International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 12, December 2015, PP 26-34
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Genetic Diversity of Free Living Filamentous Cyanobacteria Isolated from a Variety of Coal Mining Areas of Jaintia Hills District, Meghalaya, India

Nangkyntiewbor Jungai, Samrat Adhikari*

Department of Biotechnology, St. Edmund's College, Shillong, India.
stedmundc.btisnet@nic.in

Abstract: *Cyanobacteria are the microscopic photosynthetic cell factories that are known of its ubiquitous occurrence and sustainability in a variety of habitats. Due to these characteristics, they signify themselves as a potential candidate for varieties of biotechnological applications. The present study envisages with preliminary investigation of cyanobacterial diversity in coal mining areas of Jaintia Hills District, Meghalaya. A total of 116 samples were collected from 12 different ecosystems and analyzed. Most of the cyanobacterial isolates belong to the genera Anabaena sp, Nostoc sp, Calothrix sp, Tolypothrix sp and Roholtiella sp with Nostoc sp being the most abundant among all the cultures segregated in a variety of coal mining habitats. Diversity analysis indicated maximum Shannon's diversity index (H) in the mining areas and further supported by Simpson's Diversity of each genus. Furthermore, a total of selected 39 samples from these mining areas were subjected to molecular study which includes amplification of the 16S rRNA gene and procurement of the Accession numbers from GenBank. Genetic diversity among strains tested was determined with the sequences which were compared with those of representative heterocystous cyanobacteria available in databases. Phylogenetic tree was inferred by neighbor joining (NJ) distance method. The clusters are supported by bootstrap analysis and partly reflect the morphological similarity of the organisms.*

Keywords: *Cyanobacteria, 16s rRNA, diversity, coal mines, sequencing, phylogenetic relationship.*

1. INTRODUCTION

Cyanobacteria are the simplest and the oldest prokaryotic organisms to have evolved on earth. They can be used as experimental and model strains for studying the diversification of prokaryotic cells and the physiological processes occurring within the cell (Berrendero *et al.,* 2011). They are classified under gram negative bacterial phyla and occupy the diverse range of habitats. They show a wide range of morphological diversity ranging from unicellular to colonial and filamentous. Cyanobacteria are photosynthetic microorganisms by which they are capable to grow photo-autotrophically in a manner similar to those of eukaryotic algae and plants. They also have the unique ability to fix atmospheric nitrogen (Stanier, 1979; Gorl *et al.,* 1998). Cyanobacterial ecology can be best understood by matching isolated strains and their counterparts in nature. However, many species of cyanobacteria in culture produce anomalous morphologies that differ from those that are characteristic in nature (Rajaniemi *et al.,* 2005). Thus, classifications based on phenotypic characteristics do not exactly represent natural grouping when analyzed at the genetic level. To study the taxonomy of cyanobacteria, the base composition is an important genetic character. At all taxonomic levels above species, the sequence analysis of genes encoding small-subunit ribosomal RNA (16S rRNA) is currently the most promising approach for the phylogenetic classification of cyanobacteria (Wilmotte, 1994). The comparative analysis of 16S ribosomal RNA sequence has been used for the identification and construction of cyanobacterial phylogeny (Giovannoni *et al.,* 1988).

Meghalaya is one of the 8 states of the Northeastern India lying between 25°5´N and 16°10´N latitudes 89°47 E and 92°47´E longitudes covering an area of 22,429 sq.km. Physio- geographically the region consists of hilly terrains. Diverse terrestrial and aquatic ecosystems including hot springs and coal mining areas are predominantly available in this state. The Jaintia hill district of Meghalaya located at latitude of 25.5021272 and longitude of 92.341887 has been associated with concurrent establishment of many cement factories which uses coal and lime stones available in the nearby area for mining activities to sustain smelting operations in these factories. These uncontrolled and unscientific coal mining methods have deleteriously affected the environment and also the microbial

community in particular by discharging harmful chemicals in the environment. The microbial community existed in the vicinity of these coal mining areas predominantly participates in many bioremediation processes. Among these microbial community cyanobacteria plays a pivotal. Therefore, in the present study, coal mining areas were selected and cyanobacterial cultures were isolated from various ecosystems. These cultures were morphologically characterized and further analyzed based on the phylogeny of the 16S rRNA sequences.

2. MATERIALS AND METHODS

2.1. Study Area

According to the Indian Bureau of Mines the geological reserves of coal in Meghalaya is 460 million tons (IBM, 2005). However, recent explorations and other unofficial sources indicate a total reserve of about 600 million tons. The Jaintia Hills alone have about 40 million tons of coal (Krishnan, 1982). There are nine significant coal deposits – Bapung, Lakadong, Lumshnong, Malwar Musiang Lamare, Mutang, Sutnga, Jarain Shkentalang, Ioksi and Khliehriat (Raja Rao, 1981). The coal bearing areas of the district present a panorama of flat topped low hills, low vegetation and plateau of rolling grasslands intersected by river valleys. Coal mining is privately controlled by small-scale ventures and being the most profitable business rampant and scattered mining is going on in this area. Due to unscientific mining methods various environmental problems have cropped up. Samples were collected randomly from different types of ecosystem: terrestrial ground and aquatic (Table No. 2). The sites were selected carefully to include diverse environments in terms of altitude, pH and temperature, moist or water logged conditions as well as hilly or low lying terrain. Aquatic samples were collected in specimen collection tubes. Soil samples were collected by scrapping the surface of the soil.

2.2. Isolation, Purification and Cultivation of Cyanobacteria

Axenic clonal cultures of cyanobacteria collected from different habitats were grown in $BG11_0$ medium or solid medium (with 1.5% agar) in an air conditioned chamber at 24 ± 2^oC with a photon fluence rate of 50µmol m^{-2} s^{-1} and control illumination of 12 hours light and 12 hours dark (Rippka *et al.*, 1979). Pure culture of cyanobacterial isolates were obtained from processed samples by serial dilution technique and direct streak plate method. Axenic cultures of cyanobacteria were obtained by treating the cells with standard antibiotics namely Streptomycin, Nalidixic acid and Ampicillin (Packer and Glazer, 1988).

2.3. Morphological Analysis, Richness and Diversity of Cyanobacteria

The cyanobacterial species isolated from coal mining areas were identified based on Morphology, cell specialization, colony/filament morphology (Desikachary, 1959). All the above mentioned parameter were studied using Olympus 45× light microscope with a digital camera. Morphological characteristics includes shape, polarity, dimensions, division planes, color, shrath and motility. Cell specialization studies include heterocyst formation, akinetes, baecytes and necridia formation. Colony/filament studies includes cells shapes & symmetry, trichome type, shape of the terminal ell, terminal hairs, false/true branching and presence and distribution of specialized cells. Total number of strains under each genus was counted for estimating diversity and richness of cyanobacteria in study area. Genetic diversity was estimated by Shannon –Weiner Index (H) (Shannon and Weaver, 1949) and Simpson's Diversity (1-D) (Simpson, 1949) using the following formula

Shannon –Weiner Index, $H= -Spi \ In \ pi$

Simpson's Dominance, $D = S (pi)^2$

Simpson's Diversity Index, $1-D= 1- S (pi)^2$

Where pi is the total no. of strains of genus i/total no. of all strains

Besides these, the frequency of occurrence of the common cyanobacterial strains was further analysed through R software (R Core Team, 2015) and a heat map, using gplots package (Gregory et al, 2015), for the same is generated.

2.4. DNA Extraction, Agarose Gel Electrophoresis and DNA Recovery

Selected cyanobacterial cultures from different coal mining areas were selected based on the abundance occurrence for DNA extraction. Total DNA was extracted by harvesting cultures within the late exponential phase of growth. DNA was extracted from cell pellets by using DNA Purification

kit (Promega Corporation Inc, USA) following the manufacturer's protocol. Extracted DNA was stored in 10 mM Tris at -20^{o}C. Agarose gel electrophoresis was performed following standard protocol (Sambrook and Russel, 2001) using 1.0 X TAE buffer (44.5mM Tris, 44.5mM glacial acetic acid and 1.0 mM EDTA). The DNA was visualized with fluorescent dye ethidium bromide by direct examination of the gel under UV light. λDNA digested with Hind III was used as a marker throughout.

2.5. Amplification of the 16S rRNA

The 16S rRNA gene was then amplified using three sets of universal primers 16S1F & 16S740R (Seo and Yokota, 2003), CYA359F, ÇYA781(A)R & CYA781(B)R (Nubel *et al.,* 1997), CYA359F & 16S149R (Tillett *et al.,* 2001) in separate PCR reactions. The sequence of the primers and the approximate length of the PCR amplicons are given in Table 2. PCR amplifications were performed with a MultiGene Mini Thermal Cycler with reaction mixture (25 µl) consisting of 100ng of genomic DNA, 1.5mM $MgCl_2$, 10mM of each dNTP, 1x Taq buffer, 10µM of each primer and 1 unit of Taq polymerase. Samples were subjected 5 min of initial denaturation at 94°C and 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C, 1 min extension at 72°C, followed by final extension of 10 minutes at 72°C. Visualisation of the amplified DNA product was performed using agarose gel electrophoresis s described previously.

2.6. Direct Sequencing of the Amplified DNA

The amplified fragments were directly sequenced at 1st BASE DNA Sequencing Services, Malaysia. The contiqs obtained using the three sets of primers were checked for overlaps and assembled in DNA Baser Software v4.20.0. The assemble sequences were further trimmed for removal of ambiguous bases using Sequencher 4.0 software and finally submitted to GenBank database in NCBI.

2.7. Phylogenetic Analyses

The 16S rRNA gene sequences obtained were initially compared with sequences available in the National Center for Biotechnology Information database using BLAST network services (http://www.ncbi.nlm.nih.gov/BLAST) to determine their approximate phylogenetic affiliations (Altschul *et al.,* 1997). Multiple sequence alignments were generated using the CLUSTAL W program (Thomson *et al.,* 1994). Phylogenetic distance trees were then inferred by neighbor joining (NJ) (Saitou and Nei, 1987) using the Jukes-Cantor model (Jukes and Cantor, 1969) in MEGA 6.06 (Tamura *et al.,* 2013).

3. RESULTS AND DISCUSSIONS

Role of cyanobacteria in improving soil health and fertility, bioremediation potential has been well known and various reports are available, but the distribution of cyanobacterial species in the coal mining areas needs to be explored. While exploring the occurrence of the cyanobacteria species diversity in the coal mining areas, a coordinated effort between laboratory and field level research was needed. Region-specific cyanobacterial isolates could be more effective in such applications as they are pre-acclimatized to the existing environmental conditions. Hence, a region specific biodiversity study of the coal mining areas is important for deriving optimum benefits from these indigenous strains. Moreover, knowledge of cyanobacterial species diversity in this region would render them as potential candidates in decontamination of metals associated with rampant mining and also in other biotechnological applications. Thus, this investigation of cyanobacterial diversity is ecologically significant as it enumerates diversity, abundance, dominance and richness of various cyanobacteria in the coal mining region. Furthermore, such findings are important as they pinpoint biological strains that can be used to improve quality and enhance further exploration for bioremediation activities in those areas.

One hundred and sixteen samples were collected from twelve independent sites located in different major coal mining areas of Jaintia hills district of Meghalaya (Table 1). Almost majority of sites constitutes uncontrolled and unscientific mining operations and samples collection was concentrated to rice fields/soil and water samples (Table 2). Based on the morphological characteristics, the cultures isolated from these various sites were observed and concluded that they belong to heterocystous group of cyanobacteria with 50 % cultures belonging to the genera *Nostoc* and 25.8 % of the cultures belong to the Genera *Anabaena*. Predominance of the genera *Nostoc* in all the

collection sites irrespective of other influencing factors including terrain, water logging, limestone smelting discharges, temperature, and moisture content indicates towards their versatility, competitiveness and ability to occupy diverse ecological adaptations. Other cultures which are less predominantly prevalent belong to the genera *Calothrix*, *Roholtiella* and *Topylothrix* consisting of 7.7 %, 9.4 % and 6.8 % of the total cultures isolated for enunciating the biodiversity of the region. The frequency of occurrence of the five predominant genera is depicted in the form of Heat map (Fig 1) which the colour intensities signifying the frequencies. The dendrogram in the heat map also shows the relationship of the cyanobacterial genera which can be seen in the formation of the two distinct clusters. Khliehriat, Bataw, Lumshnong and Ladrymbai primarily have large amount of coal deposit in compared to the other sites of the Jaintia Hills (Raja Rao, 1981). Statistical analysis revealed high species richness and high Shannon Diversity indices in these two coal belt areas. Such values suggest that extreme unscientific coal mining has no effect on the cyanobacterial diversity and they could resist the harsh release of chemicals exhibit out from the mining. Also, the widespread of the cultures *Nostoc* and *Anabaena* in these belts indicates their resilience and high adaptability in varied type of ecosystem. However, *Calothrix*, *Roholtiella* and *Topylothrix* of cyanobacteria were not found in abundance in all the areas as mentioned in Table 1, but they do indicate the fact that these cultures also contributes to the their individual adaptability in extreme environments. In context to the diversity study richness based on Shannon Weiner Index, Simpson index and Simpson Dominance the data in Table 1 relates to the abundance of different Genera/species of a group of organism in an area and stands an accurate measurement of different kinds of Genera/species in that particular area. In context to the present study, low generic richness was observed in Bapung, Sohkymphor, Shangpung area whereas average mild generic richness were observed in the Kongong, Sutnga, Rymbai, Bataw, Shkentalang and Iooksi area. This particular type of diversity strongly depends on the various environmental factors pertaining to the coal mining areas.

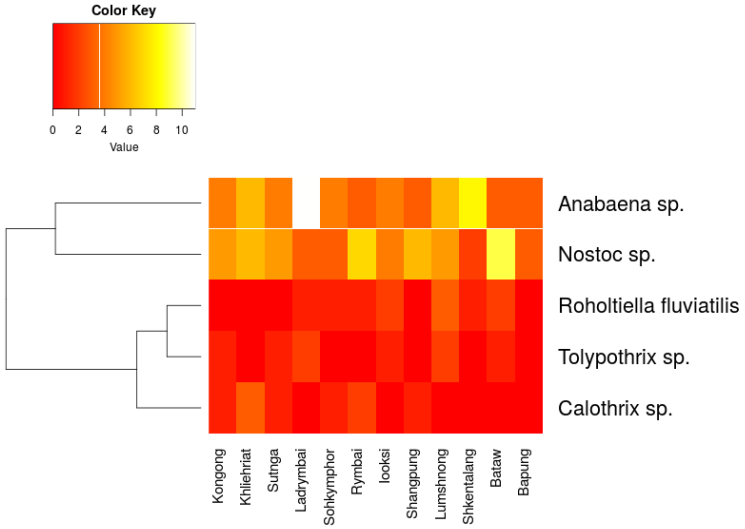

Fig1. *Distribution and frequency (Heat map using R Package) of the isolated cyanobacterial samples in the coal mining sampling sites of Jaintia Hills District of Meghalaya.*

Table1. *Distribution and relative frequency of cyanobacteria in the coal mining sampling sites of Jaintia Hills District of Meghalaya and also the ecological attributes of cyanobacterial communities calculated with average values obtain*

	Sampling Sites (Coal Mining areas)												
Cultures	*Kongong*	*Sutnga*	*Khliehriat*	*Ladrymbai*	*Sohkymphor*	*Rymbai*	*Bataw*	*Shangpung*	*Lumshnong*	*Shkentalang*	*Iooksi*	*Bapung*	*Total*
Anabaena sp.	*4*	*4*	*6*	*11*	*4*	*3*	*3*	*3*	*6*	*8*	*4*	*3*	*30*
Nostoc sp.	*5*	*5*	*6*	*3*	*3*	*7*	*9*	*6*	*5*	*2*	*4*	*3*	*58*
Calothrix sp.	*1*	*1*	*3*	*0*	*1*	*2*	*0*	*1*	*0*	*0*	*0*	*0*	*9*
Roholtiella fluviatilis	*0*	*0*	*0*	*1*	*1*	*1*	*2*	*0*	*3*	*1*	*2*	*0*	*11*
Topylothrix sp.	*1*	*1*	*0*	*2*	*0*	*0*	*1*	*0*	*2*	*0*	*1*	*0*	*8*
TOTAL	*11*	*11*	*15*	*17*	*9*	*13*	*15*	*10*	*16*	*11*	*11*	*6*	*116*
Shannon Weiner Index	*2.87*	*2.28*	*3.57*	*3.21*	*2.2*	*2.41*	*2.98*	*2.10*	*2.98*	*2.71*	*2.7*	*1.94*	
Simpson index	*0.78*	*0.89*	*0.88*	*0.87*	*0.68*	*0.89*	*0.98*	*0.56*	*0.85*	*0.75*	*0.79*	*0.84*	
Simpson Dominance	*0.48*	*0.29*	*0.40*	*0.39*	*0.39*	*0.26*	*0.31*	*0.32*	*0.28*	*0.31*	*0.24*	*0.24*	

Table2. *Cyanobacterial Collection sites from Coal Mining areas in Jaintia Hills, Meghalaya*

Area/Locality	Location		No. of Samples Collected	Type of ecosystems
	Latitude	**Longitude**		
Khliehriat	25˚ 35'67" North	92˚ 36'40" East	*10*	*Soil, Water*
Sutnga	25˚ 37'61" North	92˚ 44'55" East	*11*	*Rice fields, stagnant pond*
Kongong	25˚ 35'03" North	92˚ 36'14" East	*11*	*Soil, water*
Bapung	25˚ 38'31" North	92˚ 31'55" East	*06*	*Water, rice field*
Sohkymphor	25˚ 41'60" North	92˚ 35'91" East	*05*	*Rice fields, stagnant pond*
Rymbai	25˚ 33'21" North	92˚ 32'33" East	*08*	*Soil, Water*
Bataw	25˚ 62'04" North	92˚ 88'87" East	*11*	*Water, rice field*
Shangpung	25˚ 48'12" North	92˚ 34'92" East	*09*	*Soil, Water*
Lumshnong	25˚ 34'65" North	92˚ 18'35" East	*10*	*Soil, Water*
Shkentalang	25˚ 32'53" North	92˚ 14'26" East	*11*	*Water, rice field*
Iooksi	25˚53'18" North	92˚ 54'29" East	*12*	*Rice fields, stagnant pond*
Ladrymbai	25˚ 37'08" North	92˚ 32'58" East	*14*	*Water, rice field*

Total no. of samples collected = 116

Microscopic identification of cyanobacteria is rapid and also at the same time sensitive and forms the basis of preliminary identification (Echlin, 1966). However, with skilled and experienced operators, identification is possible only at the genus level. Thus, molecular approaches are particularly useful in the detection and identification of specific strains especially for those cultures which are morphologically identical at the species level. Genetic identification has been used to discriminate between toxic and nontoxic strains of cyanobacteria like *Microcystis*, *Anabaena* and *Cylindrospermopsis* (Wilmotte and Golubic, 1991). Phylogenetic analysis based on the 16s rRNA gene take considerable time and effort yet they provide certainty in the identification of Genera using DNA sequences. In an approach to have a compressive data on species identification of the cyanobacterial cultures isolated from the coal mine areas, a 16s rRNA fingerprinting approach has been followed in the present study based on the primers reported by Nubel *et al.* (1997). On the basis of published 16s rRNA sequences, the versatility of these primers pairs was thoroughly reviewed, and after extensive databases searches and cross referencing, the primer pairs as listed in Table 3 were determined which have been used for amplifying the 16S rRNA of the cyanobacterial isolates. The primers selected primer have been categorized into three regions RI, RII and RIII with targeted amplified amplicons of 730 bp, 740 bp and 1040bp. 39 cyanobacterial isolates from 12 different coal mining regions were selected from the total 116 as majority of the cultures belonging to the *Nostoc* and *Anabaena* genera. The 16s rRNA sequences of 39 cyanobacterial isolates selected from different coal mining areas were initially compared with the sequences available in the National Centre for Biotechnology Information database using BLAST network services to determine their approximate affiliations (Altschul *et al.*, 1997) and the sequence data were submitted to GenBank (KR709104-KR709143). The average lengths of the amplicons with corresponding Strain numbers and GenBank Accession numbers are depicted in Table 4. Furthermore, based on the similarity in the BLAST hits, a phylogenetic tree based on 16S rRNA sequences collected from the 12 coal mining sites was constructed using MEGA 6.0 software (Figure 2). The evolutionary history was inferred by using the Neighbor-Joining method to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The phylogenetic tree of the 16s rRNA were inferred based on the sequences obtained from the selected cyanobacterial species from the coal mining sites and analysis reveals that they fall into the 5 classes of cyanobacteria (*Nostoc* sp, *Anabaena* sp, *Tolypothrix* sp, *Calothrix* sp *and Roholtiella* sp) which are predominantly distributed in various geographical locations in coal belt. Maximum of the coal belt areas shows the presence of *Nostoc* sp, *Anabaena* sp and *Tolypothrix* sp whereas areas like Shangpung and Shkentalang show presence of *Calothrix* sp and *Roholtiella* sp which on the other hand confirms the morphological identification. Further analysis of the sequences shows nucleotide frequencies of 28.09% (A), 16.99% (T/U), 21.20% (C), and 33.72% (G) including the codon positions of 1st+2nd+3rd+Noncoding. The

equality of evolutionary rate between sequences of (*E.coli* (M25588.1)) and (*Anabaena_variabilis*_SN416(KR709104)*), with sequence of (*Anabaena*_sp._SN417(KR709105)*) used as an outgroup in Tajima's relative rate (Tajima, 1993) test statistic was 420.23 (P <0.05) .

Table3. *Primers used for the amplification of 16S rRNA gene*

Region	Primer	Sequence	Length of PCR amplicon
16S rRNA I region	16S1F	5'- AGAGTTTGATCCTGGCTCAG-3'	730bp
	16S740R	5'-TCTACGCATTTCACCGCTAC-3'	
16S rRNA II region	CYA359F	5'- GGGGAATTTTCCGCAATGGG-3'	740bp
	ÇYA781(A)R	5'- GACTACTGGGGTATCTAATCCCATT-3'	
	CYA781(B)R	5'- GACTACCAGGGGTATCTAATCCCTTT-3'	
16S rRNA III region	CYA359F	5'- GGGGAATTTTCCGCAATGGG-3'	1040bp
	16S149R	5'- GTACGGCTACCTTGTTACGAC-3'	

Table4. *List of 39 cultures with GenBank Accession Numbers.*

rganism	Length in bp	GenBank Accession Numbers	Location
Anabaena variabilis SN416	1440	*KR709104*	*Kongong*
Nostoc muscorum SN434	1433	*KR709122*	*Kongong*
Topylothrix sp. SN439	1126	*KR709127*	*Kongong*
Anabaena variabilis SN452	1433	*KR709140*	*Kongong*
Anabaena sp. SN417	1439	*KR709105*	*Sutnga*
Nostoc sp. SN440	1448	*KR709128*	*Sutnga*
Nostoc sp. SN449	1449	*KR709137*	*Sutnga*
Anabaena variabilis SN424	1528	*KR709112*	*Shangpung*
Roholtiella fluviatilis SN435	1432	*KR709123*	*Shangpung*
Nostoc sp. SN426	1460	*KR709114*	*Bapung*
Nostoc sp. SN432	1440	*KR709120*	*Bapung*
Anabaena variabilis SN441	1443	*KR709129*	*Bapung*
Anabaena sp. SN430	1427	*KR709118*	*Khliehriat*
Anabaena variabilis SN443	1449	*KR709131*	*Khliehriat*
Nostoc muscorum SN420	1506	*KR709108*	*Khliehriat*
Anabaena variabilis SN433	1445	*KR709121*	*Bataw*
Nostoc sp. SN418	1432	*KR709106*	*Bataw*
Tolypothrix tenuis SN436	1458	*KR709124*	*Ladrymbai*
Anabaena variabilis SN427	1445	*KR709115*	*Ladrymbai*
Anabaena sp. SN442	1421	*KR709130*	*Ladrymbai*
Anabaena sp. SN431	1449	*KR709119*	*Ladrymbai*
Anabaena sp. SN453	1136	*KR709141*	*Ladrymbai*
Nostoc muscorum SN455	1440	*KR709143*	*Ladrymbai*
Nostoc commune SN444	1447	*KR709132*	*Shkentalang*
Calothrix sp. SN438	1448	*KR709126*	*Shkentalang*
Anabaena sp. SN428	1435	*KR709116*	*Shkentalang*
Nostoc muscorum SN445	1442	*KR709133*	*Iooksi*
Anabaena sp. SN425	1434	*KR709113*	*Iooksi*
Anabaena variabilis SN448	1450	*KR709136*	*Iooksi*
Topylothrix sp. SN446	1436	*KR709134*	*Lumshnong*
Anabaena sp. SN447	1446	*KR709135*	*Lumshnong*
Nostoc sp. SN419	1435	*KR709107*	*Lumshnong*
Anabaena flos-aquae SN429	1447	*KR709117*	*Lumshnong*
Nostoc carneum SN437	1435	*KR709125*	*Rymbai*
Nostoc commune SN450	1443	*KR709138*	*Rymbai*
Anabaena sp. SN422	1441	*KR709110*	*Rymbai*
Anabaena sp. SN421	1385	*KR709109*	*Sohkymphor*
Nostoc muscorum SN451	1441	*KR709139*	*Sohkymphor*
Nostoc sp. SN454	739	*KR709142*	*Sohkymphor*

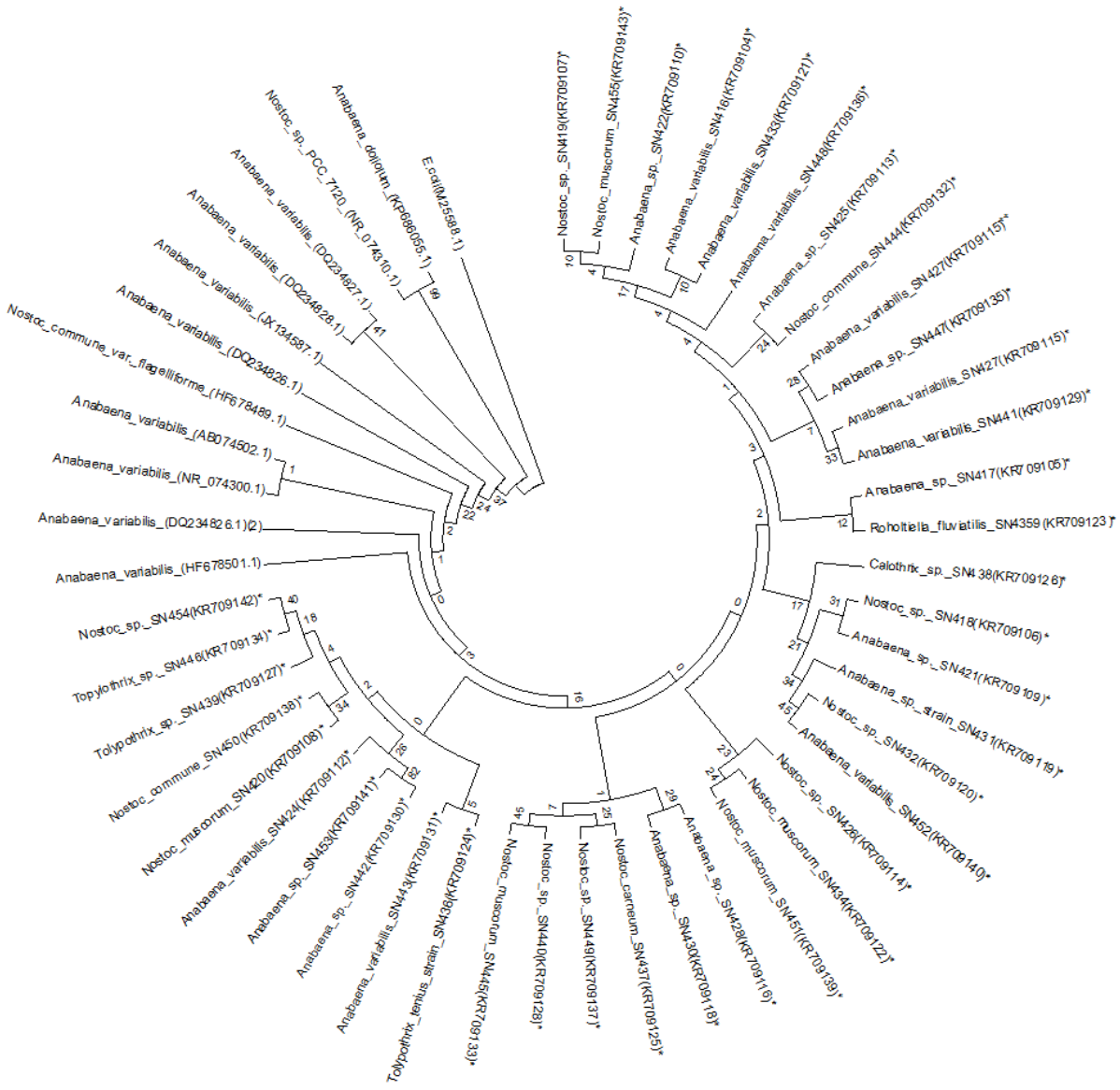

Fig2. *Neighbour Joining (NJ) phylogenetic tree for the 16S rRNA gene sequences of the 39 cyanobacterial cultures with other sequences available in public databases. Bootstrap Values are indicated in the point at the nodes.*

4. CONCLUSION

The study reveals the abundance of the genera *Nostoc, Anabaena, Calothrix, Tolypothrix and Roholtiella* in the study area. It can be seen that cyanobacteria could thrive even in environments with high levels of toxic chemicals as in coal mining areas. Also, it shows their versatility and high adaptability in different types of ecosystem. The 16S rRNA analysis shows the resemblance between the cyanobacterial strains isolated and their relationship with a variety of the heterocystous cyanobacterial species. More studies are required to further investigate, evaluate and explore the potentiality of these cyanobacteria.

ACKNOWLEDGEMENT

We take this opportunity to acknowledge the funding received from the Department of Biotechnology, Govt. of India, for setting up Institutional Biotech Hub under NER BPMC programme with a sanction number Order No BT/22/NE/2011. This work has been done in collaboration with the Bioinformatics Centre, St. Edmund's College, Shillong. The authors are also grateful to Dr Sylvanus Lamare, Principal, St. Edmund's College, Shillong for his valuable support towards the work.

REFERENCES

[1] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lippman, D.J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research, 25, 3389-3402.

[2] Berrendero, E., Perona, E. and Mateo, P. (2011). Phenotypic variability and phylogenetic relationships of the genera *Tolypothrix* and *Calothrix* (Nostocales, Cyanobacteria) from running water. International Journal of systematic and Evolutionary Biology, 61, 3039-3051.

[3] Desikachary, T.V. (1959). Cyanophyta, Indian Council of Agricultural Research, New Delhi. 686 pp

[4] Echlin, P. (1966). The Blue-Green Algae. Scientific American, 214(6):74-81.

[5] Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791

[6] Gregory, R. W., Ben, B., Lodewijk, B., Robert, G., Wolfgang, H., Andy, L., Thomas, L., Martin, M., Arni M., Steffen, M., Marc, S. and Bill, V. (2015). gplots: Various R Programming Tools for Plotting Data. R package version 2.17.0. http://CRAN.R-project.org/package=gplots

[7] Geological Survey of India. (1981). Coalfields of northeastern India, in Raja Rao, C.S., ed., Coalfields of India, volume I: Calcutta, India, Geological Survey of India Bulletin Series A, no. 45, 76 p., 15 pls.

[8] Giovannoni, S.J., Turner, S., Olsen, G.J., Barns, S., Lane, D.J. and Pace, N.R. (1988). Evolutionary Relationships among Cyanobacteria and Green Chloroplasts. Journal of Bacteriology, 170, 3584-3592.

[9] Gorl, M., Sauer, J., Baier, T. and Forchhammer, K. (1998). Nitrogen-starvation-induced chlorosis in Synechococcus PCC 7942: Adaptation to long-term survival. Microbiology, 144, 2449-2458.

[10] Indian Minerals Yearbook. (2005). Indian Bureau of Mines, Nagpur, (coal) pp. 1-22

[11] Jukes, T.H. and Cantor, C.R. (1969). Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, pp. 21-132, Academic Press, New York.

[12] Krishnan, M.S. (1982). Geology of India and Burma. 6th Edition, CBS Publishers and Distributers, Delhi, 536p.

[13] Nubel, U., Garcia-Pichel, F. and Muyzer, G.(1997). PCR Primers To Amplify 16S rRNA Genes from Cyanobacteria. Applied And Environmental Microbiology, 63(8), 3327-3332.

[14] Packer, L. and Glazer, A.N. [Eds] (1988). Cyanobacteria. Methods in Enzymology. Volume 167, Academic Press, New York, 915 pp.

[15] Rajaniemi. P., Hrouzek, P., Kastovaska, K., Willame, R., Rantala, A. and Hoffman, L. (2005). Phylogenetic and morphological evaluation of the Genera Anabaena, Aphanizomenon, Trichormus and Nostoc (Nostocales, Cyanobacteria). . International Journal of systematic and Evolutionary Biology, 55, 11-26.

[16] R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[17] Raja Rao, C.S. (1981). Coalfields of India, vol.I- Coalfields of North Eastern India, Bulletin Series A, No. 45, Geol. Surv. India, 76p.

[18] Rippka, R., Derueless, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979). Generic Assignment, strain histories, and properties of pure cultures of cyanobacteria. Journal of General Microbiology, 111, 1-61

[19] Sambrook, J. and Russel, D.W. (2001). Molecular Cloning: A Laboratory Manual, Vol 1, pp 5.4. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

[20] Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

[21] Seo, P.S. and Yokota, A. (2003). The phylogenetic relationships of cyanobacteria inferred from 16S rRNA, *gyrB*, *rpoC1* and *rpoD1* gene sequences. Journal of General and Applied Microbiology, 49, 191-203

[22] Shannon, C.E. and Weaver, W. (1949). The Mathematical Theory of Communication. Urbana: University of Illinois Press.

[23] Simpson, E.H. (1949). Measurement of Diversity. Nature 163:688

[24] Sneath, P.H.A. and Sokal, R.R. (1973). Numerical Taxonomy. Freeman, San Francisco.

[25] Stanier, R.Y. (1979). Generic Assignments: Strain histories and properties of pure cultures of cyanobacteria. Microbiology, 110, 1-61.

[26] Tajima, F. (1993). Simple methods for testing molecular clock hypothesis. Genetics 135:599-607.

[27] Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution30: 2725-2729.

[28] Thomson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. Nucleic Acid Research, 22 4673-4680.

[29] Tillett, D., Parker, D.L. and Neilan, B.A. (2001). Detection of Toxigenecity by a Probe for the Microcystin Synthetase A Gene *(mcyA)* of the Cyanobacterial Genus *Microcystis*: Comparison of Toxicities with 16S rRNA and Phycocyanin Operon (Phycocyanin Intergenic Spacer) Phylogenies. Applied And Environmental Microbiology, 67(6), 2810-2818.

[30] Wilmotte, A. (1994). Molecular evolution and taxonomy of the cyanobacteria, p. 1-25. *In* D. A. Bryant (ed.), The molecular Biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.

[31] Wilmotte, A. and Golubic, S. (1991). Morphological and genetic criteria in the taxonomy of Cyanophyta/Cyanobacteria. Algological Studies (Stuttgart) 64: 1-24.

AUTHORS' BIOGRAPHY

Nangkyntlewbor jungal, was born in shillong, India in 1988. He has obtained a master degree in biotechnology at North Eastern Hill University, Shillong, Department of Biotechnology and Bioinformatics (2011). His areas of research are in the fields of genetic engineering, cyanobacterial biotechnology, cancer and cell cultures and traditional medicinal plants too. At present, he is working as a senior research fellow (SRF) in the Institutional Biotech Hub, St. Edmund's College, Shillong under the sponcership of Department of Biotechnology (DBT), Government of India (GOI). He has also participated in various seminars and workshops at the national level.

Samrat Adhikari, was born in shilling, Meghalaya in 1979. He has obtained the doctoral degree from North Eastern Hill University, Shillong (2011) in the field of environmental biotechnology and the masters with specialization in molecular microbiology (2002) from Bangalore university, Bangalore. He has received the Biotech Industrial Training Programme fellowship (2003) and also has a vast teaching and research experience for 12 years. Currently he is an assistant professor & the head of the Biotechnology Department, St. Edmund's College, Shillong. He has published 5 papers in peer reviewed journals and 3 in national & international conferences. He has undergone many training and also has organized many workshops for young researchers. He is also the reviewer of many academic journals. He has supervised for 3 M. Tech thesis and is currently handling 3 projects sponsored by the different funding agencies of Govt. of india. He is presently a member of many academic bodies in universities. At present, he is working on environmental biotechnology & bioinformatics.