatoms thus produce and represent the main input into the marine food web. They are not only strongly involved in fixation of CO₂, but also in cycling of soluble silicates by integrating them into the shells and by releasing parts of them after decomposition at the bottom of the oceans (Bidil and Azam, 1999). It is not yet fully understood why diatoms filled aquatic niches so successfully, even though they have the capability to grow at a wide range of light intensities (Falkowski et al., 2004) and the apparent ability to perform C₄ photosynthesis (Reinfelder, 2004).

Cell Wall Structure, Cell Cycle and Sexual Reproduction

The diatom cell wall consists of two halves of identical structure, although one half (epitheca) is slightly larger and overlaps the other half (hypotheca). Together the
epitheca and hypotheca completely enclose the protoplast. Each theca is composed of a valve displaying ornate structures with often elaborate pore patterns, and several girdle bands exhibiting generally uniform pore patterns. The terminal girdle band of each theca is termed the pleural band and is located in the region where the epitheca and the hypotheca overlap (Fig 1).

**Fig 1**: Schematic overview of the siliceous components diatom cell wall

Diatoms can be divided into three major groups according to their shape and symmetry of their cell walls: radial centrics, polar centrics, and pennates. Radial centrics have a petri dish-like architecture with a circular center of symmetry (annulus) in the middle of the valve, and rows of pores (striae) radiating from the annulus. Polar centrics have bi or multipolar valves with an elongated or distorted annulus. Pennate diatoms are bilaterally symmetrical, and instead of an annulus possess a rib (sternum) running along the longitudinal axis of each valve.

In raphid pennates the sternum contains a slit (raphe) that is interrupted by the central nodule in the center of the valve. Raphid pennates are able to move on surfaces by means of traction generated by adhesive mucilage (polysaccharides or proteoglycans) that is secreted through the raphe (Edgar and Pickett-Heaps, 1984). The traction force appears to be generated by an actin-myosin based system that is located
precisely underneath the raphe on the cytosolic side of the plasma membrane (Poulsen et al., 1999). Araphid pennates, radial centrics, and polar centrics are nonmotile.

The rigidity and architecture of the silica cell wall imposes restrictions on the mechanism of cell division and growth. The valves can only be formed during cell division, and the increase in cell volume during interphase requires the stepwise synthesis of girdle bands in order to avoid the occurrence of gaps within the cell wall as the distance between the epitheca and hypotheca increases (Fig 2). Furthermore, because new valves always develop within the confines of the parental cell wall, the sibling cell that inherits the parental hypotheca is usually smaller than the parental cell.

![Diagram](https://via.placeholder.com/150)

**Fig 2:** Schematic overview of mitotic cell division and hypovalve and girdle band formation. N, Nucleus; MC, microtubule center; SDV, silica deposition vesicle.

The key events occurs during mitotic cell division are as follows: a) the nucleus of each daughter cell moves to the side of the cell where the hypotheca will be formed. (b) A microtubule center (MC) positions itself between the nucleus and the plasma
membrane above which the hypotheca will eventually be placed. (c) A specialized vesicle known as the silica deposition vesicle (SDV) forms between the MC and the plasma membrane in a region that becomes the “pattern center.” (d) The SDV elongates into a tube and then spreads along one side of the cell. (e) A new valve is formed within the SDV by the targeted transport of silica, proteins, and polysaccharides. During this process, the SDV becomes acidic as a result of the silica polymerization process (Vrieling et al., 1999). Some of the organic components eventually form a coat around the silica framework, whereas others are involved in silica deposition. (f) Once valve biogenesis is complete, it is exocytosed by fusion of the SDV membrane (the silicalemma) with the plasma membrane. As a consequence, the inner face of the silicalemma is thought to become the new plasma membrane. (g) Following separation, the daughter cells can expand unidirectionally along the cell division axis by the biogenesis of girdle bands. These structures are also formed within SDVs in an analogous way.

As a consequence, the average cell size in a diatom population gradually decreases with continued vegetative growth (the MacDonald-Pfizer rule). Ultimately, this size reduction would result in cells too small to be viable and the diatom population would die. The only way to escape this fate is by sexual reproduction (Chepurnov et al., 2004). During this process meiotic cell divisions occur, and the resulting gametes shed their cell walls. Immediately after gamete fusion a specialized zygote (termed auxospore) is formed, which is covered by an organic cell wall, and undergoes an enormous increase in volume within a relatively short time (hours to a few days).

Already during the first division of the auxospore, silicified valves are formed that exhibit the species-specific shape and silica patterns. The molecular details of this amazing process are almost completely unexplored with the exception of a study by Armbrust, who discovered a new gene family (Sig) that is expressed during the onset of sexual reproduction in *T. weissflogii* (Armbrust, 1999). Sig genes have also been identified in other *Thalassiosira* species, yet the function of the Sig-encoded proteins is still unknown (Armbrust and Galindo, 2001).
The size of some of the best-studied diatoms growing in laboratory culture (e.g., *Cylindrotheca fusiformis*, *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*) remains constant, and sexual stages of these organisms have never been observed. The mechanism by which these species avoid size reduction was not yet fully understood, but appears to involve the ability to form expandable girdle bands (Hildebrand *et al.*, 2007).

**Organic components of the cell wall**

Early light-microscopy studies of diatom cell walls using ruthenium red presented evidence that organic material is intimately associated with the silica structures (Pickett-Heaps *et al.*, 1990). Later, imaging of ultrathin-sections after anhydrous hydrogen fluoride (HF) treatment (i.e., dissolution of silica) followed by transmission electron microscopy indicated the presence of organic material both on the silica surface and inside the silica (Pickett-Heaps *et al.*, 1990, Volcani, 1981). Based on the ruthenium red staining, it was assumed that the silica-associated organic components are polysaccharides (pectin).

Indeed, to date a plethora of extracellular polysaccharides have been characterized from diatoms, which appear to play a role in cell adhesion to surfaces, gliding of pennate diatoms, and protection against desiccation (Hoagland *et al.*, 1993). While these polysaccharides are relatively loosely attached to the diatom cell wall, and can often be extracted by treatment with hot water or mildly alkaline solutions, polysaccharides have recently been characterized that are much more tightly bound to or even embedded within the diatom silica.

These silica-associated polysaccharides are composed of 3-linked mannans that are highly polyanionic due to the attachment of numerous uronic acid residues and or sulfate groups (Chiovitti *et al.*, 2003). It was unclear if the polyanionic mannans are involved in silica biosynthesis as no functional studies have been performed. Furthermore, the polyanionic mannans might be protein-linked in vivo, since silica-embedded highly mannosylated and sulphated glycoproteins have been identified from

**Cyanobacteria**

Cyanobacteria or cyanoprokaryota are oxygen producing, photosynthesizing, gram-negative prokaryotes which had a main part in the evolution of the Earth’s atmosphere. Cyanobacteria thrive in a wide range of mostly aquatic habitats. The cellular organization and basic functions regarding growth and photosynthesis have been comprehensively described (Sandgren, 1988) and since in late 1990s, the sequencing of entire genomes of or smaller specific regions of within a genome such as toxin production has allowed more extensive understanding of their unique lifestyle and metabolic traits (Kaneko *et al.*, 2001).

While cyanobacteria generally have relatively simple basic metabolic requirements consisting of carbon dioxide, light, water, and inorganic nutrients and they possess efficient uptake and retention mechanisms for phosphate, nitrate and bicarbonate. In addition to the above mechanisms, cyanobacteria are capable of producing high affinity iron chelators, known as siderophores, under iron deficient conditions to alleviate iron stress and some filamentous cyanobacterial genera possess heterocysts, cells specialized to fix atmospheric nitrogen. In total, cyanobacteria bear unique traits that allow them to dominate many phytoplankton communities as a result of the interactions between their unique physiological traits and the physical and chemical characteristics of the aquatic system itself (Ritchie *et al.*, 1997).

**Biological diversity**

Although cyanobacteria probably evolved as a group of organisms about 2,000 million years before the advent of eukaryotes, they comprise fewer taxa than eukaryotic microalgae (Bisby, 1995). The concept of species in the cyanobacteria has, however, no distinct boundaries. The situation is similar for most organisms, except for those that are sexually reproductive depending on the classification system used; the number of species recognised varies greatly. Based on the International Code of Botanical Nomenclature the class Cyanophyceae, for example, contains about 150 genera and
2,000 species (Hoek et al., 1995). Chemotaxonomic studies include the use of markers, such as lipid composition, polyamines, carotenoids and special biochemical features. The resulting data support the more traditional examinations of phenotypic and ecological characteristics. Physiological parameters are conveniently studied using laboratory cultures (Packer and Glazer, 1988). The diversity of cyanobacteria can be seen in the multitude of structural and functional aspects of cell morphology and in variations in metabolic strategies, motility, cell division, developmental biology, etc. The production of extracellular substances and cyanotoxins by cyanobacteria illustrates the diverse nature of their interactions with other organisms (i.e. allelopathy) (Rizvi and Rizvi, 1992).

A molecular approach to the systematics of cyanobacteria may be most fruitful for inferring phylogenetic relationships. Macromolecules, such as nucleic acids and proteins, are copies or translations of genetic information. The methods applied involve direct studies of the relevant macromolecules by sequencing, or indirectly by electrophoresis, hybridisation, or immunological procedures (Wilmotte, 1994). Nucleic acid technologies, especially the polymerase chain reaction (PCR), have advanced to the point that it is feasible to amplify and sequence genes and other conserved regions from a single cell. To date, 16S rRNA has given the most detailed information on the relationships within the cyanobacteria (Rudi et al., 1997). However, the molecular results obtained should be integrated with other characteristics as the base for a polyphonic taxonomy (Vandamme, et al., 1996). A considerable morphological, as well as a genotypical, polymorphy exists in the cyanobacteria, although as data from rRNA sequencing indicates they are correlated to a high degree.

The phylogenetic relationship of cyanobacteria is the rationale behind the meaningful systematic groupings. However, it is difficult to set up a system of classification that serves both the everyday need for practical identification, and offers an expression of the natural relationship between the organisms in question (Mayr, 1981). Meanwhile, it will be necessary to use the available manuals and reference books to help in these investigations and with the proper identification of the cyanobacteria. They are photoautotrophs, cyanobacteria can be grown in simple
mineral media. Vitamin $B_{12}$ is the only growth factor that is known to be required by some species. Media must be supplemented with the essential nutrients needed to support cell growth, including sources of nitrogen, phosphorus, trace elements, etc. Toxigenic strains of cyanobacteria are deposited in international-type culture collections (Rippka, 1988; Sugawara et al., 1993). Clonal cultures are distributed for research, taxonomic work and teaching purposes.

**Molecular Systematics:**

Microbial Systematics has long remained an enigma. Conceptual advances in microbiology during the twentieth century included the realization that a discontinuity exists between those cellular organisms that are prokaryotic (i.e. whose cells have no nucleus) and those that are eukaryotic (i.e. more complexly structured cells with a nucleus) within the organization of their cells. The microalgae investigated by phycologists under the International Code of Botanical Nomenclature (ICBN) (Greuter et al., 1994) included organisms of both eukaryotic and prokaryotic cell types. The cyanobacteria (Geitler, 1932) constituted the largest group of the latter category. The prokaryotic nature of these organisms and their fairly close relationship with eubacteria made work under provisions of the International Code of Nomenclature of Bacteria (ICNB) (Sneath, 1992) more appropriate (Rippka et al., 1979; Waterbury, 1992).

The prevailing systematic view is that comparative studies of the genetic constitution of the cyanobacteria will now contribute significantly to the revision of their taxonomy. Relevant classification should reflect as closely as possible the phylogenetic. The integration of phenotypic, genotypic and phylogenetic information renders possible a consensus type of taxonomy known as polyphasic taxonomy (Vandamme et al., 1996). The names "cyanobacteria" and "blue-green algae" (Cyanophyceae) are valid and compatible systematic terms. This group of microorganisms comprises unicellular to multicellular prokaryotes that possess chlorophyll $a$ and perform oxygenic photosynthesis associated with photosystems I and II (Castenholz and Waterbury, 1989).
Occurrence in nature

The majority of Cyanobacteria are aerobic photoautotrophs. Their life processes require only water, carbon dioxide, inorganic substances and light. Photosynthesis is their principal mode of energy metabolism. In the natural environment, however, it is known that some species are able to survive long periods in complete darkness. Furthermore, certain cyanobacteria show a distinct ability for heterotrophic nutrition (Fay, 1965). Cyanobacteria are often the first plants to colonize bare areas of rock and soil. Adaptations, such as ultraviolet absorbing sheath pigments, increase their fitness in the relatively exposed land environment.

Many species are capable of living in the soil and other terrestrial habitats, where they are important in the functional processes of ecosystems and the cycling of nutrient elements (Whitton, 1992). The prominent habitats of cyanobacteria are limnic and marine environments. They flourish in water that is salty, brackish or fresh, in cold and hot springs, and in environments where no other microalgae can exist. Most marine forms (Humm and Wicks, 1980) grow along the shore as benthic vegetation in the zone between the high and low tide marks. The cyanobacteria comprise a large component of marine plankton with global distribution (Wille, 1904; Gallon et al., 1996).

A number of freshwater species are also able to withstand relatively high concentrations of sodium chloride. It appears that many cyanobacteria isolated from coastal environments tolerate saline environments (i.e. are halotolerant) rather than require salinity (i.e. are halophilic). As frequent colonisers of euryhaline (very saline) environments, cyanobacteria are found in salt works and salt marshes, and are capable of growth at combined salt concentrations as high as 3-4 molar mass (Reed et al., 1984). Freshwater localities with diverse trophic states are the prominent habitats for cyanobacteria.

Numerous species characteristically inhabit, and can occasionally dominate, both near surface epilimnic and deep, euphotic, hypolimnic waters of lakes (Whitton, 1973). Others colonize surfaces by attaching to rocks or sediments, sometimes forming mats that may tear loose and float to the surface. Cyanobacteria have an impressive
ability to colonize infertile substrates such as volcanic ash, desert sand and rocks (Jaag, 1945; Dor and Danin, 1996). They are extraordinary excavators, boring hollows into limestone and special types of sandstone (Weber et al., 1996). Another remarkable feature is their ability to survive extremely high and low temperatures. Cyanobacteria are inhabitants of hot springs (Castenholz, 1973), mountain streams (Kann, 1988), Arctic and Antarctic lakes (Skulberg, 1996a) and snow and ice (Kol, 1968; Laamanen, 1996). The cyanobacteria also include species that run through the entire range of water types, from polysaprobic zones to katharobic waters (Van Landingham, 1982).

Cyanobacteria also form symbiotic associations with animals and plants. Symbiotic relations exist with, for example, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms (Rai, 1990). The hypothesis for the endosymbiotic origin of chloroplasts and mitochondria should be mentioned in this context. The evolutionary formation of a photosynthetic eukaryote can be explained by a cyanobacteria being engulfed and co developed by a phagotrophic host (Douglas, 1994). Fossils of what were almost certainly prokaryotes are present in the 3,450 million year old Warrawoona sedimentary rock of north-western Australia.

Cyanobacteria were among the pioneer organisms of the early earth (Brock 1973; Schopf, 1996). These photosynthetic micro-organisms were, at that time, probably the chief primary producers of organic matter, and the first organisms to release elemental oxygen into the primitive atmosphere. Sequencing of deoxyribonucleic acid (DNA) has given evidence that the earliest organisms were thermophilic and thus able to survive in oceans that were heated by volcanoes, hot springs and bolide impacts (Holland, 1997).

**Organization, function and behavior:**

The structure and organization of cyanobacteria are studied using light and electron microscopes. The basic morphology comprises unicellular, colonial and multicellular filamentous forms. Unicellular forms, for example in the order Chroococcales, have spherical, ovoid or cylindrical cells. They occur singly when the daughter cells separate after reproduction by binary fission. The cells may aggregate in
irregular colonies, being held together by the slimy matrix secreted during the growth of the colony. By means of a more or less regular series of cell division, combined with sheath secretions, more ordered colonies may be produced.

A particular mode of reproduction, which may supplement binary fission, distinguishes cyanobacteria in the order Chamaesiphonales and Pleurocapsales. In the Chamaesiphonales exospores are budded off from the upper ends of cells. In the second order, the principal mode of replication is by a series of successive binary fissions converting a single mother cell into many minute daughter cells (baeocytes or endospores).

Filamentous morphology is the result of repeated cell divisions occurring in a single plane at right angles to the main axis of the filament. The multicellular structure consisting of a chain of cells is called a trichome. The trichome may be straight or coiled. Cell size and shape show great variability among the filamentous cyanobacteria. Species in the order Oscillatoriales, with uniseriated and unbranched trichomes, are composed of essentially identical cells. The other orders with a filamentous organization (orders Nostocales and Stigonematales) are characterized with trichomes having a heterogeneous cellular composition. Vegetative cells may be differentiated into heterocysts (having a thick wall and hyaline protoplast, capable of nitrogen fixation) and akinetes (large thick-walled cells, containing reserve materials, enabling survival under unfavourable conditions). In the order Stigonematales, the filaments are often multiseriated, with genuine branching. Both heterocysts and akinetes are present. The only means of reproduction in cyanobacteria is asexual. Filamentous forms reproduce by trichome fragmentation, or by formation of special hormogonia. Hormogonia are distinct reproductive segments of the trichomes.

They exhibit active gliding motion upon their liberation and gradually develop into new trichomes. In contrast to eukaryotic microalgae, cyanobacteria do not possess membrane-bound sub-cellular organelles; they have no discrete membrane-bound nucleus; they possess a wall structure based upon a peptidoglycan layer; and they contain 70 S rather than 80 S ribosomes (Fay and Van Baalen, 1987; Bryant, 1994).
The photosynthetic pigments of cyanobacteria are located in thylakoids that lie free in the cytoplasm near the cell periphery. Cell colours vary from blue-green to violet-red.

The green of chlorophyll $a$ is usually masked by carotenoids (e.g. beta-carotene) and accessory pigments such as phycocyanin, allophycocyanin and phycoerythrin (phycobiliproteins). The pigments are embodied in phycobilisomes, which are found in rows on the outer surface of the thylakoids (Douglas, 1994). All cyanobacteria contain chlorophyll $a$ and phycocyanin. The basic features of photosynthesis in cyanobacteria have been well described (Ormerod, 1992). Cyanobacteria are oxygenic phototrophs possessing two kinds of reaction centres, PS I and PS II, in their photosynthetic apparatus. With the accessory pigments mentioned above, they are able to use effectively that region of the light spectrum between the absorption peaks of chlorophyll $a$ and the carotenoids.

The ability for continuous photosynthetic growth in the presence of oxygen, together with having water as their electron donor for $\text{CO}_2$ reduction, enables cyanobacteria to colonize a wide range of ecological niches (Whitton, 1992). Phycobiliprotein synthesis is particularly susceptible to environmental influences, especially light quality. Chromatic adaptation is largely attributable to a change in the ratio between phycocyanin and phycoerythrin in the phycobilisomes. Thus, cyanobacteria are able to produce the accessory pigment needed to absorb light most efficiently in the habitat in which they are present.

Cyanobacteria have a remarkable ability to store essential nutrients and metabolites within their cytoplasm. Prominent cytoplasmic inclusions for this purpose can be seen with the electron microscope (e.g. glycogen granules, lipid globules, cyanophycin granules, polyphosphate bodies, carboxysomes) (Fay and Van Baalen, 1987). Reserve products are accumulated under conditions of an excess supply of particular nutrients. For example, when the synthesis of nitrogenous cell constituents is halted because of an absence of a usable nitrogen source, the primary products of photosynthesis are channeled towards the synthesis and accumulation of glycogen and lipids. Dinitrogen fixation is a fundamental metabolic process of cyanobacteria, giving
them the simplest nutritional requirements of all living organisms. By using the enzyme nitrogenase, they convert N₂ directly into ammonium (NH₄⁺) (a form through which nitrogen enters the food chain) and by using solar energy to drive their metabolic and biosynthetic machinery, only N₂, CO₂, water and mineral elements are needed for growth in the light.

Nitrogen-fixing cyanobacteria are widespread among the filamentous, heterocyst forming genera (e.g. *Anabaena, Nostoc*) (Stewart, 1973). However, there are also several well documented examples of dinitrogen fixation among cyanobacteria not forming heterocysts (e.g. *Trichodesmium*) (Carpenter *et al.*, 1992). Under predominantly nitrogen limited conditions, but when other nutrients are available, nitrogen fixing cyanobacteria may be favored and gain growth and reproductive success. Mass developments (often referred to as "blooms") of such species in limnic (e.g. eutrophic lakes) and marine environments (e.g. the Baltic Sea) are common phenomena world-wide. Many species of cyanobacteria possess gas vesicles. These are cytoplasmic inclusions that enable buoyancy regulation and are gas-filled, cylindrical structures. Their function is to give planktonic species an ecologically important mechanism enabling them to adjust their vertical position in the water column (Walsby, 1987). To optimize their position, and thus to find a suitable niche for survival and growth, cyanobacteria use different environmental stimuli (e.g. photic, gravitational, chemical, thermal) as clues.

Gas vesicles become more abundant when light is reduced and the growth rate slows down. Increases in the turgor pressure of cells, as a result of the accumulation of photosynthate, cause a decrease in existing gas vesicles and therefore a reduction in buoyancy. Cyanobacteria can, by such buoyancy regulation, poise themselves within vertical gradients of physical and chemical factors. Other ecologically significant mechanisms of movement shown by some cyanobacteria are photomovement by slime secretion or surface undulations of cells (Häder, 1987; Paerl, 1988).
Cyanobacterial Taxonomy:

A characteristic cyanobacterial membrane lipid has been extracted from late Archean sedimentary rocks dated to 2.65 Ga. The microfossils found at the Apex Chert in Western Australia, believed to be cyanoprokaryotes, are even 800 million years older. A minimum date for the evolution of heterocytic forms is set to 1.5 billion years ago; to the period the oldest fossils interpreted as akinetes have been dated (Summons et al., 1999).

For more than 150 years cyanobacteria were considered to be eukaryotic algae, with botanists and phycologists placing them into the Cyanophyceae or cyanobacteria. Thus, initial classifications followed the International Code of Botanical Nomenclature. Traditional techniques for cyanobacteria identification and systematics have relied essentially on the observation of morphological characteristics. The confirmation by molecular methods that these denominated cyanobacteria, where in fact photosynthetic bacteria and their transfer from Cyanophyceae to Cyanobacteria were of most importance (Rasmussen and Svenning, 1998).

Evolutionary Markers:

The assessment of the phylogeny of organisms through gene sequence analysis has increased dramatically since the advent of PCR and automated sequencing. A number of genes have been used as evolutionary markers for inferring phylogenetic relations and delineation of cyanobacterial taxonomy, being the 16S rDNA gene analyzed most extensively (Nubel et al., 1997).

Phylogenetic studies using this marker helped to clarify the phylogenetic relationships among cyanobacteria, revealed the structure and intraspecific diversity of cyanobacterial communities and provide further evidence to the cyanobacterial origin of chloroplasts and the existence of a divergent evolutionary pathway among bacteria (Bergsland & Haselkorn, 1991). Bacterial rRNA genes are commonly organized in an operon in the order 16S rRNA - 3S rRNA - 5S rRNA, each rRNA gene being separated by an internal transcribed spacer (ITS) region. The amplification of the 16S - 23S rRNA internal transcribed spacer (ITS) in cyanobacteria have shown to present
different sizes and also to be more variable in sequence even within closely related taxonomic groups than the 16S rRNA (Iteman et al., 2002).

Other Markers

In some cases other sequences have also been used for phylogenetic inferences: the non-coding intergenic spacer of the phycocyanin operon (PC-IGS); other DNA-dependent RNA polymerase regions, rpoB and rpoD; the gene encoding a serine-type of protease with a regulatory role in the differentiation process of heterocysts (hetR); nitrogen fixation (nif) genes; carbon-fixation-associated gene (RubisCO spacer) (rbcLX); and the subunit B protein of DNA gyrase (gyrB) (Robertson et al., 2001).

C-Phycocyanin gene:

Cyanobacteria are the only microorganisms to produce significant quantities of Phycocyanin (PC) and its derivative Allophycocyanin. This distribution of PC in aquatic microorganisms makes the study of PC gene sequence heterogeneity ideal for the classification of freshwater cyanobacteria. The entire PC operon contains genes coding for two bilin subunits and three linker polypeptides. The intergenic spacer (IGS) between the two bilin subunit genes, designated b (cpcB) and a (cpcA), of the PC operon was chosen as a potentially highly variable region of DNA sequence useful for the identification of cyanobacteria to the strain level. C-phycocyanin is one of the major photosynthetic biliproteins and also one of the important components in the electron transfer of photosynthesis. It has some functions that are good for human health, such as antioxidant, radical scavenging, anti-inflammatory and anti-cancer properties, and also used in the food, biotechnology, and cosmetic industry because of their color, fluorescent and antioxidant properties. So in recent years it has been drawn more and more attention (Reddy et al., 2003, Sekar and Chandramohan, 2007).

The Phycobilisome components (phycobiliproteins) are responsible for the blue-green pigmentation of most cyanobacteria. All phycobiliproteins are water-soluble and therefore cannot exist within the membrane as do carotenoids, but aggregate forming clusters that adhere to the membrane called phycobilisomes. Phycocyanin absorbs orange and red light, particularly near 620 nm (depending on which specific type it is),
and emits fluorescence at about 650 nm (also depending on which type it is). Allophycocyanin absorbs and emits at longer wavelengths than Phycocyanin. A major advantage of using molecular techniques instead of microscopic methods is the ability to enhance the taxonomic resolution from genus-level to genotype-level, which is not possible to attain through other methods (Ouellette and Wilhelm, 2003).

The C-phycocyanin gene sequence was first reported (Pilot & Fox, 1984), in which the oligonucleotide synthesis of alpha and beta subunits of cpc gene of a freshwater cyanobacterum Agmenellum quadruplicatum was described and also reported the cpcA and cpcB gene sequence in Spirulina platensis. The cpc gene sequences of some Spirulina strains are used as a site to analyze their phylogenetic relationships. There is no more detailed information about the regulatory sequence and cpc operon (Yu et al., 2002).

Molecular characterization of ten marine cyanobacterial isolates belonging to the order Oscillatoriales was carried out using the phycocyanin locus (cpcBA-IGS) and the 16S-23S internally transcribed spacer region. DNA sequences from the phycocyanin operon discriminated ten genotypes, which corresponded to seven morphotypes identified by traditional microscopic analysis. The cpcB coding region revealed 17% nucleotide variation, while cpcA exhibited 29% variation across the studied species. Phylogenetic analyses support the conclusion that the Phormidium and Leptolyngbya genera are not monophyletic. The nucleotide variations were heterogeneously distributed with no or minimal informative nucleotides. Our results suggest that the discriminatory power of the phycocyanin region varies across the cyanobacterial species and strains. The DNA sequence analysis of the 16S - 23S internally transcribed spacer region also supports the polyphyletic nature of the studied Oscillatorian cyanobacteria. This study demonstrated that morphologically very similar strains might differ genotypically. Thus, molecular approaches comprising different gene regions in combination with morphological criteria may provide better taxonomical resolution of the order Oscillatoriales (Premanandh et al., 2006).
Cyanobacterial Features:

Cyanobacterial morphology ranges from simple unicellular, colonial and multicellular filamentous forms. The vegetative cell wall is of gram-negative type but in some species the peptidoglycan layer is considerably thicker than in other bacteria. Minute pores are present in regular or scattered order in the wall of all cyanobacteria, but the arrangement varies greatly. Many unicellular and filamentous cyanobacteria possess an “envelope” outside the lipopolysaccharide (LPS) “outer membrane”, which is called: sheath, glycocalyx, or capsule.

The photosynthetic apparatus of cyanobacteria contains photosystem I and photosystem II as found in higher plants with Chlorophyll $a$ and specific accessory pigments, including Allophycocyanin, Phycocyanin and Phycoerythrin. Cyanobacteria possess the ability to use low light intensities effectively, since they are able to produce the accessory pigments needed to adsorb light most efficiently in the habitat in which they are present, providing them a great advantage for the colonization of their wide range of ecological niches. Phycobiliprotein synthesis is particularly susceptible to environmental influences, especially light quality. The chromatic adaptation is largely attributable to a change in the ratio between Phycocyanin and Phycoerythrin in the Phycobilisomes. The photosynthetic pigments are located in thylakoids that are free in the cytoplasm near the cell periphery. Cell colours vary from blue green to violet-red due to the chlorophyll $a$ masking by the carotenoids and accessory pigments. The pigments are involved in phycobilisomes, which are found in rows on the outer surface of the thylakoids.

Cyanobacteria get their name from the bluish pigment phycocyanin, which they use to capture light for photosynthesis. In some cyanobacteria, the color of light influences the composition of phycobilisomes. In green light, the cells accumulate more phycoerythrin, whereas in red light they produce more phycocyanin. Thus the bacteria appear green in red light and red in green light. This process is known as complementary chromatic adaptation.
Cyanobacteria are also able of storing essential nutrients and metabolites within their cytoplasm. The occurrence of fimbriae (pili) is abundant in many cyanobacteria with varying patterns. Some filamentous forms are also able of gliding (sliding), using mucilaginous excretions as propellant. Some cyanobacteria have evolved specialized cells for nitrogen fixation (heterocytes), survival in stressed conditions (akinetes), and dispersion (hormogonia).

Cyanobacteria have many fascinating features such as buoyancy, photosynthesis, fixation of atmospheric nitrogen and production of a wide variety of bioactive compounds. In addition, cyanobacteria form symbiosis with several eukaryotic hosts such as plants, fungi, and protists. Probably owing to their physiological flexibility and long evolutionary history, cyanobacteria inhabit a large variety of terrestrial and aquatic habitats from deserts to lakes as well as hot springs and glaciers. Cyanobacteria form biofilms (microbial mats) on shores and on the surface of stones, plants, and artificial objects. Cyanobacterial blooms are frequently toxic and thus pose a health risk for humans and animals, cause an aesthetic problem, and reduce the recreational value of water (Mur et al., 1999).

Limitations of phenotypic characters in cyanobacterial identification led to the development of molecular techniques, including DNA base composition, DNA and RNA hybridizations, gene sequences and PCR fingerprinting methods for cyanobacteria taxonomy. However, it has been difficult to define taxonomic or phylogenetic relationships within the cyanobacteria because of the scarcity of distinct, consistent characters that support a taxonomic scheme. The problems of cyanobacterial taxonomy are name changes of some strains, besides the misidentification issue of others. Consequently, an ever changing classification system and a lack of a consensus phylogeny are the proofs of the unresolved evolutionary relationships among cyanobacteria.

A molecular approach to the systematic of cyanobacteria may be most fruitful for inferring phylogenetic relationships. Macromolecules, such as nucleic acids and proteins, are copies or translations of genetic information. The methods applied involve
direct studies of the relevant macromolecules by sequencing, or indirectly by electrophoresis, hybridization, or immunological procedures (Wilmotte, 1994). Nucleic acid technologies, especially the polymerase chain reaction (PCR), have advanced to the point that it is feasible to amplify and sequence genes and other conserved regions from a single cell. However, the molecular results obtained have integrated with other characteristics as the base for polyphasic taxonomy (Vandamme et al., 1996).

Rapid developments in genomic and other molecular research technologies and developments in information technologies have combined to produce a tremendous amount of information related to molecular biology by the application of bioinformatics. Common activities in bioinformatics include mapping, analyzing DNA and protein sequences, then for aligning different DNA and protein sequences to compare, create and to view 3-D models of protein structures. Major research efforts in the field include sequence alignment, gene finding, genome assembly, drug design, drug discovery, protein structure alignment, protein structure prediction, prediction of gene expression and protein-protein interactions, genome-wide association studies and the modeling of evolution. Software tools were used for sequence analysis and structure prediction includes BLAST, protein structure viewing, NCBI, EMBL etc (Mount, 2002).

Protein has three main structures: primary structure which is essentially the linear amino acid sequence and usually represented by a one letter notation. Alpha helices, beta sheets, and loops are formed when the sequences of primary structures tend to arrange themselves into regular conformations; these units are known as secondary structure. Protein folding is the process that results in a compact structure in which secondary structure elements are packed against each other in a stable configuration. This three-dimensional structure of the protein is known as the protein tertiary structure. However, loops usually serve as connection points between alpha-helices and beta-sheets, they do not have uniform patterns like alpha-helices and beta-sheets and they could be any other part of the protein structure rather than helices or strands (Kendrew et al., 1960).
In Bioinformatics, BLAST is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A blast search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. Molecular phylogenetics is the analysis of hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationships. The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree. Molecular phylogenetics is one aspect of molecular systematics, a broader term that also includes the use of molecular data in taxonomy and biogeography.

Molecular modelling is used to model the molecules. Both theoretical and computational techniques are used in molecular modelling. Molecular modelling methods are used to investigate the structure, dynamics and thermodynamics of inorganic, biological, and polymeric systems. The types of biological activity that have been investigated using molecular modelling include protein folding, enzyme catalysis, protein stability, conformational changes associated with biomolecular function, and molecular recognition of proteins, DNA, and membrane complexes. Cyanobacteria, our model organisms, are regarded as an origin of producing phycocyanin pigments; therefore they are considered as excellent candidates to study molecular techniques to resolve many of the issues and problems in cyanobacterial taxonomy. Thus, the work is mainly focused on the following objectives.

**Molecular Modeling of C-Phycocyanin:**

Molecular modeling is a powerful methodology for analyzing the three dimensional structure of biological macromolecules. There are many ways in which molecular modeling methods have been used to address problems in structural biology. It is not widely appreciated that modeling methods are often an integral component of structure determination by NMR spectroscopy and X-ray crystallography. The overall aim of modeling methods will often be to try to relate biological activity to structure. An important step towards this goal is to be able to compute the potential energy of the molecule as a function of the positions of the constituent atoms. The common feature of
molecular modeling techniques is the atomistic level description of the molecular systems; the lowest level of information is individual atoms or a small group of atoms. This is in contrast to quantum chemistry which is also known as electronic structure calculations, where electrons are considered explicitly. The benefit of molecular modeling is that it reduces the complexity of the system, allowing many more atoms to be considered during simulations (Foster, 2002).

**Structural modeling of the gene:**

Alpha-helix is spiral turns of amino acids while a beta-sheet is flat segments or strands of amino acids formed usually by a series of hydrogen bonds. As the polypeptide chain coils in, the CO and NH groups of residues form hydrogen bonds which stabilize the helix. Most of the residues in a helix are bonded in this way, making it somewhat a rigid unit of structure with a little free space in its core. A helix and can have 4 - 50 residues and makes a whole turn every 3.6 residues.

Beta-strands are the most regular form of extended polypeptide chain in protein structures. Like alpha-helices, beta-sheets are stabilized by hydrogen bonds between CO and NH groups, but they are distantly separated along the chain. Because of the geometry of the peptide backbone, the amino acid side chains of beta-strands alternate on either side of the sheet.

Loops usually serve as connection points between alpha-helices and beta-sheets, they do not have even patterns like alpha-helices and beta-sheets and they could be any other part of the protein structure. They are recognized as random coil and not classified as protein secondary structure. When the polypeptide chain makes very sharp changes in direction using as few as four residues by means of hydrogen bond, it forms turns. These secondary structures commonly contain proline or glycine or both residues (Hutchinson and Thornton, 1994).

**Tertiary Structure:**

The three-dimensional structure of the protein, which is formed from the secondary structures as subunits elements, is known as the protein’s tertiary structure.
Protein folding is the process that results in a compact structure in which secondary structure elements are packed against each other in a stable configuration.

Hydrogen bonds, van der Waals forces, and oppositely charged amino acid side-chains are other interactions that help to stabilize the fold. Folds are considered as sets of connected secondary structure elements, so they are known as topologies. Longer polypeptide chains that are usually clearly distinguished by a naked eye as self-contained units of structure, and have distinct hydrophobic cores, are known as domains. Homology modeling can produce high-quality structural models when the target and template are closely related, which has inspired the formation of a structural genomics consortium dedicated to the production of representative experimental structures for all classes of protein folds (Dill, 1990).

The Crystal structure of the light-harvesting protein phycocyanin from the Cyanobacterium Cyanidium caldarium with novel crystal packing has been solved at 1.65-Å resolution. The structure has been refined to an $R$ value of 18.3% with excellent backbone and side-chain stereochemical parameters. In crystals of phycocyanin used in this study, the hexamers are offset rather than aligned as in other phycocyanins that have been crystallized to date. Analysis of this crystal’s unique packing leads to a proposal for phycobilisome assembly in vivo and for a more prominent role for chromophore b-155. This new role assigned to chromophore b-155 in phycocyanin sheds light on the numerical relationships among and function of external chromophores found in phycoerythrin and phycoerythrocyanin (Stec et al., 1999).

**Impact of Molecular Methods on Cyanobacterial Taxonomy:**

Molecular methods have had a great impact on every level of cyanobacterial taxonomy. Prochlorophytes, which were considered a special group of oxyphototroph prokaryotes for the lack of phycobiliproteins and the presence of chlorophyll b, have been shown to be polyphyletic according to the 16S rDNA and scattered throughout the cyanobacterial lineage. But contradictory to the general concept, a phycobiliprotein gene, similar to that of the marine *Synechococcus*, has been detected in *Prochlorococcus marinus* CCMP 1375 strain (Hess et al., 1996).
Another important task solved by 16S rDNA sequencing was the origin of plastids that were proven to have descended from cyanobacteria. This was later confirmed also by *tufA* gene sequence. Plastids form a monophyletic group within cyanobacterial lineage, but no strong candidate for the sister taxon to plastids exists at present (Delwiche *et al.*, 1995 and Turner, 1997). Heterocytic cyanobacteria were confirmed and the orders Chroococcales and Oscillatoriales were shown to be polyphyletic. The baeocyte-forming order Pleurocapsales seemed to be monophyletic at first, but it turned out to be polyphyletic too, with *Chroococcidiopsis thermalis* being a close relative of heterocytous cyanobacteria. The same may be stated for heterocytous order Stigonematales - it has been shown recently, on the basis of both 16S rDNA and *nif* genes, that it is polyphyletic as well (Giovannoni *et al.*, 1988 and Zehr *et al.*, 1997).

The situation on the generic and subgeneric level is even more confused. The ecological studies concerning diversity of cyanoprokaryotes in various habitats are many, but it is often not clear what is meant under the taxonomic designations assigned to the studied organisms.

Many misidentified strains in culture collections are available and no morphological data for a bulk of available cyanobacterial sequences. However, some work has been done on the cytomorphological and polyphasic characterization of chroococcalian "*Synechocystis*, "*Synechococcus*" and a few other unicellular strains as well as of heterocytous *Aphanizomenon/Anabaena/Nostoc* strains. The studies on filamentous "*Phormidium*" and "*Oscillatoria*" genera are few. Although there is often no correlation between morphological and molecular traits, especially for taxa with very simple morphology, some morphologically well defined genera were shown to be monophyletic. These include *Microcystis, Planktothrix* or marine *Trichodesmium* species. Notwithstanding the unreliability of traditional morphological criteria, some cytomorphological and ultra structural characters were found to correlate well with molecular data. This concerns e.g. the cell division type and especially the thylakoid arrangement, which seems to have substantial taxonomic value. Some other traits, such as perforation-patterns in the cell wall of cyanobacteria may prove useful on certain taxonomic levels (Pfeifer and Palinska, 2002 and Palinska and Krumbein, 2000).
**Relationship between Genotypic and Phenotypic Characters**

Cyanobacteria are morphologically diverse in comparison to the rest of bacteria. Nevertheless, only a quite restricted number of morphotypes can be recognized. Molecular methods enabled revelation of cryptic genetic, physiological and ecological diversity among them. Rather broadly defined species *Phormidium retzii* was shown to be quite variable on molecular level. Although there were some morphological distinctions between individual populations, they did not correlate with genetic similarity. Strains morphologically corresponding to the genus *Geitlerinema* sp. generated very different restriction patterns (Casamatta *et al.*, 2003).

The studies on cyanobacterial communities of both geothermal springs in the Philippines and Lake Fryxell in Antarctica revealed significantly higher degree of diversity by molecular methods than by light microscopy. Genetically distinct toxic *Microcystis* and *Planktothrix* populations were found in different parts of the same lake. However, it is also possible that the strains, closely related on molecular level, show substantial phenotypic variability, as shown for several *Merismopedia* isolates. Co-existing *Prochlorococcus* ecotypes were detected, almost identical genetically, but possessing very different light-dependent physiologies (Taton *et al.*, 2003, Palinska *et al.*, 1996, Moore *et al.*, 1998).

Interesting topic is the correlation between phylogenetic relatedness and ecological or ecophysiological characters. Unicellular cyanobacteria from hypersaline habitats in various geographical regions form a well defined cluster on the basis of their 16S rDNA that was denominated *Halothecce* cluster. Moderately halophilic, benthic strains with very thin trichomes also form a distinct cluster in the phylogenetic tree and were assigned to a new genus *Halomicronema* (Garcia-Pichel *et al.*, 1998).

An interesting fact that the only available 16S rDNA sequences of cyanobacteria from stone surfaces of buildings group with those of desert strains from distant geographic region, while sequence homology with the strains from other
habitats is quite low. The sequence similarity somehow reflects the capacity to survive in such extreme environments (Crispim and Gaylarde, 2005).

The analysis of the hli gene family, which has to do with adaptation to high light intensities, revealed that some groups of this gene family are specific either for marine or freshwater cyanobacteria. A nice example of ecological divergence of morphologically and phylogenetically closely related, but distinct genera Trichodesmium and Hydrocoleum (Blennothrix) was documented. The first one is planktonic, the latter is the most common mat-forming cyanobacterium in tropical oceans (Bhaya et al., 2002 and Abed et al., 2006).

**Biotechnological Applications of cyanobacteria:**

Cyanobacteria constitute a resource for several applications such as aquaculture, food, feed, fuel, fertilizer, medicine, industry and even in combating pollution (WHO 1999).

Cyanobacterial bioactive compounds Cyanobacteria have been identified as a new and rich source of bioactive compounds (Abarzua et al. 1999; Shimizu 2003; Bhadury et al. 2004; Dahms et al. 2006). Isolated compounds belong to groups of polyketides, amides, alkaloids, fatty acids, indoles and lipopeptides (Abarzua et al. 1999; Burja et al. 2001).

The literature review showed that to date up to 19 cyanobacterial strains produce more than 20 different bioactive compounds. Most of the bioactive compounds isolated from cyanobacteria tend to be lipopeptides, i.e. they consist of an amino acid fragment linked to a fatty acid portion. The range of biological activity of secondary metabolites isolated from cyanobacteria includes antibacterial, antifungal, antialgal, antiprotozoan, and antiviral activities.

Only few cyanobacteria produce bioactive compounds that show a broad spectrum of biological activities. For example, the cyanobacterium Phormidium sp. has been reported to inhibit growth of different Grampositive and Gram-negative bacterial
strains, yeasts, and fungi (Bloor and England 1989). Another example is *Lyngbya majuscula* (Burja et al. 2001) that produces numerous chemicals including nitrogen-containing compounds, polyketides, lipopeptides, cyclic peptides and many others (Shimizu 2003).

The biological activity of these compounds is also diverse and includes protein kinase C activators and tumour promoters, inhibitors of microtubulin assembly, antimicrobial and antifungal compounds and sodium-channel blockers. Secondary metabolites with antibacterial activity are widely produced by cyanobacteria (Dahms et al. 2006). These compounds are effective against Gram positive and/or Gram-negative bacteria. Both toxic and nontoxic strains of cyanobacteria are producers of antibacterial compounds that are distinct from cyanotoxins. Antifungal compounds include fisherellin A, hapalindole, carazostatin, phytoalexin, tolytoxin, scytophycin, toyocamycin, tjipanazole, nostocyclamide and nostodione produced by cyanobacteria belonging to Stigonematales, Nostocales and Oscillatoriales. Additionally, cyanobacteria produce a broad spectrum of antialgal compounds, which may be used to control algal blooms.

Cyanobacteria probably use these compounds in order to out-compete other microorganisms. Antialgal compounds produced by cyanobacteria inhibit growth of algae, their photosynthesis, respiration, carbon uptake, enzymatic activity and induce oxidative stress (Dahms et al. 2006). In contrast to the large amount of antibacterial and antialgal compounds isolated from cyanobacteria there are only a few compounds that show antiviral properties, although 2–10% of extracts of different cyanobacterial species have been shown to have antiviral activity (Cohen 1999). These include acetylated sulfoglyco-lipids from *Oscillatoria raoi* (Reshef et al. 1997) and spirulan from *Spirulina platensis* (Hayashi et al. 1991). The compounds isolated from *Lyngbya lagerhaimanii* and *Phormidium tenue* has been shown to have anti-HIV activity (Rajeev and Xu 2004). Gamma linolenic acid (GLA) found rich in *S. platensis* and *Arthrospira* sp. is medically important since it is converted in the human body into arachidonic acid and then into prostaglandin E2. This compound has a lowering action on blood pressure and plays an important role in lipid metabolism. Some of the marine
cyanobacteria constitute potential sources for large-scale production of vitamins, such as vitamins B and E (Plavsic et al. 2004).

Piechula et al. (2001) demonstrated that some cyanobacteria can produce thermostable enzymes. Out of 21 endonucleases from *Phormidium*, four enzymes that catalyse the hydrolysis of DNA and RNA were active in a wide range of temperatures from 15 to 60°C. Phenyltransferases enzymes catalysing the consecutive condensation of homoallylic diphosphate of isopentenyl diphosphates at temperatures above 60°C have been isolated from the thermophilic cyanobacterium, *Synechococcus elongates* (Ohto et al. 1999). Thermostable polyphosphate kinase from the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 was successfully employed in an ATP regeneration system that could be used at high temperatures for the effective production of d-amino acid dipeptides (Sato et al. 2007). These enzymes and other heat stable bioactive compounds are of great interest in biotechnology. So far none of the isolated compounds have demonstrated anti-larval activity and inhibition of settlement of larvae and algal spores (Dobretsov et al. 2006).

Cyanobacteria have been used to synthesize isotopically labelled compounds such as sugars, lipids and amino acids, which are nowadays commercially available (Patterson 1996). This is achieved by growing the cyanobacteria in photobioreactors and allowing them to photosynthetically transform simple labelled compounds such as $^{14}$CO$_2$, $^{13}$CO$_2$, $^{33}$H$_2$O and $^{15}$NO$_3$ into complex organics cyanobacteria as a healthy food source. Strains of *Spirulina*, *Anabaena* and *Nostoc* are consumed as human food in many countries including Chile, Mexico, Peru and Philippines. *Arthrospira platensis* (misidentified as S. platensis) is grown in large scale using either outdoor ponds or sophisticated bioreactors but marketed in the form of powder, flakes, tablets and capsules. It is used as a food supplement because of its richness in nutrients and digestibility. It contains more than 60% proteins and is rich in beta-carotene, thiamine and riboflavin and is considered to be one of the richest sources of vitamin B$_{12}$. *Nostoc commune* is rich in fibres and proteins and can play an important physiological and nutritional role in the human diet. *Aphanizomenon* sp. is collected from natural blooms in the Lake Klamath (Oregon, USA) to be used as healthy food. Marine nitrogen-fixing
cyanobacteria have also been tested to feed fishes in aquacultures. The Tilapia fish showed high growth rates when fed with marine cyanobacteria in indoor and outdoor cultures (Mitsui et al. 1983). *Phormidium valderianum* has been used in India to serve as a complete aquaculture feed source based on its nutritional value and nontoxic nature. In view of the cyanobacterial significance as a food source, very little research has been performed and published about this.

Cyanobacterial alternative energy sources Cyanobacteria have been used to produce hydrogen gas that constitutes an alternative future energy source to the limited fossil fuel resources (Dutta et al. 2005). The advantages of using biological hydrogen as a fuel are its eco-friendly nature, efficiency, renewability and the absence of carbon dioxide emission during its production and utilization (Lindblad 1999).

Cyanobacteria produce hydrogen either as a byproduct of nitrogen fixation, when nitrogenase-containing heterocystous cyanobacteria are grown under nitrogen limiting conditions, or by the reversible activity of hydrogenases enzymes. Heterocystous cyanobacteria are thus more efficient in hydrogen production than nonheterocystous types (Pinzon-Gamez et al. 2005). More than 14 cyanobacterial genera including *Anabaena, Calothrix, Oscillatoria, Cyanothece, Nostoc, Synechococcus, Microcystis, Gloeobacter, Aphanocapsa, Chroococcidiopsis* and *Microcoleus* are known for their ability to produce hydrogen gas under various culture conditions (Lambert and Smith 1977; Sveshnikov et al. 1997; Masukawa et al. 2001). *Anabaena* spp. is able to produce significant amounts of hydrogen. Nitrogen-starved *Anabaena cylindrica* cells produce the highest amount of hydrogen (30 ml of H₂ per lit culture per hour) (Jefferies et al. 1978). *Gloeocapsa alpicola* showed increase in hydrogen production under sulfur starvation (Antal and Lindblad 2005) whereas *S. platensis* could produce hydrogen under complete dark and anoxia (Aoyama et al. 1997).

Large-scale hydrogen production by *Spirulina* and *Anabaena* spp. has been tried using different types of bioreactors that included vertical column reactor, tubular type and flat panel photobioreactor (Dutta et al. 2005). These reactors were designed to
make use of solar light for illumination, to maximize the area for incident light (high surface to volume ratio) and to allow sterilization and hydrogen collection with convenience and ease. Nevertheless, the reactors are subjected to continuous modification in order to increase their productivity and to decrease costs of maintenance and production. So far, these modifications succeeded in bringing down the cost of biologically produced hydrogen to $25 per m$^3$ compared to $170$ per m$^3$ for the hydrogen produced by splitting of water (Block and Melody 1992).

Increased production of hydrogen was also achieved by genetically modifying the nitrogenase enzyme in hydrogen-producing strains. The ongoing research on hydrogen production by cyanobacteria focuses on finding new strains with higher potential to produce hydrogen, optimizing the mass production of hydrogen in bioreactors and modifying the physiology and the genetic system of H$_2$-producing cyanobacteria to ensure maximum production of hydrogen Cyanobacteria as biofertilizers. Heterocystous cyanobacteria and several nonheterocystous cyanobacteria are known for their ability to fix atmospheric nitrogen (Capone et al. 2005). The fertility of many tropical rice field soils has been mainly attributed to the activity of nitrogen-fixing cyanobacteria.

Species of cyanobacteria, namely strains of *Spirulina (Arthrospira) platensis*, *Nostoc commune* and *Aphanizomenon flos-aquae*, are currently used as food and supplements due to their amounts of proteins (e.g. up to 70% of *Spirulina* dry weight), lipids, chlorophyll, carotenoids, vitamins, minerals, unique pigments and also due to their potential prebiotic effects. *Spirulina* is also used as an aquaculture and animal feed source. As a crop, *Spirulina* has the advantage of growing well in saline ponds in arid environments, places that few other crops could support (Pulz and Gross, 2004).

Several fine chemicals such as pigments (carotenoids and phycobiliproteins), vitamins (B-complex group and vitamin E) and enzymes with varied applications from cyanobacteria are being commercialized. The pigments are used as natural food colourants, food additives, and cosmetics and as enhancers of ornamental fish colour. Isotopically labelled ($^{14}$C, $^{15}$C, $^3$H and $^{15}$N) cyanobacterial metabolites such as sugars,
lipids and amino acids are commercially available. Several endonucleases produced from cyanobacteria are being marketed, e.g. Acyl (Anabaena cylindrica).

Cyanobacteria produce numerous bioactive compounds. Some secondary metabolites are potentially of therapeutical importance, such as antiviral compounds, immunomodulators, inhibitors, or cytostatics. N$_2$-fixing cyanobacteria are used as biofertilizers, for instance in the rice paddies where nitrogen to support the rice plants comes from fixation by free-living and symbiotic cyanobacteria, besides being also used in other tropical and subtropical agriculture.

Reports concerning the advantageous effects of cyanobacteria inoculation on other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chili and lettuce have been described (Thajuddin and Subramanian, 2005). It has been reported that the marine cyanobacterium, Oscillatoria formosa NTDM02 was efficiently decolorized textile dyes and laboratory dye (MubarakAli et al., 2010).
MATERIALS AND METHODS

Study area

![Sample Locations](image)

Fig. 3 Map showing the sample collection site at Mandapam, Kurusadai Island and Rameshwaram, India

Sample collection

The samples were collected from rock surfaces in Kurusadai Island, during the month of December 2009 of monsoon season and the diversity of Cyanobacteria were observed initially by the use of light microscope (Optika, California). Diatom samples were collected from stagnant water in Palkalaiperur, during the month of December to February (2010) of monsoon season and the diversity of diatoms were observed by the use of light microscope (Optika, California).
**Isolation of diatoms**

The diatoms were isolated by using enriched sea water medium F/2 (Guillard and Ryther, 1962) by serial dilution, capillary or micropipette method and streak plate method. The cultures were grown under the fluorescent light of 1500 lux, 12:12 light:dark (L:D) condition at 25±2°C. After incubation the cultures were examined at regular intervals under light microscope.

**Isolation of Cyanobacteria:**

The Cyanobacteria were isolated by using enriched sea water medium MN and ASN-III (Rippika, 1988) by serial dilution and streak plate method. The cultures were grown under the fluorescent light of 1500 lux, 12:12 light: dark (L: D) condition at 25±2°C. After incubation the cultures were examined at regular intervals under light microscope.

**Morphological characterization of diatom and marine Cyanobacteria**

The isolated diatom and marine Cyanobacteria were examined carefully by using light microscope and microphotograph was taken (Optika Photomicroscope Unit) to determine the morphology. The identification of Cyanobacteria in marine water samples was achieved using standard monograph (Geitler, 1932; Desikachary, 1959). The diatom strains were genus was identified based on frustules morphology by using the atlas of diatoms (Desikachary, 1989). The isolated cyanobacterial strains were maintained as axenic cultures in the germplasm of Department of Microbiology, Bharathidasan University.

**Molecular characterization of diatom**

**DNA extraction from diatom** (Grachev et al., 2006)

A grown culture was centrifuged to obtain about 50μl of packed biomass. Cells were suspended in 100μl of a lysis buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 2% CTAB, 20mM EDTA), homogenized with a pestle at 60°C for 10 minutes, combined with 150μl of the lysis buffer, and incubated at 60°C for 30 minutes with occasional shaking. Cell debris was removed by centrifugation (3 minutes), and the supernatant was transferred into another tube. The pellet was washed with 100μl of the lysis buffer,
and the supernatant was added to the first one. The pooled supernatant was combined with 7.5µl of RNase, incubated at 60ºC for 30 minutes, combined with 3µl of proteinase K, incubated again at 60ºC for 30 minutes, and combined with 1ml of 1% CTAB (aqueous solution) at room temperature.

The resulting suspension was stirred and frozen-thawed. The pellet was collected by centrifugation (15 minutes) and washed twice with 300µl of 1% cetavlon (aqueous solution). The supernatant was carefully removed by overturning the tube on filter paper. The pellet was extracted twice with 400µl of ethanol at 60ºC for 10 minutes. The supernatant (ethanol solution of the CTAB salt of DNA) was collected by centrifugation (5 minutes) and combined with 80µl of 3M sodium acetate and kept at -20ºC overnight. The precipitate (DNA sodium salt) was collected by centrifugation, washed with ethanol, and air-dried. DNA was dissolved in 50µl of a PCR buffer and stored at -20ºC.

The extracted DNA were estimated directly from ethidium bromide fluorescence in agarose gel (0.8% in 1X TBE buffer) against a standard marker, (Finzymes, Finland) by using a gel documentation with associated software.

**Oligonucleotide primers and PCR conditions:**

All PCR reactions were carried out in a 40µl volume containing (i)20µl of 2x prime Taq premix ( GENET BIO, Korea) which contains 1unit Taq DNA Polymerase, 20mM Tris-Hcl, 80mM KCl, 4mM MgCl₂, enzyme stabilizer, sediment, loading dye, pH 9.0, 0.5mM of each dATP, dCTP, dGTP, dTTP. (ii) 1µl of upstream primer (10 pmoles/µl) (iii) 1µl of downstream primer (10pmoles/µl) (iv) 1µl (50ng) of extracted DNA from diatom (v) 7 µl of sterilized distilled water.

PCR amplification of the small subunit of 18S rRNA gene (SSU rDNA) was done in a thermal cycler gradient (Applied biosystems, Germany) with the primer pairs 1F and 1528R (Medlin et al. 1988). Conditions for PCR were as follows: initial denaturation at 94°C for 5 mins, followed by 36 cycles of 94°C for 2 min, 54°C for 4 min, and 72°C for 2 min, and a final extension at 72°C for 10 min.
18S rDNA gene amplification:

A fragment of 18S rDNA gene was amplified as mentioned above (Ulf Karsten *et al.*, 2006). After the reaction was completed, 10µl of amplified DNA was separated on 1.2% agarose, stained with ethidium bromide and recorded using a CCD camera in UVP Imager (UVP Bioimaging, England). A ready to use DNA size standard samples (Finzymes, Finland) was added in the gel to determine the molecular weight of the marker.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′→3′</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>AAC CTG GTT GAT CCT GCC AGT</td>
<td>Medlin <em>et al.</em>, 1988</td>
</tr>
<tr>
<td>1528 R</td>
<td>TGA TCC TTC TGC AGG TTC ACC TAC</td>
<td></td>
</tr>
</tbody>
</table>

Molecular characterization of marine Cyanobacteria:

DNA extraction:

The extraction of genomic DNA from cyanobacterial isolates were carried out (Smoker and Barnum 1988). Briefly, 1ml of overnight grown cultures was centrifuged at 10,000 rpm for 5 minutes and the pellet was collected, then collected pellet was washed using STE buffer (NaCl – 50mM; Tris-HCl - 50mM; EDTA - 5mM) and suspended in 500µl of STE buffer. Followed by 20µl of lysozyme (10mg/ml) was added and incubated in water bath at 55°C for 30 minutes. After incubation, 10µl of proteinase K (10mg/ml) and 20µl of 10% SDS was added and incubated in water bath at 55°C for 30 minutes. The mixture was then cooled in ice and extracted with equal volume of phenol: chloroform: Isoamyl alcohol mixture (25:24:1) and centrifuged at 12,000 rpm for 10 minutes. After centrifugation, the supernatant was separated with care and equal volume of 4M Ammonium acetate and two volumes of Isopropanol were added. Mixture was again centrifuged at 14,000 rpm for 15 minutes and the supernatant was decanted and the pellet was washed with 70% ethanol, dried. Finally, the DNA pellet was dissolved in 100µl of TE buffer and stored at 20°C until further use. The loading dye was added to each DNA samples and the samples were transferred to separate wells in 0.8% agarose gel and after loading, the electrophoresis was carried...
out using 1X TAE buffer at a constant supply of 100 V for 30 minutes. The bands were documented under transilluminator.

**16S rDNA gene amplification:**

PCR amplification was performed for the respective two samples of purified DNA using CYA 106 (5’ - CGG ACG GGT GAG TAA CGC GTGT- 3’) and CYA 781 (5’ - GAC TAC TGG GGT ATC TAA TCC CA T - 3’) primers. The polymerase chain reactions conditions include initial denaturation of template DNA was achieved at 94°C for 3 minutes. Further denaturation was carried out at 94°C for 45 sec; annealing at 56°C for 30 sec, elongation at 72°C for 1 min and a final elongation at 72°C for 7 min for 30 cycles. Amplified products were isolated by electrophoresis on 1.2% agarose gel using 1X TAE buffer at a constant supply of 100 V for 30 minutes. Again, resolved bands were documented under Uv transilluminator.

**C- Phycocyanin (PC) Gene amplification:**

PCR amplification of C-Phycocyanin was performed for the respective two samples of purified DNA using PCβ F (GGC TGC TTG TTT ACG CGA CA) and PCα R (CCA GTA CCA CCA GCA ACT AA) primers (Neilan, 2002). The polymerase chain reactions conditions include initial denaturation of template DNA was achieved at 94°C for 5 minutes. Further denaturation was carried out at 94°C for 2 mins; annealing at 55° C for 1 min, elongation at 72°C for 3 mins and a final elongation at 72°C for 8 min for 40 cycles. The reaction mixture of amplification of PC-IGS region contains 40µl.

**Sequencing and Construction of phylogeny:**

Sequencing was done with amplified samples with respective forward and reverse primers and sequences. The query sequence was uploaded in alignment box and the query was submitted to BLAST analysis for pair-wise sequence alignment. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site and it was computed in MEGA 5 software.
Molecular modeling of C-Phycocyanin gene:
Prediction of primary, secondary and tertiary structure of C-Phycocyanin and determination of conserved domains of the C-phycocyanin sequences were analyzed using online and offline softwares for molecular modeling of the protein.

Tertiary Structure:
Retrieval of target protein sequences from NCBI in Fasta format and save as in notepad. The template structure was searched through Blastp algorithm against PDB database. Target sequence alignment and template protein structure were derived for Structure prediction using spdV software. The modelled structures were subjected to fit the models and to optimize the stereochemistry atoms of molecules. The final modelled structure to predict 3D structure profiles and it was visualized.

Conserved Domain determination (CDD):
CDD is a protein annotation resource that consists of a collection of well-annotated multiple sequence alignment models for ancient domains and full-length proteins. These are available as position-specific score matrices (PSSMs) for fast identification of conserved domains in protein sequences via RPS-BLAST. CDD content includes NCBI-curated domains, which use 3D-structure information to explicitly define domain boundaries and provide insights into sequence / structure / function relationships, as well as domain models imported from a number of external source databases (Pfam, SMART, COG, PRK, TIGRFAM)

Identify the putative function of a protein sequence, identify the amino acids in a protein sequences that are putatively involved in functions such as binding or catalysis, as mapped from conserved domain annotations to the query sequences. View a query protein sequence embedded within the multiple sequence alignment of a domain model, interactively views the 3D structure of a conserved domain, Find other proteins with similar domain architecture and interactively view the phylogenetic sequence tree for a conserved domain model of interest with or without a query sequence embedded (Marchler-Bauer et al. 2011).
RESULTS AND DISCUSSION

Totally, 50 cyanobacterial species and 38 diatom species were recorded. They were identified taxonomically with standard monographs. The schematic representation of collection and marine cyanobacterial diversity were shown (Fig. 4). There are five family from cyanobacterial diversity such as Chroococcaceae (7), Nostacaceae (4), Oscillatoriales (37), Pseudanabaenaceae (1) and Mastigocladaceae (1) respectively, whereas in diatom were found to be in thirteen families such as Achnanthaceae (2), Bacillariaceae (6), Catenulaceae (1), Cymbellaceae (2) Desmidiaceae (1), Fragilariaceae (4), Gomphonemataceae (1), Mastogloiaceae (2), Naviculaceae (8), Pinnulariaceae (4), Pleurosigmaaceae (4), Stauroneidaceae (1), Stephanodiscaceae (2) (Table 1a & b).

The morphological evidence of the cyanobacteria and diatom were shown (Fig. 5a & b and 6). Cyanobacterial taxonomy follows the International Code of Nomenclature of Bacteria (Stainer et al., 1978) and published a taxonomy of the cyanobacteria that was based on physiological, morphological and some genetic criteria (Rippka et al., 1979). The revision of the status of the nomenclature of Cyanobacteria under the Bacteriological Code, only 13 names of cyanobacterial species have been proposed and since the corresponding genera name had not been validly published according to the International Code of Nomenclature of Bacteria. Although at the present there has been an attempt to classify cyanobacteria using both Botanical and Bacteriological Code of Nomenclature (Tindall, 2006).

The traditional observation of the morphological characters by light microscopy requires considerable expertise to identify species. Moreover, some phenotypic features, such as gas vacuoles or akinetes etc. may vary with environmental or growth conditions and even their features may lost during repeated subcultures (Rudi et al., 1997). Although cyanobacterial identification at genus level is easier to be reached, particularly where morphological characteristics are significantly different from other genera, there are some cases where this separation is not clear, namely the delineation of Microcystis from Synechocystis, Anabaena from Nostoc, as well as Anabaena from Aphanizomenon and Nodularia.
Figure 4 Schematic representation of cyanobacterial sample collection and morphological divergence of marine Cyanobacteria
### Table 1a. Microbial diversity of Cyanobacteria

**Chroococcaceae**

1. Chroococcus minor Kutzing
2. Chroococcus sp. Kutzing
3. Gleocapsa sp. Kutzing
4. Synechococcus elongatus Nageli
5. Synechocystis sp. Sauvageau
6. Microcystis marginata Menedhini
7. Merismopedia convoluta Brebisson

**Nostacaceae**

8. Nodularia spumigena Mertens
9. Nodularia sp. Mertens
10. Anabaena spiroides Klebahn
11. Anabaena cylindrica Lemmermann

**Oscillatoriaceae**

12. Phormidium tenue Gomant
13. Phormidium valderianum Gomant
14. Phormidium chlorinum Kutzing
15. Phormidium tenue Meneghini
16. Phormidium fragile Gomant
17. Phormidium subtilissma Kutzing
18. Phormidium angustissimum
19. Phormidium corium Agardh
20. Phormidium tenue Gomant
21. Oscillatoria subrevis Schmidle
22. Phormidium willei Gardner
23. Oscillatoria formosa Vincent
24. Oscillatoria boryana Bory
25. Oscillatoria subrevis Schmidle
26. Oscillatoria princeps Rabenhorst
27. Oscillatoria tenius Agardh
28. Oscillatoria amphibium Anagnostidis
29. Oscillatoria laete-viridens Crouan
30. Oscillatoria limosa Agardh
31. Oscillatoria princeps Roberhorst
32. Oscillatoria limosa Agardh
33. Oscillatoria curviceps Agardh
34. Oscillatoria boryana Bory and Gomant
35. Oscillatoria princeps Rabenhorst
36. Oscillatoria rubencens De Candolle
37. Oscillatoria tenus Gomant
38. Oscillatoria sp. Bory and Gomant
39. Oscillatoria chalybya Metens
40. Oscillatoria acuminata Gomant
41. Oscillatoria salina Biswas
42. Jaaginema pseudogeminatum Schmid
43. Spirulina subsalsa Oersted
44. Spirulina princeps West and West
45. Spirulina meneghini Zanardini
46. Lyngbya majuscula Harvey
47. Lyngbya allergii Kutzing
48. Lyngbya confervoides Agardh

**Pseudanabaenaceae**

49. Pseudoanabaena sp Lauterborn

**Mastigocladaceae**

50. Hapalosiphon welwitschii West and West
<table>
<thead>
<tr>
<th>Table 1b. Microbial diversity of Diatoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Achnanthaceae</strong></td>
</tr>
<tr>
<td>1. Achnanthes gibberula Bory De Saint</td>
</tr>
<tr>
<td><strong>Bacillariaceae</strong></td>
</tr>
<tr>
<td>3. Denticula vanheurcki Brun</td>
</tr>
<tr>
<td>8. Nitzchia sigmoidea Smith</td>
</tr>
<tr>
<td><strong>Catenulaceae</strong></td>
</tr>
<tr>
<td><strong>Cymbellaceae</strong></td>
</tr>
<tr>
<td>11. Cymbella affinis Agardh</td>
</tr>
<tr>
<td><strong>Desmidaceae</strong></td>
</tr>
<tr>
<td><strong>Fragilariaceae</strong></td>
</tr>
<tr>
<td>13. Fragilaria construens Grunow</td>
</tr>
<tr>
<td>14. Fragilaria sp. Lyngbye</td>
</tr>
<tr>
<td>15. Synedra ulna Ehrenberg</td>
</tr>
<tr>
<td>16. Synedra acus Ehrenberg</td>
</tr>
<tr>
<td><strong>Gomphonemataceae</strong></td>
</tr>
<tr>
<td><strong>Stephanodiscaceae</strong></td>
</tr>
</tbody>
</table>
In addition, it is difficult to accurately identify cyanobacteria of the order Oscillatoriales. The identification problems increase further at species level, being of course impossible to differentiate strains through this approach. Sometimes, even for someone with experience, misidentification can be obtained, as occurred with an *Aphanizomenon* strain, originally classified as *Aphanizomenon flos-aquae* and later reclassified as *Aph. issatschenkoi* with the help of the 16S rRNA phylogenetic positioning (Neilan, 2002).

Molecular characterization of the marine cyanobacteria and diatoms were done with 16S rDNA and 18S rDNA sequence. Amplification of 18S rDNA and 16S rDNA gel picture was shown (Fig. 7), in which clear resolved bands at 2.1kb and 1.6kb were found in diatom and cyanobacteria respectively. The evolutionary relationships of the isolates were found out with phylogenetic tree, in which the isolated strain were closely associated with marine forms of cyanobacteria and some of new reports were found in case of diatom (Fig 8 & 9).

Molecular phylogenetics is the analysis of hereditary differences in DNA which gave the information on an organism's evolutionary relationships. The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree. The first molecular and phylogenetic characterization of two species of the genus *Nostoc* - *Nostoc linckia* and *Nostoc punctiforme* based on the cpcB-IGS-cpcA locus of the phycocyanin operon (Teneva et al., 2005). The phylogenetic position of these two species within order *Nostocales* as well as within division *Cyanoprokaryota* has been determined. The results indicate that genus *Nostoc* is heterogeneous. Analysis of the IGS region between cpcB and cpcA showed that *Nostoc* and *Anabaena* are distinct genera, while the taxonomic status of the genera *Aphanizomenon* and *Anabaena* is still unclear, since the similarity is up to 96.3%. Therefore, additional analyses and revision are required. Reported molecular and phylogenetic data will be useful to solve other problematic points in the taxonomy of genera *Aphanizomenon*, *Anabaena* and *Nostoc*.

The ribosomal sequences were deposited to GenBank (NCBI) to record our isolated diatom and cyanobacterial strains. Accession numbers for the each organism
Figure 7 Amplification of ribosomal rDNA: (a) diatom (18S rDNA) and (b) cyanobacteria (16S rDNA); marker (M); 1-5 represents selected diatom and cyanobacterial isolates.
Figure 8 Evolutionary relationship of diatom based on the 18S rDNA sequences. Organisms indicated by red color are the selected isolates while black colored ones are retrieved from GenBank (NCBI). Differences between sequences are indicated by the sum of the horizontal length; the scale bar is in fixed nucleotide substitutions per sequence position.
Figure 9 Evolutionary relationship of marine Cyanobacteria based on the 16S rDNA sequences by Maximum Likelihood Method. Organisms indicated by red color are the selected isolates while black colored ones are retrieved from GenBank (NCBI). Differences between sequences are indicated by the sum of the horizontal length; the scale bar is in fixed nucleotide substitutions per sequence position.
Table 2 Selected cyanobacterial 16S rDNA sequences were submitted to GenBank (NCBI) with Accession numbers mentioned below.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organisms</th>
<th>Sequence</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oscillatoria willei NTDM01</td>
<td>16S rDNA</td>
<td>GU812858</td>
</tr>
<tr>
<td>2.</td>
<td>Phormidium tenue NTDM05</td>
<td>16S rDNA</td>
<td>GU585847</td>
</tr>
<tr>
<td>3.</td>
<td>Leptolyngbya valderianum NTDM10</td>
<td>16S rDNA</td>
<td>JQ867392</td>
</tr>
<tr>
<td>4.</td>
<td>Oscillatoria boryana NTDM11</td>
<td>16S rDNA</td>
<td>JQ867393</td>
</tr>
<tr>
<td>5.</td>
<td>Spirulina subsalsa NTDM21</td>
<td>16S rDNA</td>
<td>GU585848</td>
</tr>
</tbody>
</table>
Table 3 Selected diatom 18S rDNA sequences were deposited to GenBank (NCBI) with Accession numbers mentioned below.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>Sequence</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Synedra sp. NTDMN01</td>
<td>18S rDNA</td>
<td>HM635776</td>
</tr>
<tr>
<td>2.</td>
<td>Synedra sp. NTDMD01</td>
<td>18S rDNA</td>
<td>HM635777</td>
</tr>
<tr>
<td>3.</td>
<td>Amphora sp. NTDMN02</td>
<td>18S rDNA</td>
<td>HM635778</td>
</tr>
<tr>
<td>4.</td>
<td>Stauroneis sp. NTDMD02</td>
<td>18S rDNA</td>
<td>HM635779</td>
</tr>
<tr>
<td>5.</td>
<td>Cymbella tumida NTDMN03</td>
<td>18S rDNA</td>
<td>HM585048</td>
</tr>
</tbody>
</table>
have received from GenBank were tabulated (Table 2 & 3). In addition, C-phycocyanin gene sequence also been deposited to GenBank with translated protein sequence (Table 4).

Protein conformational structures are primary, secondary and tertiary structures were predicted. Among which tertiary structure gives three-dimensional structures in different isoforms or different structural elucidation such ribbon model, ball and stick model, space-filling model and so on. A representative of the molecular modeling studies ribbon model and ball and stick models were elucidated with amino acid sequence (Fig. 11). In this protein model, sequence start with Met amino acids and ends with Ile amino acids.

Conservation domains were analysed in which the query sequence highly matched with phycobilisome superfamily (Fig 12). It was further analyzed for the identity with cyanophyceae and chlorophyceae phycocyanin family. Flow-chart for the determination of cyanobacterial Phycocyanin gene was shown (Fig 13). Molecular modelling methods are now routinely used to investigate the structure, dynamics, surface properties and thermodynamics of inorganic, biological and polymeric systems. The types of biological activity that have been investigated using molecular modelling include protein folding, enzyme catalysis, protein stability, conformational changes associated with biomolecular function, and molecular recognition of proteins, DNA, and membrane complexes (Foster, 2002). The query sequence was closely identical with cyanobacterial species with 75 % identity, whereas 37% of identity was observed chlorophyceae algal groups; hence, perhaps it may be used as genetic marker for development of DNA barcodes for the Cyanobacteria (Fig 14). After performing the structure prediction of SPDBV software, the final 3D Structure calculated for modelled structure reactivity, which can provide valuable information of protein structure and function. The in silico design of drug formulations, plays crucial roles in the biomedical research. Computational research on biomedics has generated a lot of information concerning its protein structure and reactivity.
Table 4 Selected cyanobacterial phycocyanin gene sequences were deposited to GenBank (NCBI) with Accession numbers mentioned below.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>Sequence</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Phormidium tenue</em> NTDM05</td>
<td>C-PC gene</td>
<td>JF421685</td>
</tr>
<tr>
<td>2.</td>
<td><em>Phormidium animale</em> NTMP03</td>
<td>C-PC gene</td>
<td>JF421686</td>
</tr>
<tr>
<td>3.</td>
<td><em>Oscillatoria acuminata</em> NTDM04</td>
<td>C-PC gene</td>
<td>JF421687</td>
</tr>
<tr>
<td>4.</td>
<td><em>Spirulina subsalsa</em> NTDM21</td>
<td>C-PC gene</td>
<td>Bankit No 1421025</td>
</tr>
<tr>
<td>5.</td>
<td><em>Oscillatoria chlorina</em></td>
<td>C-PC gene</td>
<td>Bankit No 1431046</td>
</tr>
<tr>
<td>6.</td>
<td><em>Oscillatoria salina</em></td>
<td>C-PC gene</td>
<td>Bankit No 1431047</td>
</tr>
</tbody>
</table>
Figure 10 Amplification of Cyanobacterial Phycocyanin (C-PC) gene: marker (M); 1-5 represents selected cyanobacterial isolates
Figure 11 Three-Dimensional structure of C-Phycocyanin gene: ribbon model (a) and Ball and stick model with 63 amino acid alpha chain (b)
Figure 12 Determination of conserved domains of the cyanobacterial phycocyanin gene sequence from Phycobilisome superfamily
Figure 13 Flowchart showing the multiple sequence alignment and determination of conserved domains of the cyanobacterial phycocyanin gene sequence.
Figure 14 Comparative sequence analysis of cyanobacterial phycocyanin gene with PDB database: 75% similarity with 1JBO (a) and 37% similarity with 3BRP (b)