SUMMARY

North eastern states of India which falls under Indo-Burma biodiversity hotspots has been a favourable home for numerous algae like cyanobacteria due to abundant rainfall with temperate to tropical climate. Systematic exploration and exploitation of this rich bioresource are needed for commercial purpose. In the present study, a brief introduction has been highlighted on cyanobacteria, oscillariales, diversity, carotenoids and its application along with the objectives of the thesis in the first chapter. A review of literature on oscillariales classification and systematic, chemotaxonomy, diversity, carotenoids structure, carotenoids functions, carotenoids applications, production and enhancement along with molecular characterization of oscillariales using 16S rRNA and RAPD techniques were described in the chapter two. In the third chapter, materials and methods for isolation, identification, biochemical estimations and molecular characterizations based on 16S rRNA and RAPD were mentioned. The fourth chapter provides complete results of the objective mentioned. In the fifth chapter, discussion based on the results were described and analyzed. Appendix and cited references were mentioned separately.

Total 244 Oscillatoriales cyanobacteria were encountered from different ecological habitats of 08 states of north eastern region of India viz. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura during the period of study. These strains belong to 11 genera from the group of Oscillatoriales namely; Phormidium, Limnothrix, Microcoleus, Oscillatoria, Leptolyngbya, Lyngbya, Hydrocoleum, Trichodesmium, Porphyrosiphon, Pseudanabaena and Plectonema. Lyngbya, Plectonema, Oscillatoria and
*Phormidium* were isolated from the north eastern region of India. Morphology, habitats and locations were recorded of each and every strain including GPS data.

One hundred ten (110) Oscillatoriales strains were encountered from Manipur, twenty six (26) from Meghalaya, twenty five (25) from Assam, twenty four (24) from Sikkim, seventeen (17) from Tripura, fourteen (14) from Mizoram, eighteen (18) from Nagaland and ten (10) from Arunachal Pradesh and established in culture. The cultural studies of each strain along with filament/trichome structure, sheath behavior, motility and granulation along with apices of filaments were investigated for all encountered strains. Diversity based on the altitude differences was studied.

Total eighteen (18) species from genus *Phormidium* were found to be distributed at different altitudes in north eastern region of India. 3 species of *Phormidium* were recorded from lowland to subalpine regions viz. *Phormidium molle* occurred between 5 to 1396 MSL; *P. articulatum* between 770 to 1396 MSL; *P. tenue* between 6 to 1353 MSL. Some species were found to be grown at the lowland to montane regions viz. *P. valderianum* was recorded between 24 to 213 MSL; *P. mucosum* between 24 to 213 MSL; *P. crassior* between 8 to 213 MSL; *P. purpurascens* between 19 to 769 MSL.

Total 11 species were found at the specific altitudes viz. *P. okenei* was occurred at 769 MSL, *P. taylori* at 103 MSL, *P. dignetti* at 770 MSL, *P. uncinatum* at 760 MSL, *P. griceoviolaceum* at 885 MSL, *P. subincrustatum* at 775 MSL, *P. bohneri* at 788 MSL, *P. chlorinum* at 775 MSL, *P. cf. terebriforme* at 103 MSL, *P. roseum* at 92 MSL, *P. cortinum* at 34 MSL and *P. fragile* at 24 MSL.

Different species of *Limnothrix* were less abundant and found to be grown at the montane regions viz. *Limnothrix vacuolifera* was occurred at the height of 792 MSL while *L.
_brachynema_, _L. redekei_ and _L. borgertii_ were at 769 MSL. 2 species from the genus _Plectonema_ viz. _Plectonema radiosum_ occurred between 89 to 792 MSL and _P. notatum_ between 47 to 793 MSL. _P. tomasianum_ occurred between 205 to 1353 MSL and _P. wollei_ between 213 to 1396 MSL. 3 species from the genus _Oscillatoria_ were found to be grown at montane regions viz; _Oscillatoria princeps_ at 793 MSL, _O. leavittae_ at 760 MSL and _O. okeni_ at 769 MSL. 4 species were found to be abundant in the lowlying plains viz; _O. willei_ at 2 MSL; _O. acuta_ between 8 to 24 MSL; _O. geitleriana_ at 8 MSL and _O. chlaybea_ at 89 MSL.

4 species from genus _Lyngbya_ were recorded from lowland and sub alpine regions viz; _Lyngbya spiruloides_ between 5 to 1106 MSL; _L. giuseppei_ between 3 to 1363 MSL ; _L. patrickiana_ at 1396 MSL; _L. arbusitva_ at 1361 MSL. 8 species from genus _Lyngbya_ were recorded from montane regions viz; _L. thermalis_; _L. heironymusii_ were occurred at 793 MSL; _L. martensiana_ between 89 to 769 MSL; _L. arboricola_ between 773 to 802 MSL; _L. sordida_ between 329 to 910 MSL; _L. agardhii_ between 775 to 910 MSL; _L. bergei_ between 888 to 920 MSL; _L. agardhii_ between 775 to 910 MSL; _L. aestuarii_ between 92 to 910 MSL. 5 species were recorded at the lowlands viz. _L. maior_ at 89 MSL; _L. dendoria_ at 50 MSL; _L. truncicola, L. allorgei_ and _L. ambiguum_ at 24 MSL. _Porphyrosiphon notariscii_ was recorded at 725 MSL, _Hydrocoleum lyngbyaceum_ at 782 MSL, _Pseudanabena catenata_ at 769 MSL, _Trichodesmium n_ sp. at 790 MSL and _Microcoleus_ sp. 24 MSL. Genus _Leptolyngbya_ was observed at both lowland and montane regions between 5 to 873 MSL.

Diversity indices for the 11 genera distributed in the entire north eastern region of India were studied. Genus _Oscillatoria_ showed highest diversity as shown by the Shannon index (1.92) and Simpson’s index (0.83) due to both species richness and high evenness in the entire north eastern region of India. It may be also due to their adaptability and presence of laminated sheath
for their growth at the temperate to tropical climate with the temperature ranges from 1-35°C with annual rainfall ranges from 210 cm to 250 cm. Species richness was more in genus *Phormidium* in the north eastern region of India but the diversity was less due to uneven abundance of species proportion resulting to less evenness. 4 genera were found to be less abundant in the entire north eastern region of India viz. *Hydrocoleum, Microcoleus, Porphyrosiphon, Trichodesmium* which showed 0.00 of Shannon index, Simpson’s index 0.00, evenness of 1.00 and equitability of 0.00.

Assam harbored highest diversity based on Shannon index (1.65), Simpson’s index (0.77) and evenness (0.74) and equitability of 0.84 whereas diversity of Oscillatoriales in entire north eastern region of India was found to be lowest in Sikkim having Shannon index (1.38) and Simpson’s index (0.75) and evenness (0.59) only. Species richness was high in Manipur (110 strains) but with least evenness (0.47). The result inferred that species richness may not be a function of diversity in this region due to uneven distribution of the strains.

Chemotaxonomy based on fatty acid profiling were conducted for the selected 5 strains viz. *Phormidium animale* BTA 258, *Leptolyngbya* sp. BTA 287, *Leptolyngbya* sp. BTA 477, *Leptolyngbya tenerrima* BTA 766 and *Phormidium tenue* BTA 803. It was found that *Phormidium animale* BTA 258 produced maximum myristic acid (C14:0) out of 20 fatty acids obtained. *Leptolyngbya* sp. BTA 287 produced maximum pentadecanoic acid (C15:0) out of 19 fatty acids obtained. *Leptolyngbya* sp. BTA 477 produced maximum pentadecanoic acid (C15:0) out of 12 fatty acids obtained. *Leptolyngbya tenerrima* BTA 766 produced maximum pentadecanoic acid (C15:0) out of 13 fatty acids obtained. *Phormidium tenue* BTA 803 produced maximum myristoleic acid (C14:1) out of 21 fatty acids obtained. Chemotaxonomy based on
quantitative analysis of carotenoids could not figure out the correct identification due to overlapping of data.

*Leptolyngbya* sp. BTA 287 produced highest carotenoids (25.38 μg/mg), while *Plectonema indica* BTA 238, *Lyngbya* sp. BTA 259 and *Phormidium autumnale* BTA 338 produced least but similar quantity of carotenoids (0.01 μg/mg). *Limnothrix redekei* BTA 657, *Phormidium tenue* BTA 803, *Oscillatoria acuta* BTA 354, *Lyngbya biebliana* BTA 790 *Leptolyngbya* sp. BTA 287, *Pseudanabaena* sp. BTA 767, *Plectonema* sp. BTA 598 were produced good percentage of carotenoids during investigations. 5 high yielding carotenoids strains viz. *Phormidium animale* BTA 258, *Leptolyngbya* sp. BTA 287, *Leptolyngbya* sp. BTA 477, *Leptolyngbya tenerrima* BTA 766 and *Phormidium tenue* BTA 803 were screened for detailed characterization including process optimization and molecular identification. Production of total carotenoids was found to be strain dependent.

The selected Oscillatoriales were subjected for 16S rRNA aligned pairwise using BLASTN and compared with the NCBI database. BTA 258 showed 88% similarity with the *Phormidium animale* M8 bearing accession no. KC768847.1. BTA 287, BTA 356 and BTA 477 showed 96% similarity with the *Leptolyngbya* sp. CRL 19 bearing accession no: EF545635. BTA 503 showed 95% similarity with the *Phormidium terebriforme* UAM 409 and UAM 408 bearing accession no: JN382220.1 and JN382221.1. BTA 507 showed 99% similarity with the *Phormidium* sp. MMG-4 bearing accession no: JN382232.1. BTA 605 showed 85% similarity with *Phormidium tenue* bearing accession no.: AB042971.1. BTA 657 showed 98% similarity with the *Limnothrix redekei* bearing accession no: FM177493.1. BTA 766 showed 99% similarity with the *Leptolyngbya tenerrima* UTCC 77 bearing accession no: AF218368.1. BTA 803 showed 81% similarity with the *Phormidium tenue* ISC 24 bearing accession no:
The similarity was supported by the neighbour joining and maximum parsimony bootstrap consensus tree which got 80-100% in maximum parsimony method using Kimura 2-parameter model. Morphological descriptions of the strains identified by traditional methods also resembles with the strains identified from the GenBank of the 16S rRNA sequences. *Phormidium* sp. BTA 605 of Arunachal Pradesh showed divergent from the rest of the strains which can be considered as less evolved with least substitution may be inferred as ancestor from the rest of the strains. *Phormidium tenue* BTA 803 of Mizoram showed divergent and found to be less substituted from the rest of the clusters. *Leptolyngbya* sp. BTA 356 of Nagaland, *Leptolyngbya* sp. BTA 287 of Tripura and *Leptolyngbya* sp. BTA 477 of Assam showed highest similarity in their characters and genetic distance though they belong to different location and habitats. *Phormidium* cf. *terebriforme* BTA 503 of Arunachal Pradesh and *Phormidium* sp. BTA 507 from Mizoram was found to be closely related sharing same ancestor with same genetic distance. *Leptolyngbya tenerrima* BTA 766 of Mizoram was found to be related with *Phormidium animale* BTA 258 of Arunachal Pradesh and found to have less substitution as determine by the phylogenetic tree. Environment and location may effect the genetic relation between the same genus/species. The 05 strains viz. *Phormidium animale* BTA 258, *Leptolyngbya* sp. BTA 287, *Leptolyngbya* sp. BTA 477, *Leptolyngbya tenerrima* BTA 766 and *Phormidium tenue* BTA 803 selected for highest carotenoids production did not determine their phylogenetic relatedness as 16S rRNA gene sequences not correlate with chemical variability among samples.

RAPD analysis results showed 88 bands out of which 13 showed monomorphism. It showed that the carotenoids production correlate with the geographical distribution variations and depends on the habitat it belongs. *Phormidium animale* BTA 258 and Linnothrix sp. BTA
obtained from Arunachal Pradesh with the exception of *Phormidium* sp. BTA 507 from Mizoram, though they lie at the same cluster their carotenoids production differs as their genetic distance varied. *Leptolyngbya* sp. collected from different location and habitats belong to Tripura and Mizoram but *Leptolyngbya* sp. BTA 287 showed high presence of carotenoids. Though they are genetically similar, carotenoids production were different. *Phormidium tenue* BTA 356, *Phormidium* cf. *terebriformis* BTA 503 and *Phormidium* sp. BTA 605 where as *Phormidium tenue* BTA 356 and *Phormidium* cf. *terebriformis* BTA 503 showed high similarity obtained both from moist soil which belong to Nagaland and Arunachal Pradesh but showed different carotenoids productions. *Leptolyngbya* sp. BTA 477 of Assam showed high similarity to *Phormidium tenue* BTA 803 of Mizoram but they produced carotenoids with a difference of 1.68 µg/ml.

Optimization of carotenoids production was carried out under the influence of three major factors viz. pH, nitrate and phosphate which were considered to be played a vital role in the growth and photosynthesis. 05 potent cyanobacterial strains were selected in depth investigation for carotenoids production viz. *Phormidium animale* BTA 258, *Leptolyngbya* sp. BTA 287, *Leptolyngbya* sp. BTA 477, *Leptolyngbya tenerrima* BTA 766 and *Phormidium tenue* BTA 803. *Phormidium animale* BTA 258 produced highest carotenoids in pH 7.5, normal nitrate concentration (1.5 gm/L) and phosphate concentration of 5N (0.20 gm/L). *Leptolyngbya* sp. BTA 287 strain produced highest carotenoids in pH 9.0, nitrate concentration of 5N (7.5 gm/L) and phosphate concentration of 5N (0.20 gm/L). *Leptolyngbya* sp. BTA 477 produced highest carotenoids in pH 6.5, nitrate concentration of N (1.5 gm/L) and phosphate concentration of 2N (0.20 gm/L). *Leptolyngbya tenerrima* BTA 766 produced highest carotenoids in pH 6.5, nitrate concentration of 2N (3.0 gm/L) and phosphate concentration of 4N (0.16 gm/L).
*Phormidium tenue* BTA 803 produced highest carotenoids in pH 8.0, nitrate concentration of N (1.5 gm/L) and phosphate concentration of 2N (0.08 gm/L).

*Phormidium animale* BTA 258 produced highest biomass in pH 6.5, 3N (4.5 gm/L) of nitrate and 2N (0.08 gm/L) of phosphate. *Leptolyngbya* sp. BTA 287 produced highest biomass in pH 9.0, N (1.5 gm/L) of nitrate and 2N (0.08 gm/L) of phosphate. *Leptolyngbya* sp. BTA 477 produced highest biomass in pH 6.0, 3N (4.5 gm/L) of nitrate and 2N (0.08 gm/L) of phosphate. *Leptolyngbya tenerrima* BTA 766 produced highest biomass in pH 8.0, 3N (4.5 gm/L) of nitrate and 4N (6.00 gm/L) of phosphate. *Phormidium tenue* BTA 803 produced highest biomass in pH 7.0, 4N (6.0 gm/L) of nitrate and N (0.04 gm/L) of phosphate.

*Phormidium animale* BTA 258 produced highest phycoerythrin (PE) and phycocyanin (PC) in pH 6.5 and allo-phycocyanin (APC) in pH 6.0. It indicated that this particular strain produced good percentage of phycobiliproteins in the acidic pH. *Leptolyngbya* sp. BTA 287 produced highest phycoerythrin (PE) and allo-phycocyanin (APC) in pH 8.0 while phycocyanin (PC) in pH 6.0. It showed that this particular strain performed better in the alkaline for PE and APC whereas PC in the acidic pH. *Leptolyngbya* sp. BTA 477 produced highest phycoerythrin, phycocyanin and allo-phycocyanin (APC) in pH 8. This strain was found to be best suited at the alkaline pH. *Leptolyngbya tenerrima* BTA 766 produced highest phycoerythrin in pH 6.5 and allo-phycocyanin in pH 6.0 whereas to the contrary phycocyanin was maximum in pH 8.5. *Phormidium tenue* BTA 803 produced highest phycoerythrin in acidic pH 6.5, but phycocyanin and allo-phycocyanin in pH 7.0 and pH 9.0 respectively.

Effect of nitrate on the phycobiliproteins productions also showed strain dependent. *Phormidium animale* BTA 258 produced highest phycoerythrin and phycocyanin in 5N and allo-phycocyanin in 2N concentration. *Leptolyngbya* sp. BTA 287 produced highest phycoerythrin,
phycocyanin and allo-phycocyanin in control (N). *Leptolyngbya* sp. BTA 477 produced highest phycoerythrin and allo-phycocyanin in 3N. *Leptolyngbya tenerrima* BTA 766 produced highest phycoerythrin, phycocyanin and allo-phycocyanin in 2N. *Phormidium tenue* BTA 803 produced highest phycoerythrin in 5N and allo-phycocyanin and phycocyanin in 3N.

Effects of phosphate on the production of phycobiliproteins showed strain dependent. *Phormidium animale* BTA 258 yielded highest phycoerythrin, phycocyanin and allo-phycocyanin in 3N. *Leptolyngbya* sp. BTA 287 yielded highest phycoerythrin and allo-phycocyanin in 4N. *Leptolyngbya* sp. BTA 477 yielded highest phycoerythrin, phycocyanin and allo-phycocyanin in 2N. *Leptolyngbya tenerrima* BTA 766 yielded highest phycoerythrin, phycocyanin and allo-phycocyanin in 5N. *Phormidium tenue* BTA 803 yielded highest phycoerythrin and allo-phycocyanin in 3N and phycocyanin in 4N.

Therefore, it may be concluded that the process optimization for enhancement of carotenoids production varied from strain to strain and factor to factor. The findings of this research work have highlighted the Oscillatoriales diversity in north eastern region of India. Lipid profiling and fatty acid composition, total carotenoids, process optimization through modification of certain environmental and nutritional factors and of course molecular identification was the key findings and investigated successfully.