General Introduction

The north eastern region (NE region) of India (located between 87°32'E to 97°52'E longitudes and 21° 34'N to 29°50'N latitudes) is a genetic treasure house of plant, animal and microbial resources and also the Biogeographical Gateway to the India that finds place in part of two-biodiversity hotspots in India (Mittermeier *et al.*, 2003). The north eastern region of India comprises of 08 states namely; Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura. The NE region of India has a wide range of physiographic and eco-climatic conditions (Singh *et al.*, 2009). The forests in these regions are extremely diverse in structure and composition, combining tropical, sub tropical and temperate forest types, alpine meadows and cold deserts. The temperature ranges from sub-zero to 36ºC and the annual rainfall ranges from 250 mm to 2100 mm. The region forms a distinctive part of the Indo-Burma hotspots which ranks the 8th among the 34 biodiversity hotspots of the world (Hughes, 2012) and is a prime one among the two identified for the Indian sub-continent.

The region also falls in the bio-geographic tri-junction of the Indian, the Himalayas and the oriental landmass. This active centre of speciation is a centre of gene diversity of the domesticated crops and a secondary centre for several economically important plant and animal species. The Indo-Burma biodiversity hotspots is home to 13,500 plant species (2.3% of the global agencies) of which 7000 are endemics and 2,185 vertebrates (1.9 % of the global species) of which 528 are endemics (Myers, 2000). Avian fauna is represented by 1,170 species with 140 as endemics. Mammalian diversity constitutes 329 species with 73 endemics. The species richness in terms of number of reptiles and amphibians are 484 and 202 with 201 and 114 endemics respectively. Of the total 40,000 species of algae reported so far, about 6,500 species are found in India. Of this 2,000 species belong to 150 genera of cyanobacteria (Litavitis, 2002).
The gene pools of the NE region are invaluable genetic resources and can be put to use for the economic growth of this region in particular and to the nation in general. Biotechnological interventions are needed for conservation and sustainable uses of the bioresources.

Cyanobacteria are oxygenic photosynthetic prokaryotes possessing the ability to synthesize chlorophyll-a. Cyanobacteria have been characterized by their ability to form the phycobilin pigment, where it gives the characteristic blue green colour at high concentration, hence commonly referred as blue green algae. The cells constituting the thallus are prokaryotic, lack flagella and motility of gliding or jerky type (Singh et al., 2009). On the basis of the most recent 16S rDNA sequence analysis, cyanobacteria are most closely related to the bacteria. Cyanobacteria are usually made up of trichomes referring to the row of cells or filaments made up of row of cells covered by sheath even though a similar morphology occurred (Koijam et al., 2009). The cyanophytes have a large and diverse array of intracellular inclusions: those of regular or usual occurrence (DNA, ribosomes, plasmids, thylakoids and the associated phycobilisomes, polyphosphate bodies, glycogen, cyanophycin and lipid granules, carboxysomes); and those of irregular or sporadic occurrence (gas vesicles, different granules, tubules, filaments, various bodies and inclusions etc.).

Cyanobacteria occur in the vast array of habitats by its several general characters and other special adaptive features. Many species tolerate a great range of environmental conditions, including extremes that usually or often exclude eukaryotic algae. Cyanobacterial diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkalophilic, planktonic to barophilic, freshwater to marine including hypersaline (Devi et al., 2010). It is presumed that cyanobacteria evolved in the Precambrian well before the Paleozoic
boundary and this is borne out by the existence of microfossils of the middle and late Proterozoic that are nearly identical morphologically to some living cyanobacteria (Knoll, 1985; Schopf, 1996). Cyanobacteria are the primary colonizers of exposed moraines after the retreat of glaciers and they are widely distributed throughout the soils of both polar regions. Various types of rock environment provide a favourable habitat for cyanobacteria.

The communities form dark crust over the rock (epiliths) and blue-green coloured biofilms under translucent stones (subliths or hypoliths). Well developed communities can also grow within rock fissures (chasmololiths) or in the interstitial spaces beneath the surface of porous rocks, particularly sandstones (cryptoliths). During the course of evolution they adapted to almost every ecological niches, including the most extreme ones (Schopf, 2000). They can be found in freshwater lakes, hot and cold springs examples of which are: the Arctic ice (Torsvik et al., 1996), deserts (Garcia-Pichel et al., 2001), oceans, that is marine cyanobacteria (Thajuddin and Subramanian, 2005) and even the Arctic hot springs (Rios et al., 2007) and Antarctic granite rocks (Roeselers et al., 2007).

Order Oscillatoriales of class cyanophyceae have filamentous forms without heterocysts and akinetes. The genera of the Oscillatoriales are traditionally distinguished from one another primarily on the basis of presence or absence of sheath. In addition to the type of sheaths, the appearance of the filament, false branching and the colour of cells (pigments) have been used as generic characters. At the species level, the cell size, constrictions at the cross walls, cellular inclusions (granulations) and cell shape (especially of the terminal cells of the trichomes) have been the main taxonomic criteria by the traditional methods. According to Desikachary (1959), filamentous and non heterocystous strains belong to family oscillatoriaceae of order Nostocales under class cyanophyceae which have 16 genera viz. Crinalium, Microcoleus, Sirocoleus,
Polychlamydum, Dasygloea, Hydrocoleum, Schizothrix, Porphyrosiphon, Lyngbya, Symploca, Trichodesmium, Oscillatoria, Spirulina, Arthrospira, Katagnymene and Phormidium. According to Komarek and Anagnostidis (2005) under new method of classification based on molecular techniques and traditional methods, Oscillatoriales are divided into 6 families viz. pseudanabaenaceae, schizotrichaceae, borziaceae, phormidiaceae, gomontiellaceae and oscillatoriaceae. The reproduction of Oscillatorean cyanobacteria proceeds usually by hormocytes and homogonia. Hormocytes are non-motile while hormogonia are actively and facultatively motile.

Phormidium formosum, P. limosum, Pseudanabaena limnetica, Limnothrix planctonica and Oscillatoria species were abundant under Karababa Bridge-Euphrates and Goksu rivers in epiphytic flora, it was rare and in little quantity in Ataturk Dam phytoplankton. These species show better growth in stagnant and anaerobic water bodies (Prescott, 1975) and are used as water pollution indicator species (Sahin, 2002). As a result of the increase in eutrophication in Turkey, these species were found in abundance and widespread. Lyngbya was observed as widespread especially in Ataturk dam, the epilitic flora of the coastal region of Adiyaman and both in the epilitic and the epiphytic flora of Karababa-underbridge (Euphrates). Komarek and Lund (1990) and Desikachary and Jeejibai (1996) have described different species of the genus Arthrospira (A. platensis, A. fusiformis, A. jenneri, A. maxima and A. indica) considering various morphological characters and habitats.

The diversity of the alkaline lake, Alchichica showed the depth of lake affect the species diversity of Oscillatoriales (Couradeau et al., 2011). Three distinct Lyngbya species on nearshore reefs in Broward County, Florida were sampled in 2006 and 2007 (Sharp et al., 2009). The cyanobacteria community observed in lake Czarne were reported to have three filamentous
cyanobacteria: *Limnothrix planctonica*, *L. redekei* and *Planktothrix agardhii*. The *Phormidium cf. terebriformis* strain AB2002/07 was reported from a phytoplankton sample from lake Nakuru, the strain KR2003/25 from a less alkaline hot spring at the shore of lake Bogoria. The dominance of *Limnothrix bicudoi* in a shallow, turbid, mesotrophic lake in southeast Brazil were reported (Soares et al., 2009). Hindak (2001) and Krienitz et al. (2003) have described *Phormidium cf. terebriformis* from hot springs at the shore of lake Bogoria.

*Phormidium retzii* is one of the most commonly encountered lotic taxa in North America and has been recorded from a wide range of habitats worldwide (Casamatta et al., 2002). Seasonal distribution, nitrogen fixation and primary productivity of *Trichodesmium* species were intermittently studied in the coastal waters of Tanzania. The following species were encountered viz. *T. erythraeum*, *T. tenue*, *T. thiebautii* and one unidentified *Trichodesmium* sp. while *T. contortum* was rarely encountered and only as individual trichomes (Lugomela et al., 2002).

*Aphanacapsa littoralis*, *Chroococcus minutus*, *Calothrix geitonos*, *Lyngbya* sp., *Microcystis* sp., *Microcoleus chthonoplastes*, *Myxosarcina burmensis*, *Oscillatoria chlorina*, *Oscillatoria limosa*, *Oscillatoria salina*, *Oscillatoria subbrevis*, *Spirulina subsalsa*, *Phormidium fragile*, *Phormidium tenue*, *Phormidium valderianum*, *Synechococcus aeruginosus*, *Synechococcus elongatus* and *Stichosiphon sansibaricus* were reported from the salt pan sites such as Kovalam, Puthalam and Rajakamangalam in Kanyakumari district, Tamil Nadu, India (Sugumar et al., 2011) belong to twelve genera of five families. 68 species from the member of family oscillatoriaeceae were reported from freshwater of different sites of Goalpara district, Assam (Deka and Sarma, 2011) belong to genera, *Katagnymene* 1.47%, *Porphyrosiphon* 1.47%, *Polychlamydam* 1.47%, *Symploca* 1.47%, *Microcoleus* 1.47%, *Hydrocoleum* 1.47%, *Spirulina* 8.82%, *Phormidium* 17.64%, *Lyngbya* 19.12% and *Oscillatoria* 45.58%.
DNA sequences play an essential role in the reconstruction of evolutionary relationships among organisms and have led to new genetic classifications that may confirm or conflict with traditional taxonomy. Application of molecular techniques to amplify some portions of the genome in order to characterize and deduce phylogenetic relationships of cyanobacteria has increased considerably in the recent years (Neilan et al., 1995; Garcia-Pichel et al., 1996; Orcutt et al., 2002; Taton et al., 2003). At the molecular level, the rRNA genes are the most widely used markers for the identification of bacteria and cyanobacteria due to their conserved function and universal presence. Several researchers have exploited the conserved regions of the 16S rRNA gene for phylogenetic analysis of cyanobacteria (Nubel et al., 1997; Crosbie et al., 2003; Salomon et al., 2003). The following strains were examined by 16S rRNA; Leptolyngbya sp. PCC 7104 and PCC 7375; Lyngbya aestuarii PCC 7419T; Oscillatoria acuminata PCC 6304 and Oscillatoria sancta PCC 7515T; Pseudanabaena sp. PCC 6802, PCC 6903, PCC 7367, PCC 7403 and PCC 7408; Symploca sp. PCC 8002 were obtained from the Pasteur Culture Collection (PCC) and all were axenic.

The 16S rDNA phylogeny indicates that Lyngbya majuscula and Lyngbya bouillonii were genetically distinct, greater than 10% average sequence divergence. L. bouillonii can be recognized in the field by its dark red colouration and thick, net-like mats, but it showed an extremely wide variation in cell shape, cell size and trichome width. Phylogenetic relationship of Arthrospira, Phormidium and Spirulina strains were studied. Despite their differences in morphology and habitats, the 16S and PC-IGS phylogenetic trees all sequences of Kenyan and Indian Arthrospira strains (AY575923-AY575932; AY575937-AY575946) were grouped in a uniform cluster each and in pairs show similarities of 100%. Li et al. (2001) also reported 100%
sequence similarity when investigating the 16S rDNA sequences of an *Arthrospira maxima* strain (AF260509) and *Arthrospira fusiformis* strain (AF260510).

Nelissen et al. (1994) found a 16S rRNA sequence similarity of two *Arthrospira* strains (PCC7345 and PCC8005) of 99.7%. According to Castenholz et al. (2001), such a high molecular similarity makes it likely that all *Arthrospira* strains were representatives of only one species. A 16S rRNA gene sequence similarity value of more than 97.5% is regarded the level at which two bacterial strains can be congeneric or belong to the same species (Devereux et al., 1990; Stackebrandt and Goebel, 1994).

Random amplification of polymorphic DNA (RAPD) technique for the analysis of random regions has been useful in comparative genetic studies (Bowditch et al., 1993). However, the regions represent divergent markers, thus making the method reliable only for closely related organisms (Thormann et al., 1994). In this technique, a single primer is able to anneal at multiple locations throughout the genome to produce a spectrum of amplified products, characteristics of the template DNA (Welsh and McClelland., 1990; Williams et al., 1990). The analysis is advantageous as a subtyping technique, as it only requires fairly small amounts of template DNA, which need not be double stranded or highly purified (Wang et al., 1993).

Results based on RAPD showed possible relationship between *Phormidium* from different geographical places which suggested that *Phormidium* strains were firstly globally distributed in similar habitats but have later been genetically changed under pressure of different environments (Palinska et al., 2011). This is also reflected in their morphology and most pronounced in pigment composition. Scheldeman et al. (1999) and Casamatta et al. (2003) showed on examples of *Arthrospira* and *Phormidium retziï* strains that species of cyanobacteria are more localized than having global distribution. RAPD primers yielded 100% polymorphism
among the studied strains, indicating a considerable degree of intra-specific genomic heterogeneity with percent similarity between 13% and 82%. Partial 16S rDNA sequence similarity values ranged from 91% to 99% (Premanandh et al., 2009). RAPD-PCR was used to reveal DNA sequence polymorphism to a level beyond the taxonomic range approached by the 16S rRNA gene.

Carotenoids belong to the category of tetraterpenoids (i.e. they contain 40 carbon atoms). Structurally they are in the form of a polyene chain which is sometimes terminated by rings. Carotenoids have important functions in photosynthesis, nutrition and protection against photooxidative damage. Carotenoids in cyanobacteria function as accessory light harvesting antenna and as photoprotectants due to their ability to quench toxic singlet oxygen and triplet Chi (Hirschberg and Chamovitz, 1994). The spectrum of carotenoids in three species of *Phormidium* showed β-carotene as the major pigment (Healey, 1968). Similarly, carotenoid composition of three other species of *Phormidium* also showed the presence of β-carotene as the major carotenoids in all species (Hertzberg et al., 1971).

The cyanobacteria are the only photosynthetic organism in which often about one-half the total carotenoid is β-carotene (25% is a normal value for higher plants and 10% for green algae) (Goodwin, 1957). The unique carotenoid distribution of the cyanobacteria tends to support the general view that they have existed apart from the main line of evolution from a very early age (Fogg, 1956). Some of the diseases that can be prevented or cured by carotenoids (i) Cataracts, (ii) Age-related macular degeneration (ARMD), (iii) Coronary heart disease (CHD) and (iv) Anti-carcinogen. Gustafson *et al.* (1989) reported anti-HIV activity of marine cyanobacterial compounds from *Lyngbya lagerheimii* and *Phormidium tenue*. *Spirulina platensis* was shown to contain detectable levels of mercury and lead when grown under contaminated conditions.
(Slotton et al., 1989), implying that this cyanobacterium was taking up the toxic metal ions from its environment.

β-carotene rich *Dunaliella* powder has been commercially exploited in many countries since 1980s. *Dunaliella salina* produces total carotenoids of $102.5\pm33.1$ mg/m$^2$/d (β-carotene: 10% of biomass) by semi-continuous outdoor, closed tubular (55 L) at temperature 25°C and pH 7.5±0.5. The maximum biomass measured as chl-a differed from one algal strain to another. The highest production of carotenoids and phycocyanin takes place in *Spirulina platensis* (1.4 and 4.5 mg/ml). On the other hand, *Oscillatoria limnetica* produced the lowest carotenoids and phycocyanin content (0.8 and 0.18 mg/ml) (Sayda et al., 2010).

Modification of normal composition of BG-11 culture medium by altering nitrogen and carbon sources resulted in 25-38% increased in carotenoids in *Phormidium* sp. The carotenoids content showed positive increase by lowering the nitrogen concentration in which the modified BG-11 medium showed the highest value (85.2 μg/ml) in half strength. In *Synechocystis* sp., 15 and 30 days old cultures, the amount of chlorophyll-a decreased at all irradiation doses. The concentration of chl-a and carotenoids increased as the growth period increased (Trimurtulu et al., 1994). Carotenoids were found to increase in less nitrogen content. It was concluded that enhanced carotenoids synthesis in stressed conditions and it increased two ecological functions, providing photoprotection and increasing photosynthetic performance of surface cyanobacterial populations (Paerl, 1984).

The work on distribution of Oscillatoriales and their adaptation to different ecological condition is lacking particularly in NE region of India although the region falls under Indo-Burma biodiversity hotspots. Therefore, the present study was planned to study the diversity of Oscillatoriales from NE region of India and screening for carotenoids and process optimization
for enhancement of biomass and carotenoid content both. The molecular characterization of the selected Oscillatoriales based on carotenoids content was also an important aspect of the study.

Keeping in view of the inadequacies, following objectives have been taken up for the study:

- **Isolation, identification and chemo-taxonomical characterization of Oscillatoriales (cyanobacteria) of north eastern region of India.**
- **Evaluation and biochemical screening of carotenoids from selected oscillatory cyanobacteria of north eastern region of India.**
- **PCR based molecular characterization of oscillatory cyanobacteria having rich carotenoids.**
- **Optimization of carotenoids production along with fine chemicals for industrial application.**