Chapter-2

Review of Literature
Chapter II: Review of Literature

2.1 Cyanobacterial taxonomy: an overview

Algae are the important food link in the aquatic ecosystems. Being autotrophic and members of the first trophic level, their major role in water is to capture solar energy to drive the ecosystem. It is estimated that there are approximately 10 million species of algae from which only about 40,000 species have been reported. Among these, 1500 species belong to 150 genera of blue-green algae (Norton et al., 1996). Using data of algal species included in on-line taxonomic database ‘AlgaeBase’ Guiry (2012) estimated that there are 72,500 algal species, of which names for 44,000 have probably been published. Further, Guiry (2012) estimated 5000 described and 3000 undescribed cyanobacterial species though only 3300 described species of cyanobacteria are listed in AlgaeBase. However, till date (August, 2014) 4050 species of cyanobacteria are listed in AlgaeBase (http://www.algaebase.org).

Cyanobacteria occupy a twilight zone on the evolutionary scale, sharing the characters of gliding bacteria and higher plants. Researchers working on blue-green algae (cyanobacteria) have different approaches regarding their classification. The bacteriological approach put forward by Stanier et al. (1971) has been further supported by Rippka et al. (1979), Lewin (1989), Castenholz and Waterbury (1989), Castenholz (1992) and Oren (2004). The traditional approach to classify cyanobacteria favours their treatment along with other algae as plants (Geitler, 1932; Desikachary, 1959; Anagnostidis and Komárek, 1985, 1988, 1990). Therefore, cyanobacteria have been treated under the International Code of Botanical Nomenclature (ICBN) as Blue-green algae (Cyanophyta) and under the International Code of Bacteriological Nomenclature as Gram-negative oxygen evolving photosynthetic bacteria.

2.1.1 Botanical approach to classify blue-green algae (cyanobacteria)

Traditionally, cyanobacteria have been studied by botanists as algae, even after recognition of the bacterial nature of their cells. Moreover, their conspicuous though superficial resemblance to eukaryotic algae earned them the name “blue-green algae”. Although efforts have been made to classify cyanobacteria under various names in the past, the work of Gomont (1892) and Bornet and Flahault (1886) are considered the starting points for the taxonomy of blue-green algae (Rippka, 1988). Since then, there have been many attempts to classify blue-green algae in view of both new information gathered and shortcomings of the existing classification schemes (Anagnostidis and
Komárek, 1985). In the traditional classifications, blue-green algae have been placed in the phylum Cyanophyta which comprised the class Cyanophyceae. Traditional approach to classify blue-green algae was based on morphological and ecological traits, with Geitler (1932) being pioneer worker. This system of nomenclature and classification, with more or less unambiguously defined taxa, is still widely used, as it enables relatively easy identification of blue-green algae in natural samples by field Phycologists. Fritsch (1945) classified cyanobacteria on the basis of their morphology and mode of reproduction (Table 1).

**Table 1.** Classification of cyanobacteria according to Fritsch (1945).

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Status</th>
<th>Reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chroococcales</td>
<td>Chroococcaceae</td>
<td>Unicellular/Colonial, sometimes forming pseudofilamentous, no polarity</td>
<td>By cell division, endospore formation but not sporangia</td>
</tr>
<tr>
<td></td>
<td>Entophysalidaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chamaesiphonales</td>
<td>Cyanidiaceae</td>
<td>Unicellular/Colonial lithophytes/epiphytes exhibiting marked polarity</td>
<td>By endospores/exosposes</td>
</tr>
<tr>
<td></td>
<td>Chamesiphonaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dermocarpaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleurocapsales</td>
<td>Pleurocapsaceae</td>
<td>Uniform arrangement, heterotrichous forms, heterocyst lacking, no differentiation into trichome/filament.</td>
<td>Reproduction by endospores in sporangia</td>
</tr>
<tr>
<td></td>
<td>Hyellaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nostocales</td>
<td>Oscillatoriaceae</td>
<td>Non-heterotrichous filamentous forms, heterocyst commonly present, often showing false branching</td>
<td>Reproduction by hormogonia and akinetes</td>
</tr>
<tr>
<td></td>
<td>Nostocaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scytonemataceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microchaetaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rivulariaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stigonematales</td>
<td>Capsosiraceae</td>
<td>Heterotrichous filamentous forms mostly with heterocysts, showing true branching</td>
<td>Reproduction mostly by hormogonia and rarely by heterocysts and akinetes</td>
</tr>
<tr>
<td></td>
<td>Nostochopsisaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mastigocladopsidaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mastigocladaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stigonemataceae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A completely different concept was proposed by Drouet (1968, 1973, 1978, 1981), who presumed that the morphological diversity of blue-green algae is the result of diverse environmental conditions on the restricted number of genotypes. Therefore, he reduced dramatically the number of genera, but it turned out that he had strictly underestimated the existing genetic variability among cyanobacteria (Anagnostidis and Komárek, 1985; Castenholz, 1992). The main morphological features used in the taxonomy of blue-green algae are: (i) growth form- unicellular, colonial, filamentous;
(ii) compactness and shape of colonies; (iii) shape of filaments; (iv) sheath-presence, absence, shape; (v) cell differentiation—presence or absence of heterocysts and akinetes; (vi) size and shape of vegetative cells, heterocysts and akinetes; (vii) branching—presence or absence, false or true, when false "y" shaped or geminate; and (viii) nature of true branches: uniseriate or multiseriate. Combinations of these characters have been used for designing traditional classification of blue-green algae (Anagnostidis and Komárek, 1985).

The most recent exhaustive reorganization of cyanobacterial classification system under ICBN, based on morphological, ecological, genetic and ultrastructural information on both cultivated and uncultivated cyanobacteria, was made by Anagnostidis and Komárek (1985, 1988, 1990) and Komárek and Anagnostidis (1986, 1989, 2005). The four orders were recognized: Chroococcales, Oscillatoriales, Nostocales and Stigonematales, subdivided into families, subfamilies, genera and species (Table 2). An attempt has been made to reconcile the differences between botanical and bacteriological codes. Besides the taxonomic criteria based on the botanical code, when required even the information obtained from bacteriological methods including molecular markers was also used. Many objective features and characteristics have been defined for distinguishing various genera and species. The concomitant application of both ICBN and bacteriological code has resulted in proper classification of cyanobacteria (Garcia-Pichel et al., 1998; Sarma, 2013).

All unicellular and coccoid taxa have been classified in the single order, Chroococcales, with clearly distinguishable and definable families. In earlier classifications, Oscillatoriaceae has been placed under the order Nostocales but Anagnostidis and Komárek (1988) have revised cyanobacterial classification on the basis of some non-traditional features (type of cell division, occurrence of aerotopes, motility, type of trichome disintegration, etc.) and introduced a separate order Oscillatoriales. The species concept of the genera, Phormidium, Oscillatoria and Lyngbya (LPP-group A) (Rippka et al., 1979, 1981) has been revised by the introduction of some non-traditional features along with the traditional features already used for classification by various workers namely Geitler (1932), Elenkin (1936), Fritsch (1945), Desikachary (1959), Starmach (1966), Kondrateva (1975), Bourrelly (1979). Several traditional and recently defined genera, including Arthrospira, Borzia, Katagnymene, Porphyrosiphon, Pseudanabaena, Pseudoscytonema, Symploca and Trichodesmium have been accepted.
Table 2. Classification of cyanobacteria according to bacteriological (Bergey’s Manual of Systematic Bacteriology) and botanical systems (Komárek and Anagnostidis; Hoffmann, Komárek and Kaštovský).

<table>
<thead>
<tr>
<th>Bacterial system</th>
<th>Botanical system</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subsection I:</strong> unicellular or colonial, division by binary fission in 1 to 3 planes or by budding</td>
<td><strong>Chroococcales:</strong> unicellular or colonial Family Merismopediae Subfamily Gomphosphaeriaceae <em>Snowella, Woronichinia</em> Subfamily Merismopedioideae <em>Merismopedia</em> Family Microcystaceae <em>Microcystis</em> Family Synechococcaceae <em>Synechococcus</em></td>
<td><strong>Gloeobacteria:</strong> coccoid, lacking thylakoids <strong>Synechococcales</strong>*: thylakoids arrange parallel to cell surface, unicellular or colonial Family Merismopediae <em>Merismopedia</em> Family Synechococcaceae <em>Synechococcus</em></td>
</tr>
<tr>
<td>Form-genus <em>Microcystis</em> Form-genus <em>Synechococcus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subsection II:</strong> unicellular or colonial, division by multiple fission or in combination with binary fission</td>
<td><strong>Chroococcales</strong>1: radial arrangement of thylakoids, unicellular or colonial Family Gomphosphaeriaceae <em>Snowella, Woronichinia</em> Family Microcystaceae <em>Microcystis</em></td>
<td><strong>Oscillatoriales</strong>2: radial arrangement of thylakoids, large filamentous <strong>Pseudoanabaenales</strong>2: thylakoids arrange parallel to cell surface, thin filamentous Family Pseudanabaenaceae <em>Limnothrix</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subsection III:</strong> filamentous, nonheterocytous</td>
<td><strong>Oscillatoriales</strong>2: radial arrangement of thylakoids, large filamentous</td>
<td><em>Note: Orders belong to four subclasses, which are not presented in correct order in this table</em></td>
</tr>
<tr>
<td>Form-genus <em>Limnothrix</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subsection IV:</strong> filamentous, heterocytous, non-branching</td>
<td><strong>Nostocales:</strong> filamentous, heterocytous, akinetes, falsebranching Family Nostocaceae <em>Anabaena, Aphanizomenon</em></td>
<td><strong>Nostocales:</strong> filamentous heterocytous cyanobacteria Family Nostocaceae <em>Anabaena, Aphanizomenon</em></td>
</tr>
<tr>
<td>Form-genus <em>Anabaena</em> Form-genus <em>Aphanizomenon</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subsection V:</strong> filamentous, heterocytous, branching</td>
<td><strong>Stigonematales:</strong> filamentous, heterocytous, akinetes, true-branching genera</td>
<td></td>
</tr>
</tbody>
</table>

*Orders Chroococcales and Oscillatoriales form subclass Oscillatoriophycideae
Orders Synechococcales and Pseudoanabaenales form subclass Synechococcophycidae
Nine new genera (*Hormoscilla, Jaaginema, Komvophoron, Leptolyngbya, Limnothrix, Planktothrix, Pseudophordium, Tychonema*) were established in this order. Genus *Blennothrix* Kutz. 1843 was also validated (Anagnostidis and Komárek, 1988). The new families Pseudanabaenaceae and Phormidiaceae were defined. In the order Nostocales, no major change was introduced except separating oscillatorian members from it and inclusion of a new genus *Trichormus* (Komárek and Anagnostidis, 1989). This genus was separated from the genus *Anabaena* with respect to apoheterocytic origin of akinetes. The order Nostocales was divided into four families. Anagnostidis and Komárek (1990) classified order Stigonematales into eight families with one new family Fischerellaceae.

Later on Komárek and Anagnostidis (1998) revised classification in the order Chroococcales at family and subfamily level and introduced changes. Eleven, instead of seven families were recognized- Gloeobacteraceae, Synechococcaceae, Merismopediaceae, Microcystaceae, Chroococcaceae, Entophysalidaceae, Hydrococcaceae, Chamaesiphonaceae, Dermocarpellaceae, Xenococcaceae and Hyellaceae. Families Synechococcaceae, Merismopediaceae, Entophysalidaceae and Hyellaceae have been divided into two subfamilies each. Aphanothecoideae and Synechococcoideae are subfamilies of the family Synechococcaceae. Merismopediaeae and Gomphospherioideae are subfamilies of Merismopediaceae. Entophysalidoideae and Siphonematoideae are subfamilies of the family Entophysalidaceae. Podocapsoideae and Hyilloideae are subfamilies of the family Hyellaceae.

Komárek and Anagnostidis (2005) on the basis of large number of reports, further implemented changes in the order Oscillatoriales. Order Oscillatoriales has been divided into six families- Pseudanabaenaceae, Schizothrichaceae, Borziaceae, Phormidiaceae, Gomontiellaceae and Oscillatoriaceae. Family Pseudanabaenaceae is further divided into four subfamilies- Pseudanabaenoidae, Spirulinoideae, Leptolyngbyoideae and Heteroleibleinioideae. Family Phormidiaceae is divided into three subfamilies- Phormidioidae, Microcoleoideae and Ammatoideoideae. Family Gomontiellaceae is divided into three subfamilies- Hormoscilloideae, Crinalioideae and Starrioidae. Family Oscillatoriaceae is divided into two subfamilies- Oscillatorioideae and Homoeotrichoideae.
2.1.2 Bacteriological approach to classify cyanobacteria (blue-green algae)

Based on the recommendations of the subcommittee on Phototrophic Bacteria of the International Committee on Systematic Bacteriology (ICSB) of the International Association of Microbiological Societies (IAMS), the cyanobacteria have been included under the I Division followed by the bacteria in the II Division in the eighth edition of Bergey’s Manual of Determinative Bacteriology (Buchana and Gibbons, 1974). The bacterial nature of the “blue-green algae” made Stanier et al. (1978) to put forward a proposal for their integration under the Bacterial Code of Nomenclature. Stanier et al. (1978) and Rippka et al. (1979) challenged the botanical approach since cyanobacteria are prokaryotes. This concept was realized in the classification system of Rippka et al. (1979) and Rippka (1988), which is based on both phenotypic and genotypic characters, restricted to the isolates under culture. Rippka et al. (1979) divided cyanobacteria into five sections. In Section I and Section II members belonging to order Chroococcales, Chamaesiphonales and Pleurocapsales have been redistributed as suggested in the Botanical Code. Section III consists of entirely non-heterocystous filamentous cyanobacteria, and Section IV and V comprised heterocystous, unbranched, and branched representatives, respectively (Table 2). The limitation of this conception is that it ignores most of the cyanobacterial diversity found in nature that immediately evoked protests of the ecologically oriented researchers (Golubić, 1979).

The scheme proposed by Rippka et al. (1979) was adopted and modified in Bergey’s Manual of Systematic Bacteriology (Boone and Castenholz, 2001). The cyanobacteria constitute a monophyletic group within Eubacteria and are closely related to purple bacteria and chlorophyll-\(b/a\) containing Prochlorales (Prochlorophyta) (Castenholz, 2001a). Gibbons and Murray (1978) suggested to treat cyanobacteria integrated with photobacteria under the order ‘Cyanobacteriales’. The name of the order was not based on a designated name of genus or species. Although special request was made by these workers to the Judicial Commission of International Committee on Systematic Bacteriology, their proposal was not accepted (Holt, 1979). In order to pave the way for the creation of this order under Bacteriological Code, Rippka and Cohen-Bazire (1983) created two genera Cyanobium and Cyanobacterium. The type species of Cyanobacterium as Cyanobacterium stanieri (strains PCC7202; ATCC29140) fulfilled the condition for
creation of the order Cyanobacterales under the Bacteriological Code. One of the intriguing aspects of this classification is the recognition of ‘form genera’ a term that has no standing either under the International Code of Botanical Nomenclature (ICBN) or Bacteriological Code (Oren, 2004). The concept of ‘form species’ has also been questioned by Whitton and Potts (2000). The number of validly published cyanobacterial genera under the Bacteriological Code is only 6 and names of 13 species have been proposed with the validation lists published by the International Journal of Systematic and Evolutionary Microbiology or International Journal of Systematic Bacteriology (Sarma, 2013).

Cavalier-Smith (2002) classified all bacteria into the Kingdom Bacteria. The ancestors for the evolution of cyanobacteria have been identified as Negibacteria (Sub-Kingdom I) in which two Infrakingdoms Ecobacteria (Infrakingdom I) and Glycobacteria (Infrakingdom II) have been recognized. Cyanobacteria are included in Division I that has been divided into two Sub-divisions, Gloeobacteria (Sub-division I; with the only known genus Gloeobacter having phycobilisomes but without thylakoids) and Phycobacteria (Sub-division II; consisting of all the traditional blue-green algae and Prochlorophytes). He formally validated all the five traditional cyanobacterial orders (valid under ICBN) under the Bacteriological Code under two classes; Class I: Chroobacteria classified into three orders Chroococcales, Pleurocapsales and Oscillatoriales, and Class II: Hormogoneae, divided into two orders Nostocales and Stigonematales.

2.1.3 Recent developments in taxonomy of cyanobacteria

The state of cyanobacterial systematics is still very complex (Komárek, 2006). The system of Castenholz (2001b) is based on the bacteriological code, while that of Komárek and Anagnostidis (1998, 2005) is mainly based on the rules of the botanical code of nomenclature. A proposal had been made for the formation of the consensus nomenclature that would be acceptable to both bacteriologists and botanists (Oren, 2004). The worst aspect of the problem probably is that many researchers do not use formal nomenclature prescriptions of either of the Codes, not to mention a common habit of assigning arbitrary names from old unrevised literature to the strains and problem of incorrect taxonomic identification (Komárek, 2006).

Recently, botanical and bacteriological approaches of classifying cyanobacteria seem to be converging; Botanical classifications have started using
genetic information in addition to morphological, cytological, ecological, and biochemical features of cyanobacteria (Hoffmann et al., 2005; Komárek and Anagnostidis, 2005). Botanical nomenclature has been used in bacteriological classification, and the division of phylum Cyanobacteria into subsections mirrors the orders used in botanical classification (Table 2). Nevertheless, the nomenclature by these two classification systems differs, despite several proposals for their unification (Oren, 2004; Oren and Tindall, 2005; Hoffmann et al., 2005). In addition, an isolated, living, pure culture of each described species is required in the bacteriological code, whereas preserved specimens together with microphotographs or drawings are preferred in the botanical code (Oren, 2004; Oren and Tindall, 2005).

Hoffmann et al. (2005) proposed a revision of the cyanobacterial classification under the botanical code on polyphasic approach by taking into account morphological, ultrastructural, ecological and genetic relationships mainly based on 16S rRNA gene sequences. This was an attempt to merge the Botanical as well as Bacteriological codes in the classification of cyanobacteria. The cyanobacteria are given the rank of a class designated as Cyanoprokaryota with three subclasses Synechococcineae, Oscillatorineae and Nostocineae. The Synechococcineae with two orders: Synechococcales and Pseudanabaenales include coccoid and filamentous forms, respectively. The second subclass Oscillatorineae comprises coccoid (Chroococcales) and filamentous forms (Orders Phormidiales and Oscillatoriales). The unification of heterocystous cyanobacteria into one subclass Nostocineae with single order Nostocales is a unique feature of this classification. The inclusion of Prochlorophyta in cyanobacterial classification system and the absence of distinction into coccoid and filamentous forms at the highest subclass level are the other highlights of this classification. The distinction into subclasses is based on the arrangement of thylakoids and the presence of differentiated cells. Chroococcales with unicellular or colonial members are characterized by radial arrangement of thylakoids. The non-heterocystous filamentous forms have been divided into three orders based on the nature of filaments and arrangement of thylakoids. Oscillatoriales included filamentous forms with radial arrangements of thylakoids while Pseudanabaenales consists of thin filamentous forms in which thylakoids are arranged parallel to the cell surface. The order Nostocales possesses filamentous, heterocystous, unbranched and branched members (Table 2).
The modern system of cyanobacteria must be based on the molecular definition of genotypes (basic clusters with a similarity index of ±95% or less using the 16S rRNA gene sequencing, considered the standard method), which correspond to the traditional taxonomic category of ‘genus’. Obligatory separation of genera must be done also by at least one diacritical phenotypic character (or autapomorphic set of characters) and ecological, ecophysiological, ultrastructural and biochemical characteristics, which are included as an integral part of the generic definition (Komárek et al., 2008). This revised system confirms in principle the traditional cyanobacterial genera, which must be continually corrected and updated (Hoffmann et al., 2005). This revised system justifies the definition of numerous new generic entities, which have arisen through genetic (and morphological) separation of existing genera, and/or are described from newly studied habitats, extreme ecosystems or by revision of cultured material (Komárek et al., 2008).

2.1.3.1 Molecular methods used in taxonomy of cyanobacteria

During the last two decades, molecular methods have become an essential tool for characterization of organisms and assessment of evolutionary relationships. Analysis of DNA/protein sequences and other molecular markers have become key methods for understanding the evolution of organisms. A similar trend evolved in the molecular systematics and population genetics of cyanobacteria (Giovannoni et al., 1988; Boyer et al., 2001; Castenholz, 2001b; Komárek, 2010).

2.1.3.1.1 PCR independent approach

The PCR independent approach includes guanine plus cytosine (G+C) content estimation, nucleic acid reassociation and hybridization and DNA microarrays. Differences in the guanine plus cytosine (G+C) content of DNA have been used to study the bacterial diversity of soil communities. It is based on the fact that microorganisms differ in their G+C content and taxonomically related groups only differ between 3 and 5% (Tiedje et al., 1999). Baudouin-Cornu et al. (2004) have examined the G+C content of planktomic species of *Anabaena* and attested to its taxonomic significance, all the cyanobacterial strains assigned to *Anabaena* species showed similar DNA base composition. In spite of its fundamental role in determination of bacterial species (Wayne et al., 1987), DNA-DNA hybridization has been rarely used for cyanobacteria (Stulp and Stam, 1984; Otsuka et al., 2001; Suda et al., 2002). The similarity values obtained by DNA-DNA hybridization do not reflect
the actual degree of sequence similarity. The phylogenetic relationships cannot be
determined by this method for strains with more than 20% divergence in genome
sequence (Rosselló-Mora and Amann, 2001). Study of allozymes (Stulp and Stam,
1984; Kato et al., 1991; Nishihara et al., 1997) or the whole-cell protein analyses
(Palinska et al., 1996; Lyra et al., 1997) are rarely used.

2.1.3.1.2 PCR-based approaches

The direct sequencing of various genes is the most common molecular method used in
cyanobacterial taxonomy. However, Restriction Fragment Length Polymorphism
(RFLP) is commonly applied, especially for detailed assessment of the genetic
variability of closely related taxa (Ernst et al., 1995; Postius et al., 1996; Bolch et al.,
1996; Lyra et al., 1997; Bolch et al., 1999; Scheldeman et al., 1999; Comte et al.,
2007) or to infer the extent of cyanobacterial diversity in nature (Lu et al., 1997;
Frias-Lopez et al., 2003; Kim et al., 2004). Randomly amplified polymorphic DNA
(RAPD) analysis is sometimes used in order to discriminate between genotypes of
close relatives (Neilan, 1995; Nishihara et al., 1997; Bolch et al., 1999; Casamatta et
al., 2003). Genomic characteristics, such as the presence and structure of tandem
repeats (Asayama et al., 1996; Lyra et al., 1997, 2005; Rasmussen and Svenning,
1998; Chonudomkul et al., 2004) or of a whole gene family (Bhya et al., 2002), have
also been shown to possess some discriminatory power on various taxonomic levels.

2.1.3.1.3 Molecular markers

Non-coding gene sequences

Various gene sequences have been used for molecular phylogenetics of the
cyanobacteria, and the 16S small subunit ribosomal RNA gene (16S rDNA) is by far
the most common gene (Neilan et al., 1997; Giovannoni et al., 1988; Honda et al.,
1999; Turner et al., 1999; Iteman et al., 2000; Ishida et al., 2001; Litvaitis, 2002;
Gugger and Hoffman, 2004). 16S rRNA gene has all the characteristics of a
phylogenetic marker gene due to the universal distribution in prokaryotes, presence of
variable and conserved regions and high information content (Woese, 1987; Ludwig
and Klenk, 2001). The significance and expansion of 16S rRNA gene can be judged
from the statistics for 16S rRNA sequences deposited in GenBank
(http://www.ncbi.nlm.nih.gov/) and processed by RDP (Ribosomal Database Project
http://rdp.cme.msu.edu/). The RDP database provides cohesive and regularly updated
ribosomal gene related data, mainly 16S rRNA gene sequences of bacteria and
archaea (Cole et al., 2009). Based on large number of 16S rDNA gene sequences available in NCBI GenBank, a modern molecular cyanobacterial classification is now available in the NCBI Gene Bank (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi) (Table 3). The conserved nature of 16S rRNA gene has been used for cyanobacterial phylogenetic studies (Giovannoni et al., 1988; Nelissen et al., 1996; Nubel et al., 1997; Honda et al., 1999; Turner et al., 1999; Willmotte and Herdman, 2001; Gugger and Hoffmann, 2004; Rajaniemi et al., 2005; Tomitani et al., 2006). Although the 16S rRNA gene contains variable regions, it is well conserved for resolving species identity or intraspecies variations (Woese, 1987; Fox et al., 1992; Ward et al., 1992).

Another useful marker used in cyanobacterial taxonomy is 16S rRNA gene-23S rRNA gene internal transcribed spacer (ITS) region. 16S-23S ITS region is variable in sequence, length and secondary structure (Gugger et al., 2002), though, a few conserved regions can also be identified (Iteman et al. 2000, Boyer et al., 2001). 16S-23S ITS region usually contains both tRNA^{Ile} and tRNA^{Ala} genes (Williamson and Doolittle, 1983), though there are several instances of 16S-23S ITS with either only tRNA^{Ile} gene (Nelissen et al., 1994) or completely without tRNA genes (Iteman et al., 2000; Boyer et al., 2001; Taton et al., 2003; Taton et al., 2006b). 16S-23S ITS region has been successfully used to distinguish the closely related species of *Arthrospira* (Scheldeman et al., 1999; Baurain et al., 2002; Ballot et al., 2004), *Phormidium* (Comte et al., 2007), *Microcystis* (Otsuka et al., 1999) and *Synechococcus* (Ernst et al., 2003; Becker et al., 2004). In addition, it has been used to address some questions concerning biogeography of the cyanobacteria (Papke et al., 2003; Gugger et al., 2005; Taton et al., 2006a).

**Protein-coding gene sequences**

Various protein coding gene sequences have also been used for inferring phylogenies in the cyanobacteria (Bolch et al., 1996; Zehr et al., 1997; Seo and Yokota, 2003; Gugger et al., 2005; Lyra et al., 2005; Rajaniemi et al., 2005). Palys et al. (1997, 2000) reported that some protein coding genes should be considered as the primary criterion for demarcating bacterial taxa since protein-coding genes evolve faster than 16S rDNA, thereby providing better resolution between bacterial species. However, there is only one 16S rRNA gene and the proteins are coded by many genes, so it is possible that a different set of protein coding genes may be used for species demarcation of different groups of bacteria (Gevers et al., 2005).

<table>
<thead>
<tr>
<th>Order</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chroococcales</td>
<td>Acaryochloris; Aphanocapsa; Aphanothece; Chamaesiphon; Chlorogloea;</td>
</tr>
<tr>
<td></td>
<td>Chondrocystis; Chroococcus; Chroogloeocystis; Coelosphaerium;</td>
</tr>
<tr>
<td></td>
<td>Crocosphaera; Cyanobacterium; Cyanobium; Cyanocystis; Cyanodictyon;</td>
</tr>
<tr>
<td></td>
<td>Cyanosarcina; Cyanothece; Dactylococcopsis; Geminocystis; Gloecapsa;</td>
</tr>
<tr>
<td></td>
<td>Gloecapsopsis; Gloeotheca; Halamuthe; Johannesbaptistia; Limnoscus;</td>
</tr>
<tr>
<td></td>
<td>Merismopedia; Microcystis; Radiocystis; Rhabdoderma; Rubidibacter;</td>
</tr>
<tr>
<td></td>
<td>Snowella; Sphaerocavum; Synechococcus; Synechocystis; Thermosynechococcus; Woronichinia; unclassified Chroococcales; environmental samples</td>
</tr>
<tr>
<td>Gloeobacterales</td>
<td>Gloeobacteria; environmental samples</td>
</tr>
<tr>
<td>Nostocales</td>
<td>Calochaete; Camptylonema; Camptylonemopsis; Coleodesmium; Fortiea;</td>
</tr>
<tr>
<td></td>
<td>Godleya; Hassallia; Microcachae; Petalonema; Raxia; Spirirestis;</td>
</tr>
<tr>
<td></td>
<td>Tolypothrix; Anabaena; Anabaenopsis; Aphanizomenon; Aulosira;</td>
</tr>
<tr>
<td></td>
<td>Cuspidothrix; Cyanospira; Cylindrospermopsis; Cylindrosporum;</td>
</tr>
<tr>
<td></td>
<td>Desmonostoc; Dolichospermum; Hydrocoryne; Mojavia; Nadularia; Nostoc;</td>
</tr>
<tr>
<td></td>
<td>Raphidiopsis; Richelia; Sphaerothricopsis; Trichormus; Wollea;</td>
</tr>
<tr>
<td></td>
<td>Rivulariaeae; Calothrix; Dichothis; Gleeocichia; Rivularia; Brasilonema; Chakia; Scytonema; Scytonematopsis; Umezakia; uncultured Nostocales cyano bacterium</td>
</tr>
<tr>
<td>Oscillatoriales</td>
<td>Arthrionema; Arthrospera; Bleonothrix; Crinalium; Geitlerinema;</td>
</tr>
<tr>
<td></td>
<td>Halomicronema; Halospirulina; Hormosicila; Hydrocoleum; Jaaginema;</td>
</tr>
<tr>
<td></td>
<td>Katagnymene; Komvophoron; Leptolyngbya; Limnothrix; Lyngbya; Microcoleus; Oscillatoria; Phormidesmis; Phormidium; Planktothricoides; Planktothrix; Plectolyngbya; Plectonemia; Pseudanabaena; Pseudophormidium; Pseudooscillatoria; Pseudoscytonema; Romeria; Schizothrix; Spirulina; Starria; Symплоca; Tapinotherix; Trichocoleus; Trichodesmium; Tychonema; Wilmottia; unclassified Oscillatoriales; environmental samples</td>
</tr>
<tr>
<td>Pleurocapsales</td>
<td>Chroococcidiopsis; Chroococcus; Dermocarpa; Dermocarpella; Hyella; Myxosarcina; Pleurocapsa; Solentia; Stanteria; Xenococcus; environmental samples</td>
</tr>
<tr>
<td>Stigonematales</td>
<td>Capsosira; Chlorogloeopsis; Fischerella; Hapalosiphon; Iphione;</td>
</tr>
<tr>
<td></td>
<td>Loriellopsis; Mastigocladosis; Mastigocladus; Mastigocoleus; Nostochopsis; Stigonema; Symphyonema; Symphyonemopsis; Westiellopsis; unclassified Stigonematales; environmental samples</td>
</tr>
<tr>
<td>Prochlorales</td>
<td>Prochloraceae; Prochlorococcaceae; Prochlorotrichaceae; unclassified Prochlorales; environmental samples</td>
</tr>
</tbody>
</table>
The protein-coding genes examined in cyanobacteria encompass those for DNA-dependent RNA polymerase- rpoB, rpoC and rpoD (Palenik and Swift, 1996; Seo and Yokota 2003; Gugger et al., 2005; Rajaniemi et al., 2005; Lyra et al., 2005; Everroad et al., 2006), Rubisco large subunit and/or chaperonin-like protein X- rbcL and rbcX (Rudi et al., 1998; Gugger et al., 2002; Shimada et al., 2003; Lyra et al., 2005; Rajaniemi et al., 2005; Tomitani et al., 2006), various regions of the phycocyanin operon (Bolch et al., 1996; Manen and Falquet, 2002; Crosbie et al., 2003; Ballot et al., 2004; Liu et al., 2005; Premanandh et al., 2006; Abed et al., 2006), nitrogenase complex- nifD, nifK and nifH (Kallas et al., 1985; Ben-Porath et al., 1993; Ben-Porath and Zehr, 1994; Zehr et al., 1997; Henson et al., 2002; Henson et al., 2004; Gugger et al., 2005; Abed et al., 2006), regulatory genes hetR of heterocyst differentiation (Janson et al., 1999; Carpenter and Janson 2001; Lundgren et al., 2005; Tomitani et al., 2006), RNase P RNA gene- rnpB (Vioque, 1997; Schon et al., 2002), DNA gyrase subunit B- gyrB (Seo and Yokota, 2003), PSII reaction centre protein D1 gene- psbA (Hess et al., 1995), elongation factor Tu gene- tufA (Delwiche et al., 1995), microcystin-coding gene- mcyA (Hisbergues et al., 2003; Yoshida et al., 2005; Rinta-Kanto et al., 2006), nodularin synthetase subunit F gene- ndaF, intergenic spacer between gas vacuole protein A genes- gvpA- IGS (Barker et al., 1999; Lyra et al., 2005), hoxH (Zhang et al., 2005) and phycoerythrin intergenic spacer region (Abed et al., 2006).

**Multilocus Sequence Typing**

A combination of several molecular markers should be used for adequate phylogenetic resolution. MLST (Multilocus Sequence Typing) was developed for typifying pathogenic bacterial strains (Maiden et al., 1998). Generally, MLST requires use of several housekeeping genes (ribosomal operon, circadian genes, and cytochrome b6 etc.) together. This approach was also found suitable for cyanobacteria. Lodders et al. (2005) studied the population structure and recombination of *Microcoleus (Coleofasciculus) chthonoplastes* based on MLST. Similarly, Acinas et al. (2009) studied Spanish and Baltic populations of *Pseudanabaena* strains and confirmed multilocus approach suitable for cyanobacteria. Regardless of the fact that there are an increasing number of cyanobacterial genomes available for searching the genetic divergence among related strains, the multi-gene analysis species is still an effective approach to determine the phylogenetic relationship and molecular diversity (Wu et al., 2011).
By application of the above molecular markers, taxonomy and phylogeny of cyanobacteria (Anabaena, Aphanizomenon, Microcystis, Microcoleus, Oscillatoria, Phormidium, Synechococcus etc.) have been investigated based on cultures and field samples. In many cases correlation has been drawn between cultures and field samples.

**Phylogenomics**

The growing number of sequences of cyanobacterial genomes in public databases, provides taxonomy and phylogeny new possibilities, but also with new challenges. Currently (June, 2014), 86 completely sequenced cyanobacterial genomes are available, (NCBI, National Centre for Biotechnology Information). Estimated genome sizes vary from $1.6 \times 10^6$ base pairs in unicellular taxa to $8.6 \times 10^6$ bp in filamentous cyanobacteria (Herdman *et al.*, 1979). With the increasing availability of genomic data and the computational power to easily compute phylogenetic trees for thousands of genes and thousands of taxa, choosing suitable loci for phylogenetic analyses is no longer limited by an artificial number of loci feasible for an analysis. Identifying suitable genes, which are present across all taxa and which represent the underlying organismic evolution, is a crucial step prior to an analysis (Stamatakis *et al.*, 2007). Schirrmeister *et al.* (2011a) constructed a phylogenetic tree based on 16S rRNA gene sequences of 1254 taxa and suggested that cyanobacteria may share a common ancestor, which was probably unicellular cyanobacteria. Phylogenetic tree, indicated that *Gloeobactor violaceus*, which has light harvesting system at the outer membrane of the cell rather than internal thylakoids, is the nearest living organism to that ancestor. Schirrmeister *et al.* (2011a) stated that evolution in cyanobacteria is not a gradual transition from simple unicellular to more complex multicellular forms as often assumed. Instead, complexity was lost several times and regained at least once. Phylogenomic approaches would be helpful to understand the genetic basis of these transitions. This will enable us to identify and reconstruct the evolution of a wide set of genes that have been responsible for morphological and biochemical adaptations in this phylum (Schirrmeister *et al.*, 2011b).

**2.1.3.2 Polyphasic approach for the cyanobacterial taxonomy**

The correct identification of microorganisms is essential to study their diversity. The relatively small size and non-distinctive appearance of microorganisms render microscopic differentiation of population (Ward *et al.*, 1998). The reliance upon
culture characteristics for identification may not provide then accurate description of microorganisms as they occur in natural habitats (Ward et al., 1992). The cultivation of microorganisms, however, is also essential for thorough characterization, understanding physiology and genetics, though inadequate culture conditions in many cases can lead to loss of morphological characteristics which make it difficult to apply taxonomic assignments based on culture characteristics to field populations (Castenholz and Waterbury, 1989; Wilmotte, 1994; Garcia-Pichel et al., 1996). The bacteriological approach and traditional botanical approach rely primarily on morphological characteristics of cell(s) and colonies and do not necessarily lead to the identification of phylogenetically coherent taxa (Wilmotte and Golubic, 1991; Castenholz, 1992). Notwithstanding the unreliability of traditional morphological criteria, some cytomorphological and ultrastructural characters were found to correlate well with molecular data. This concerns e.g. the cell division type (Palinska et al., 1996, Casamatta et al., 2003, Komárek et al., 2004) and especially the thylakoid arrangement, which seems to have substantial taxonomic value (Komárek and Caslavaska, 1991; Komárek and Kaštovský, 2003). Some other traits, such as constriction-patterns in the cell wall of cyanobacteria may prove useful on certain taxonomic levels (Palinska and Krumbein, 2000).

Komárek (2006) stated that application of modern ecological, ultra structural and molecular methods, aided by the cultivation of numerous cyanobacterial morphotypes, has substantially changed our knowledge of these organisms. It has led to major advances in cyanobacterial taxonomy and criteria for their phylogenetic classification. Molecular data provide basic criteria for cyanobacterial taxonomy; however, a correct phylogenetic system cannot be constructed without combining genetic data with the knowledge from nearly 150 years of research on cyanobacterial diversity based on morphological studies (Komárek, 2011). Thus, studies on morphological variations in naturally growing microorganisms, and modern molecular, ultrastructural, ecophysiological and biochemical characters need to be combined in a “polyphasic” approach. This approach is currently the most popular choice for classifying cyanobacteria. The genera which were previously placed under invalid taxa have now been resolved into new genera or species. However, some work has been done on the cytomorphological and polyphasic characterization of chroococcalian "Synechocystis", "Synechococcus" and a few other unicellular strains (Komárek et al., 2004) as well as of heterocystous Aphanizomenon, Anabaena and
The studies on filamentous "Phormidium" and "Oscillatoria" genera are few (Pfeiffer and Palinska, 2002; Casamatta et al., 2003; Teneva et al., 2005; Marquardt and Palinska, 2007). Although there is often no correlation between morphological and molecular traits, especially for taxa with very simple morphology (Wilmotte et al., 1992; Lee and Bae, 2001; Margheri et al., 2003), some morphologically well defined genera were shown to be monophyletic. These include Microcystis (Neilan et al., 1997; Otsuka et al., 1998), Arthrospira (Nelissen et al., 1994; Manen and Falquet, 2002; Zhang et al., 2005), Planktothrix (Suda et al., 2002) or marine Trichodesmium species (Ben-Porath et al., 1993; Abed et al., 2006).

Johansen and Casamatta (2005) give a concept for cyanobacterial species, a species as the smallest monophyletic group which can be delimited by recognizable morphology. They suggested following practical criteria for defining cyanobacterial species using “polyphasic approach”: (1) characterizing morphological differences, (2) genetic distances in 16S rRNA sequences, (3) differences in 16S-23S ITS secondary structures, (4) biochemical dissimilarity (composition of secondary metabolites), and (5) ecophysiological characteristics predominantly defined by biotope of studied strains.

### 2.2 Studies on cyanobacterial diversity: Indian scenario

India is one of the mega diversity centers and a home of 3 out of 34 reserved hotspots of the world (Myers, 1988; Myers, 2003; Ebert, 2005). Although India has only 2.4% of the total land area of the world yet it has a wide range of geographical and climate conditions (Singh, 2002). It has unique geological history, highly diverse physiography, monsoon climate, extremes of temporal and spatial variability, and high biotic diversity. India is also endowed with equally diverse aquatic habitats.

Freshwater cyanobacteria have been reported from sea level to high altitudes. A large number of reports on cyanobacterial diversity of freshwater are available. Cyanobacteria also occupy a variety of terrestrial environments, particularly in moist or waterlogged habitats. Paddy fields constitute one of the important habitats of cyanobacteria as these provide a favourable environment for growth of these organisms with respect to their requirement for light, water, temperature and nutrient availability. Montagne (1849) described the first blue-green alga Calothrix indica from Assam. Kurz’s work done in 19th century contributed significantly to our
knowledge of algae from India. Kurz’s specimens were worked out by different workers and form the matter of number of publications (Krempelhuber, 1869, Martens, 1870a, b, 1871a, b; Zeller, 1873a, b). Kirtikar (1886) was the first Indian to record any algae from India. After that Ghose (1919, 1923, 1926, 1927a-e, 1931) published a series of papers which have vastly contributed to our knowledge of Indian blue-green algae. Bharadwaja (1928) made series of contributions on the Indian blue-green algae. His studies on mode of branching in Scytonema and Tolypothrix were based on his own collections and those by Prof. Iyengar’s and Prof. Fritsch’s stand foremost (Bharadwaja, 1933, 1934). Bharadwaja (1935) and his students (Singh, 1939a, b, 1942a, b; Singh, 1941; Rao, 1935, 1936, 1937a, b, 1938b, c, 1947; Rao, 1939, 1940; Parukutty, 1939, 1940) added to knowledge on the flora of India especially of the many parts of the north India. Further additions had been made by Dixit (1936), Gonzalves and Joshi (1943a, b), Gonzalves and Gangla (1949) by publishing an account on blue-green algae of Bombay.

Iyengar who is regarded as the ‘Father of modern algology of India’, started his work in 1920. Assisted by his students Balakrishana, Desikachary, Kanthammma, Ramanathan and Subrahmanian, he described a number of new species and genera and worked out life histories of many Indian algae. Publications of Iyengar and Desikachary (1944, 1946a, b; 1953, 1954) have extended our knowledge about the blue-green algae of South India. Considerable and significant work had been done by Desikachary (1959) on the blue-green algal flora of India by writing a comprehensive monograph on the Indian Cyanophyta. Other important contributions on the floristics of blue-green algae are those of Pandey (1965), Tiwari (1972, 1975), Laloroya and Mitra (1973), Tiwari and Pandey (1976), Prasad and Mehrotra (1980), Anand and Shanthakumar Hopper (1987), Anand (1989), Pattanaik and Adhikary (2002).

Other reports from the various parts of India include freshwater algal flora from the different parts of country by Prasad (1962); Punjab (Pandhol, 1974; Grover and Pandhol, 1975; Sarma and Kanta, 1978; Gupta and Singh, 2003; Dhingra and Ahluwalia, 2009; Singh et al., 2009a,b), Jammu and Kashmir (Khan, 1957; Subba Raju, 1963; Anand, 1979; Goyal et al., 1984; Ara et al., 2002), Himachal Pradesh (Kamat, 1968; Sidhu and Ahluwalia, 2011), Uttarakhand (Singh, 1959; Khan and Mathur, 1976; Chaturvedi and Habib, 1995; Habib, 2001), West Bengal (Bruehl and Biswas, 1922; Sinha and Mukherjee, 1975; Chatterjee and Chatterjee, 1983; Santra and Pal, 1992; Sen and Gupta, 1998; Singh et al., 2001; Chatterjee and Keshri, 2005),
2.3 Cyanobacterial diversity in hot water springs

Hot water springs are widely distributed although the greatest concentration of these occurs in the Yellowstone Plateau of North America, the North Island of New Zealand, Iceland, Japan, line of Andes, Italy, Algeria-Tunisia, Greece, Turkey, portions of Central Africa, India, Central Asia, Indonesia, and Philippines (Castenholz, 1969; Brock, 1978). The microorganisms that inhabit hot water springs are adapted to extreme conditions markedly different from the surrounding environment from which their ancestors might have dispersed (Papke et al., 2003). Thermophilic cyanobacteria generally exist in the range of water temperature 45 °C - 74 ºC (Castenholz, 1969, 1973; Brock, 1978). Setchell (1903) suggested the upper limit of temperature for cyanobacteria as 65 °C to 68 ºC and Lemmermann (1970)
described it as 69 °C. Their ancestors were possibly the oldest primary producer organisms in the distant past, and they perhaps used hot water springs as refugia (Gold, 1992, 1999; Plescia et al., 2001; Adhikary, 2006; Izagiurre et al., 2006; Hindák, 2008).

2.3.1 Microbial mats in hot water springs

Microbial mats in hot springs commonly occur as gelatinous or calcareous mats of several centimeters in thickness of varied colours. The top most layer of these mats is generally composed of photosynthetic cyanobacteria and orange, yellow, red or flesh-colour, usually of filamentous bacteria make up the remaining layers (Castenholz, 1969; Ward and Castenholz, 2000). Cyanobacterial mats in geothermal springs are often exposed to high light intensity. As an adaptation, some cyanobacteria produce extracellular, UV-screening pigments such as scytonemin. This inducible pigment is known to be synthesized upon exposure to high levels of UV-A irradiance and protects cells from those short wavelengths (Garcia-Pichel and Castenholz, 1991; Ehling-Schulz and Scherer, 1999; Dillon and Castenholz, 1999; 2003). Carotenoids present in all cyanobacteria are indirectly involved in protection from high solar irradiance by their ability to quench various forms of reactive oxygen or by inhibiting production of these molecules (Sheridan, 2001).

2.3.2 Composition of cyanobacterial mats

Most of research on thermophilic microbial community composition has been focused on two hot spring systems in USA: the Hunter’s hot spring in Oregon and the Octopus hot spring in Yellowstone National Park. Cyanobacterial mats at 54-55 °C in Hunter’s spring are composed of green top layer of Synechococcus lividus, dark red-brown layer of Oscillatoria cf. terebriformis (Castenholz, 1968). Octopus Spring cyanobacterial mats at ~57 °C are made up of Phormidium and Synechococcus and mats dominated with Phormidium usually turn orange during summer (Ward and Castenholz, 2000). Rod-shaped Synechococcus spp. and filamentous green non-sulfur bacterium Chloroflexus spp. have been also reported from these mats (Ward et al., 1998, 2002, 2006). Microbial mat from Yellowstone National park was found to support Synechococcus, Phormidium, Pseudanabaena and Spirulina like cyanobacteria and this was more diverse than their higher temperature (60-80 °C) counterparts (Norris et al., 2002).
Roeselers et al. (2006) have studied Arctic hot springs in Greenland, and revealed that microbial mats are dominated by heterocystous and non heterocystous filamentous cyanobacterial species. McGregor and Ramussen (2008) described cyanobacterial composition of microbial mats from alkaline thermal spring issuing at 43 - 71 °C from tropical north-eastern Australia. Eight genera and 10 species belonging to three cyanobacterial orders were identified based on morphological characters from these hot water springs. Biogeographic study of thermophilic cyanobacteria from Zerka Ma’in hot water springs of Jordan reported that phylotypes belonging to Thermosynechococcus, Chroogloeocystis, Fischerella (Mastigocladus) and Scytonema were dominant, and Synechococcus and Mastigocladus like phylotypes were endemic to this hot water spring (Ionescu et al., 2010). Lukavsky et al. (2011) reported eight cyanobacterial species from hot water springs at Pancharevo from Bulgaria. Most studies on European hot springs are focused on Iceland, which has one of the world’s largest subsurface hot spots and large number of hot springs with diverse water chemistry (Marteinsson et al., 2001). Depending on temperature and sulfide content, Mastigocladus (Fischerella) laminosus or Phormidium laminosum were found to be the main inhabitants of the microbial mats formed in Icelandic alkaline hot springs (Sonne-Hansen and Ahring, 1997). Microbial mats collected from nine hot water springs with water temperature ranging 39 °C to 90 °C from Algeria had nineteen different cyanobacterial morphotypes belonging to Gloeocapsa, Gloeocapsopsis, Stigonema, Fischerella, Synechocystis, Microcoleus, Cyanobacterium, Chroococcus and Geitlerinema and three species of Leptolyngbya were most abundant (Yala et al., 2014).

In the Philippines, both filamentous cyanobacterial phylotypes, Fischerella spp., Oscillatoria spp. and unicellular cyanobacteria, Synechococcus spp. were observed from volcano-derived geothermal springs and intertidal geothermal vents (Lacap et al., 2005; Jing et al., 2005). Unicellular Synechococcus lividus at 40-80 °C and Synechococcus spp. as well as filamentous Phormidium boryanum at 30-60 °C were found dominant among 36 cyanobacterial species reported from the hot water springs of northern Thailand (Sompong et al., 2005). The study on phylogenetic and morphological diversity of cyanobacteria in microbial mats at 40 °C to 75 °C collected from nine hot water springs of Thailand reported that from fourteen cyanobacterial morphotypes Synechococcus spp., Phormidium cf. boryanum and Leptolyngbya spp. were dominant. The decrease in morphological diversity and
increase in molecular sequence diversity was observed with increase in water temperature among morphologically indistinguishable species (Sompong et al., 2008). Cyanobacterial diversity in microbial mats collected from hot water springs of Thailand and also in mats collected from China and the Philippines revealed genotypic diversity was more compared to morphotype diversity (Hongmei et al., 2005, 2006). Phylogenetic analysis revealed distinct thermophilic lineages from mesophilic species among Calothrix, Cyanothece, Fischerella, Phormidium, Pleurocapsa, Oscillatoria and Synechococcus. Many studies on hot springs ecosystems in Yunnan Province, China showed high diversity of cyanobacteria (Bao et al., 2002; Chen et al., 2002; Xiang et al., 2003; Xu et al., 1998). Li et al. (2004) reported rich cyanobacterial diversity profiles in hot water springs using culture independent approach and suggested that temperature was the key factor affecting composition of thermophilic cyanobacterial community.

Studies on Japanese hot water springs showed that these springs are geologically different from those in Yellowstone National Park and support different types of microbial mats including mixed Chloroflexus and cyanobacteria (Hiraishi et al., 1999; Takai and Sako 1999). The cyanobacterial mats contained large number of unicellular rod-shaped Synechococcus like cyanobacteria (Maki, 1991; Yamamoto et al., 1998; Hiraishi et al., 1999).

2.3.3 Effect of physico-chemical parameters of hot springs on cyanobacterial diversity

Factors such as temperature and water chemistry affect distribution of cyanobacteria in geothermal springs (Castenholz and Utkilen, 1984; Ward et al., 1989; Dillon and Castenholz, 2003). Cyanobacteria usually do not occur in hot springs with pH below 4.0. Synechococcus spp. has been reported from Yellowstone National Park hot springs with pH 5.2 (Ward and Castenholz, 2000). Temperature between 40-60 °C in hot springs supports a range of different cyanobacterial mats comprising filamentous and unicellular taxa. Works of Skirnisdottir et al. (2000), Nakagawa and Fukui (2002), Sompong et al. (2005) and Debnath et al. (2009) have revealed that cyanobacterial diversity and community complexity decreased with increase in temperature. The occurrence of cyanobacteria is determined by temperature plus combined nitrogen and free sulphide levels (Hongmei et al., 2005; Sompong et al., 2005; Debnath et al., 2009). Sulphide is toxic to primary metabolism, thus springs with high level of free sulphide may be dominated with sulphide tolerant/utilizing
Oscillatoria limnetica and Cyanidium caldarium species that can use sulphide as photosynthetic electron donor (Castenholz, 1969; Ward et al., 1989; Ward and Castenholz, 2000). Spring water having low concentration of nitrogen supports mats of diazotrophic cyanobacteria, usually Fischerella or Calothrix and/or Pleurocapsa at lower temperatures, whereas those rich in nitrogen support Synechococcus and Phormidium/Oscillatoria mats (Ward and Castenholz, 2000; Sompong et al., 2005; Debnath et al., 2009).

2.3.4 Cyanobacterial diversity in Indian hot water springs

Very little work has been done on cyanobacterial diversity of Indian hot water springs, although geochemical studies on Indian thermal springs have been carried out by several workers (Gupta et al., 1975; Giggenbach et al., 1983; Guha, 1986; Chandrasekharan and Antu, 1995; Pandey and Negi, 1995; Minissale et al., 2000, 2003; Alam et al., 2004; Walia et al., 2005a). These workers have observed that these waters are generally associated with tectonic belts, Mid-continental rifts, Cretaceous–Tertiary volcanism and regional fault zones. Thomas and Gonzalves (1965a, b) reported 16-32 cyanobacterial species in their series of papers (I-VII) on thermal algae of western India. Other reports on cyanobacterial diversity of hot water springs from different parts of country are as, from Bihar (Jha and Kumar, 1990; Jha, 1992), from Orissa (Adhikary, 2006) and from West Bengal (Jana, 1973; Ghosh et al., 2003; Debnath et al., 2009; Roy et al., 2014). Debnath et al. (2009) studied cyanobacterial diversity of four hot water springs from Bakreswar, West Bengal, India and reported that dominant species of Chroococcus, Gloeocladium, Oscillatoria, Phormidium, Lyngbya, Fischerella and Calothrix were dominant and divided cyanobacterial diversity of these hot water springs into two categories i) Oscillatoria amphibia, O. princes, Phormidium fragile and P. laminosum as mesothermophiles community (≤ 45°C, bathing pool and Dukhunda) and ii) S. elongatus, S. lividus, G. gelatinosa, Fischerella thermalis and Calothrix thermalis as thermophiles (45-65°C, Suryakunda and Kharkunda). Cyanobacterial diversity of the hot water spring of Panifala from West Bengal, India was represented by species belonging to Synechococcus, Chroococcus, Gloeocladium, Phormidium, Calothrix, Scytonema and Fischerella (Roy et al., 2014).

Previous studies on the Himalaya geothermal province were focused mainly on the famous thermal springs of Manikaran and Kasol along the Parvati Valley of
Himachal Pradesh with the aim to characterize the geothermal resources with respect to their suitability for hydro-power generation (Alam et al., 2004; Chandrasekharan et al., 2005). Other works were focused on radon monitoring of waters and soils for health hazard assessment and as a tool for earthquake prediction studies (Choubey et al., 1997; Virk and Walia, 2000; Walia et al., 2003, 2005a, b). No report is available in literature on diversity of cyanobacteria from north Indian hot water springs.

2.4 Cyanobacterial diversity in cold environments

Cold environments in which temperature remains below 5 °C are common on the biosphere (Margesin and Haggblom, 2007). The atmospheric temperature at altitude >3000 m is consistently <5 °C. Low temperatures is characteristics of high mountains where snow remains throughout the year. The Antarctic, Arctic, as well as high altitudes have habitats which represent cold environments. These habitats can be used as models for ecosystem studies on microbial dynamics in simple and short food web.

Microorganisms dwelling in extreme environments have attracted microbial ecologists because of the uniqueness of peculiar physiological properties (Margesin and Miteva, 2011). High mountain ecosystems are also of ecological interest because of their richness in natural resources, biodiversity, clean water, healthy climate, original soil, and diverse geology, relevant to the issues of environmental protection (Zakhia et al., 2008).

2.4.1 Cyanobacteria in cryosphere

Cyanobacteria are frequently found in the ice as well as cold desert habitats, and use a variety of strategies to mitigate the harshness of their surroundings (Vincent, 2007). Some cyanobacterial communities live inside rocks where the humidity can be high and thermal variation is buffered, while others form dark-coloured mats on or within ice that absorb sunlight, which increases temperature adequately to melt some ice in summer and provide liquid water conditions (Nienow and Friedmann, 1993; Mueller and Pollard, 2004). Cyanobacteria inhabiting the cryosphere are comprised of a few morphospecies (Nienow and Friedmann, 1993; Wynn-Williams, 2000). Molecular studies are providing evidence on the locally restricted (endemic) taxa and also on the cosmopolitan distribution of closely related genotypes (Taton et al., 2003; Jungblut et al., 2010).

Studies on the cyanobacterial diversity in the Antarctica using a culture independent molecular approach have been focused on Prydz Bay region (Bowman et
al., 2000; Smith et al., 2000; Taton et al., 2006a,b), the McMurdo Bry Valleys (Priscu et al., 1998; Gordon et al., 2000; Christner et al., 2003; de la Torre et al., 2003; Taton et al., 2003; Smith et al., 2006; de los Rios et al., 2007), James Ross Island (Komárek et al., 2008; Skácelová et al., 2013), the McMurdo ice Shelf (Jungblut et al., 2005), and Antarctic Peninsular region (Hughes and Lawley, 2003; Hughes et al., 2004). The arctic ice-based habitats can be differentiated into cryoconite holes, melt water ponds and sediment patches without continuous coverage by water (Mueller et al., 2003). Cyanobacterial diversity in the Arctic includes diverse genera like Synechococcus, Gloeocapsa, Leptolyngbya, Phormidium, Oscillatoria, Lyngbya, Microcoleus, Anabaena, Aphanocapsa, Calothrix, etc. (Sawstrom et al., 2002; Cockell and Stokes, 2004; Mueller et al., 2005; Bonilla et al., 2005; Zielke et al., 2005).

At high mountains, which are snow bound throughout the year, persistent cold temperature is often accompanied by freeze-thaw cycles, extreme fluctuations in irradiance, and large variations in nutrient supply and high salt levels. Consequently polar and alpine environments have very reduced biodiversity, with prokaryotes being a major component of the total ecosystem biomass as well as species richness (Vincent, 2000; Ramette and Tiedje, 2006; Dorador et al., 2008; Zakhia et al., 2008). The presence of cyanobacteria in these habitats has already been observed during the early explorations of the polar regions during the last decade of the 19th century (Vincent, 2007). A diverse range of cyanobacteria have been found in polar, alpine habitats as benthic mats (Vincent et al., 1993; Elster et al., 1997; McKnight et al., 1999; Fernández-Valiente et al., 2007; Zakhia et al., 2008), films in shallow thermokarst lakes (Vézina and Vincent, 1997; Rautio and Vincent, 2006; Biondi et al., 2008), ice shelf ponds of the Arctic (Vincent et al., 2004a, b; Mueller et al., 2005) and the Antarctica (Howard-Williams et al., 1989; Sabbe et al., 2004; Jungblut et al., 2005), and in ice-covered lakes (Hawes and Schwarz, 2001; Taton et al., 2006a, b; Vopel and Hawes, 2006; Vincent, 2007; Zakhia et al., 2008; Jungblut et al., 2010). The psychrophilic cyanobacterial flora is dominated by Anabaena, Aphanocapsa, Calothrix, Chroococcidiopsis, Gloeocapsa, Lyngbya, Microcoleus, Oscillatoria, Phormidium, Nostoc and Scytonema etc (Taton et al., 2003, 2006a, b; Vincent, 2007; Zakhia et al., 2008; Jungblut et al., 2010).
2.4.1.1 Ice-based habitats

Cyanobacteria dominate in microbial consortia formed in ice-based habitats such as cryoconite (literally “cold rock dust”) holes and melt water ponds. Cryoconite gives rise to vertical, cylindrically-formed holes in the ice surface that contains a thin layer of sediment overlaid by water. The formation of these habitats is initiated through the absorption of solar radiation by the sediment and subsequent ablation of the surrounding ice (Wharton et al., 1985; Zakhia et al., 2008). Studies on these holes on the Canada Glacier, McMurdo Dry Valleys showed occurrence of cyanobacteria phylogenetically related to *Chamaesiphon* (Mueller et al., 2001; Christner et al., 2003).

Another important class of ice-based habitat is the melt water ponds that form on ice shelves. These contain liquid water during the summer months that freezes completely in winter. The biota of these habitats experience extreme temperature changes, freezing and desiccation stress, and high salinities (Vincent, 2000; Jungblut et al., 2005; Zakhia et al., 2008). The ponds on the McMurdo Ice Shelf have low nutrient concentrations, especially of nitrogen due to the marine origin of the sediments (Hawes et al., 1993, Wait et al., 2006). In such habitats, thick benthic cyanobacterial mats comprise a diverse community of Nostocales and Oscillatoriales (Howard-Williams et al., 1989; Nadeau et al., 2001; Jungblut et al., 2005).

2.4.1.2 Lithic and edaphic environments

Cyanobacteria are also found in biofilms below and within the rocks at high altitude where the microclimate gives protection against environmental stresses such as high UV radiation, temperature extremes and desiccation. Such habitats can be found in variable depths below the rock surface depending on the optical characteristics of the rocks and the level of available photosynthetically active radiation. Depending on the spatial location, communities are hypolithic (beneath rocks), endolithic (in pore spaces of rocks), chasmoendolithic (in cracks and fissures of rocks), or cryptoendolithic (in the pore space between mineral grains forming sedimentary rocks) (Vincent, 1988; Hughes and Lawley, 2003; Cockell and Stokes, 2004). Cryptoendolithic communities are common in sandstone outcrops of Eureka, Ellesmere Island, similar to the cyanobacterial morphotypes as in the Antarctic rocks (Omelon et al., 2006).
Edaphic cyanobacteria have been found at several sites around the Antarctica and the Arctic, including polar deserts and more humid environments (Vincent, 1988; Quesada and Vincent, 2012). However, it has been suggested that the presence of cyanobacteria in the most arid soils is due to wind dispersion (Aislabie et al., 2006; Michaud et al., 2012). Consistent with this hypothesis, Wood et al. (2008) and Michaud et al. (2012) found that the cyanobacterial diversity in Dry Valley soils was very similar to that found in nearby microbial mats. In the Dry Valleys of Antarctica, the soils are old, weathered and have low carbon and nutrient concentrations (Vincent, 1988; Zakhia et al., 2008). Terrestrial dark crusts are found throughout Antarctica dominated by cyanobacteria (Broady, 1996; Mataloni et al., 2000; Adams et al., 2006). Cyanobacteria from the three main orders (Chroococcales, Oscillatoriales and Nostocales) are frequently reported in Antarctic soils with *Leptolyngbya* and *Phormidium* being the most common genera (Cavacini, 2001; Mataloni et al., 2000; Wood et al., 2008). The occurrence of different cyanobacterial taxa seems to be related with the extent and duration of liquid water conditions (Vincent, 1988; Quesada and Vincent, 2012). Terrestrial cyanobacteria in the Arctic are also major primary colonizers of soils and can be found within soil crusts, in symbiotic association with lichens and within rocks. Cyanobacteria are an important source of nitrogen for the nutrient limited soils of the Arctic (Zielke et al., 2005).

Cyanobacteria are also dominant components of alpine soil crusts rock-associated communities as described for endolithic communities of dolomite rocks in the Swiss Alps (Sigler et al., 2003) and soils from recently deglaciated areas in the Peruvian Andes (Nemergut et al., 2007). Cyanobacteria have been found as part of microbial mats, epiphytes on mosses and endosymbionts in lichens in stream habitats of alpine regions (McClintic et al., 2003; Rott et al., 2006).

Ancient communities of edaphic cyanobacteria have been found preserved in permafrost. These cyanobacteria are not only fossil remnants from past ages, but in some cases shown to be viable despite apparently being trapped and frozen in the permafrost for millions of years (Erokhina et al., 2000; Vishnivetskaya et al., 2003; Vishnivetskaya, 2009). Several cyanobacterial species belonging to Oscillatoriales and Nostocales have been isolated from the Arctic permafrost (Vishnivetskaya et al., 2003; Vishnivetskaya, 2009) and show a close phylogenetic similarity to cyanobacteria found nowadays in microbial mats or endolithic environments, notably *Leptolyngbya, Microcoleus, Phormidium, Nostoc* and *Anabaena* (Quesada and Vincent, 2012).
2.4.1.3 Lakes, ponds, rivers and streams
Cyanobacteria also form large bio-mass accumulations in polar ponds, lakes, rivers and streams. They often form thick, cohesive, highly pigmented mats which coat the benthic environments (Vincent, 1988). Cyanobacteria play an important role in Arctic lakes, ponds and streams and have been well studied in the Canadian High Arctic (Bonilla et al., 2005; Gibson et al., 2006). The most common groups are Oscillatoriales and Nostocales, with some Chroococcales. Planktonic picocyanobacterial communities of these lakes comprise mainly *Synechococcus* (Vincent, 2000). Studies on perennially ice-covered Lake Hoare in the McMurdo Dry Valleys have shown that photosynthetically active radiation exerts an overall control on microbial production, composition and mat structure (Vopel and Hawes, 2006). Cyanobacteria are also found in the water column of lakes, and picoplanktonic forms often dominate the plankton. The abundance of planktonic picocyanobacteria is dependent on nutrient availability and light in lakes (Vincent, 2000; Zakhia et al., 2008).

Pandey et al. (2004) have reported 35 cyanobacterial species while Singh et al. (2008) reported 109 cyanobacterial species from Schirmacher in Antarctica.

2.4.1.4 Diversity of cyanobacteria in cold desert of India
No reports are available in literature on cyanobacterial diversity from Indian cold deserts region of North-Western Himalayas.

2.4.1.5 Limnology of lakes in relation to cyanobacterial diversity
Life in aquatic environment is largely governed by physico-chemical characteristics and their stability. Limnological studies of water bodies provide information about the trophic status which may help in management and conservation of water bodies (Marchetto et al., 1995). General increases in phytoplankton biomass and an increase in frequency and duration of blooms of individual phytoplankton species have been found to be correlated with an overall increase in nutrient input to fresh-waters (Hallegraeff, 1993). Reduced organic and inorganic nitrogen forms, such as urea and ammonium, are the favoured nitrogen source for phytoplankton (McCarthy et al., 1977). Increase in phytoplankton biomass, despite low nitrogen concentration, can be explained by analyzing the availability of various forms of nitrogen and their relative rates of utilization among different phytoplankton species. High nitrate nitrogen favours the growth of eukaryotic phytoplankton, while low nitrate nitrogen concurrent
with sufficient ammonium nitrogen, either present as a pool or recycled at a sufficient rate to supply phytoplankton growth, favors some cyanobacteria (Jacoby et al., 2000; Berg et al., 2003). Typically, non-nitrogen-fixing cyanobacteria prefer ammonium nitrogen over nitrate nitrogen as nitrogen source and would be outcompeted by other phytoplankton in high nitrate nitrogen environments because of their low nitrate nitrogen assimilation rate (Kappers, 1984; Taranu et al., 2012).

Limnological studies on fresh water bodies from India have been undertaken (Rao and Mahmood, 1995; Naganandini and Hosmani, 1998; Pandey et al., 2000; Patel and Sinha, 2000; Kumar et al., 2012), but little information is available on the limnological studies on high altitude lakes of North-Western Himalayas (Kumar et al., 2012; Nautiyal et al., 2012). Various workers have studied phytoplankton dynamics in relation to water quality in the Northern Himalayan lakes (Zutshi et al., 1972; Zutshi, 1985, 1991), Kashmir lakes (Zutshi, 1989; Kaul and Handoo, 1989, 1993; Trisal et. al., 1994; Kundangar and Sarwar, 1997), and Himachal Lakes (Thakur et. al., 2013; Jindal et al., 2014).

Physico-chemical parameters of lakes like light, temperature and nutrient concentration, lake currents, and grazing by zooplankton control the phytoplankton growth and reproduction (Khan, 2003; Bhat, 2009). The diversity and density of phytoplanktons declines with increase in salinity (Williams, 1992; Kipriyanova et al., 2007). Conductivity and lake trophy have been identified as the main factors regulating species composition of benthic communities (Vincent and James, 1996; Roberts et al., 2001; Sabbe et al., 2004). Temperature, pH and nutrient level of lakes are major factors influencing distribution of cyanobacterial species in high altitudinal lakes (Singh et al., 2014).