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Fundamental Idea of Evolutionary Relationships among Cyanobacteria using 16s RRNA and RBCL Gene Sequences

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Abstract

Cyanobacteria are aquatic and autotrophic organisms. They are found all over the world, generally in terrestrial, freshwater and marine habitats. But the blooms are found in fresh water. 16S rRNA is used for identification of diversity of prokaryotic organisms as well as other organisms. So it helps in the study of phylogeny among them. Here we have collected 16S gene sequences of 122 cyanobacterial species determine the phylogeny among them. rbcL (Ribulose-biphosphate carboxylase/oxygenase) is also a gene which is widely sequenced from numerous plant taxa and the resulting database is aided in plant physiology. So we have collected rbcL gene sequences of 46 cyanobacterial species and determine the evolutionary relationship among them. The evolutionary relationships between all organisms are called phylogeny and are represented by phylogenetic tree. Phylogenetic tree not only give the relationship among the species but also gives the proper position of the species in cyanobacterial classification.

Keywords: Cyanobacteria, 16S RRNA, rbcL, Mega, Phylogenetic tree.

Introduction

Cyanobacteria are identified as the primitive and most important group of bacteria on earth¹. Cyanobacteria are formly called "blue-green algae". They are generally found in all over the world, basically in terrestrial, freshwater and marine habitates but the blooms are found in fresh water²⁻³. According to the biologists, still now about 5 to 100 million species⁴⁻⁵ of organisms are living on earth⁶⁻⁷. From the morphological and biochemical evidence and also gene sequence data, all organisms of earth are genetically related. This relationship of living organisms can be represented by evolutionary tree or phylogenetic tree⁸⁻⁹. Phylogenetic tree is the graphical representation between the relationships among the species of interest in evolutionary history¹⁰. It is a branching diagram or "tree" based upon similarities and differences in physical or genetic characteristics of different species. The term "phylogenetics" is derived from the Greek term 'phyla' and 'phylon', indicating "tribe" and "race", and the term 'genetikos', which mean "relative to birth", from genesis "origin" and "birth". Phylogenetic study of organisms helps us to explain similarity and differences among organisms. The importance of phylogenetic tree is that it provides a rigorous framework for research to guide all biological sub disciplines, and therefore it gives an ideal model for the organization of biological knowledge¹¹. The phylogenetic analysis of cyanobacteria supports the hypothesis that the capable of cell differentiation are decended from common ancestor. The evolutionary tree or phylogenetic tree represents the phylogeny of organisms¹². Molecular phylogeny has become a powerful tool which helps to elucidate evolutionary pattern and the

analysis is based on 16S rRNA sequences¹³. Two genes are analised here: 16S rRNA and rbcl which codes for the large subunit of ribulose 1,5-biphosphate carboxylase/oxygenase¹⁴⁻¹⁵. The 16S rRNA¹⁶ gene have the hyper variable region which provides a species specific signature sequence that is useful for bacterial identification process¹⁷⁻¹⁸. rbcL (Ribulose-biphosphate carboxylase / oygenase) have the slow synonymous nucleotide substitution rate of ¹⁹⁻²⁰ by comparing with nuclear genes and its functional constraint that reduce the evolutionary rate of non synonymous substitutions. *RbcL* is considered to be more useful than isozymes²¹⁻²². Here we have analyzed the sequences by MEGA 5.0^{23} . using software, version MEGA (Molecular Evolutionary Genetics Analysis) is software which is freely available in internet. It is used for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees. There are two types of sequence alignments are involved for the preparation of phylogenetic tree, Such as; global alignments and local alignments. Global alignment is a process which calculates the alignment in the form of global optimization that "forces" the alignment to span the entire length of all query sequences. Similarly, local alignment is a process which identifies the regions of similarity within long sequences that are often widely divergent. The applications of computer to perform the computational models and simulations in studies of complex biological phenomena are in silico biology. In bioinformatics, an alignment of sequence is a process of arranging sequences of DNA, RNA, or protein. It helps to identify the regions of similarity which gives the structural, functional, or evolutionary relationships among them. After alignment the nucleotide sequence or amino acid sequence residues are represented as

rows within a matrix. The inserted gaps between the residues indicate that identical or similar characters are aligned in successive column. Multiple sequence alignment (MSA) is a sequence alignment process of three or more sequences of protein, DNA, or RNA²⁴. In many cases, the sequences are assumed to have an evolutionary relationship by which they share a lineage and are monophyletic. Most multiple sequence alignment methods try to minimize the number of insertions/deletions (gaps) and, as a consequence, produce compact alignments²⁵.

Materials and Methods

The nucleotide sequences of 168 cyanobacteria and one out group, containing 16S rRNA gene and *rbcL* gene have retrieved from NCBI (National Center for biotechnology information) database in FASTA format²⁶. FASTA enables the user to compare a query sequence against large databases²⁷. Out group helps to get the independent information about the relationship among all the cyanobacteria and reduce tree search space and also root unrooted trees. Uses of an out group are a type of experimental control. An appropriate out group has a greater genetic distance results a longer branch length than any other sequences, which will appear near the root of a rooted tree.

An appropriate out group should be moderately related to the sequence of interest; too close a relationship defeats the purpose of the out group and too distant adds noise to analysis. Here we took *Thermus caliditerrae* strain YIM77925 as an out group for 16S rRNA sequences and *Thermus caliditerrae* strain YIM77777 for *rbcL* gene sequences. This *Thermus caliditerrae* undergoes the Order-Thermales of Kingdom Bacteria.

Rbcl is considered to be more useful than isozymes²⁸. To align 16S rRNA gene sequences and rbcL genes by using MEGA software; version 5.0, first we have to remove the sequences containing non-A, T, G, C character and less than 800bp length. After treaming the sequence properly, start the alignment by clustalW processs. After alignment of the sequences properly save it in fasta format. Then construct the phylogenetic tree in maximum likelihood method by using mega software; version 5.0. This Maximum likelihood method is broadly similar to maximum-parsimony method. This method uses standard statistical technique which has the possibilities to get particular phylogenetic tree. This method requires the evolution at different sites and along different lineages must be statistically independent. So it is well suited to the analysis of distantly related sequences. The 16S rRNA and rbcL gene sequences are analyzed by Mega software. The species under studied are.

Sl. No.	Family	Sc. Name	Strain	Accession No.
1	Chroococcaceae	Chroococcus turgidus	HUW 799	DQ460703.1
2	Chroococcaceae	Chroococcus turgidus	SERB 26	KM982575.1
3	Chroococcaceae	Chroococcus turgidus	AICB61	KJ746515.1
4	Chroococcaceae	Gloeocapsa sp.	KSU-WH-8	KT807480.1
5	Chroococcaceae	Gloeocapsa sp.	HKAR-9	KJ470767.1
6	Chroococcaceae	Microcystis panniformis	strain: VN354	AB666074.1
7	Chroococcaceae	Microcystis wesenbergii	-	D89034.1
8	Chroococcaceae	Microcystis viridis	-	D89033.1
9	Chroococcaceae	Microcystis aeruginosa	-	D89032.1
10	Chroococcaceae	Microcystis aeruginosa	-	D89031.1
11	Chroococcaceae	Microcystis panniformis	strain: VN425	AB666076
12	Chroococcaceae	Microcystis panniformis	strain: VN355	AB666075
13	Oscillatoriaceae	Lyngbya sp.	JW-2010a	HQ419205
14	Oscillatoriaceae	Lyngbya sp.	UTEX LB 2516'	HQ419209
15	Oscillatoriaceae	Lyngbya sp.	JW-2010b	HQ419206
16	Oscillatoriaceae	Lyngbya sp.	Ind63	JQ029931
17	Oscillatoriaceae	Lyngbya sp.	UIC 10445	KJ813000
18	Oscillatoriaceae	Oscillatoria limosa	-	AF337653
19	Oscillatoriaceae	Oscillatoria limosa	SAG 42.87	KM019961
20	Oscillatoriaceae	Oscillatoria nigroviridis	3LOSC	EU244875
21	Oscillatoriaceae	Oscillatoria acuminate	strain PCC 6304	NR 102463

Table-1

Sl. No.	Family	Sc. Name	Strain	Accession No.
22	Oscillatoriaceae	Oscillatoria acuminate	PCC 6304	KM019978
23	Oscillatoriaceae	Oscillatoria lutea	-	AB115967
24	Oscillatoriaceae	Oscillatoria sancta	PCC 7515	AF132933
25	Oscillatoriaceae	Oscillatoria kawamurae	-	AB298443
26	Oscillatoriaceae	Oscillatoria sancta	SAG 74.79	EU196639
27	Oscillatoriaceae	Oscillatoria prolifera	-	AB075993
28	Nostocaceae	Anabaena cycadae	PKGSAK6	JQ964322
29	Nostocaceae	Anabaena circinalis	AWQC131C	AF247589
30	Nostocaceae	Anabaena circinalis	AWT205B	AF247586
31	Nostocaceae	Anabaena circinalis	AWT02	AF247587
32	Nostocaceae	Anabaena circinalis	AWT204A	AF247585
33	Nostocaceae	Anabaena circinalis	AWT001	AF247584
34	Nostocaceae	Anabaena circinalis	AWQC344B	AF247583
35	Nostocaceae	Anabaena circinalis	AWQC332H	AF247582
36	Nostocaceae	Anabaena circinalis	AWQC323B	AF247580
37	Nostocaceae	Anabaena circinalis	AWQC331C	AF247581
38	Nostocaceae	Anabaena circinalis	AWQC310F	AF247579
39	Nostocaceae	Anabaena circinalis	AWQC307C	AF247578
40	Nostocaceae	Anabaena circinalis	AWQC306A	AF247577
41	Nostocaceae	Anabaena circinalis	AWQC279B	AF247576
42	Nostocaceae	Anabaena circinalis	AWOC271C	ΔE247575
43	Nostocaceae	Anabaena circinalis	AWQC173A	AF247574
43	Nostocaceae	Anabaena circinalis	AWOC150A	AF247573
45	Nostocaceae	Anabaena circinalis	AWOC134C	ΔΕ247572
45	Nostocaceae	Anabaena circinalis	AWQC114C	AF247571
40	Nostocaceae	Anabaena macrospora	-	AB047103
47	Nostocaceae	Anabaena sphaerica	_	AY157743
40	Nostocaceae	Anabaena sphaerica	UTFX 'B 1616'	GO859616
50	Nostocaceae	Anabaena sphaerica	RPAN38	FU599117
51	Nostocaceae	Anabaena sphaerica	RPAN10	EU599116
52	Nostocaceae	Anabaena sphaerica	RPAN2	EU599114
53	Nostocaceae	Anabaena sphaerica	A904	EF375612
54	Nostocaceae	Anabaena sphaerica	RPAN12 clone 3	GO466569
55	Nostocaceae	Anabaena sphaerica	RPAN12 clone 1	GO466513
56	Nostocaceae	Anabaena sphaerica var tenuis	PMC267.06	GO859615
57	Nostocaceae	Anabaena sphaerica var tenuis	PMC266.06	GO859614
58	Nostocaceae	Anabaena sphaerica var. tenuis	PMC263.06	GO859613
59	Nostocaceae	Anabaena sphaerica var tenuis	PMC246.05	GO859612
60	Nostocaceae	Anabaena sphaerica var. tenuis	PMC229.04	GO859611
61	Nostocaceae	Anabaena sphaerica var tenuis	PMC189.03	GO859610
62	Nostocaceae	Anabaena sphaerica var. tenuis	PMC188.	GO859609

Sl. No.	Family	Sc. Name	Strain	Accession No.
63	Nostocaceae	Anabaena spiroides	-	AB271212
64	Nostocaceae	Anabaena spiroides	NIES-76	AB047104
65	Nostocaceae	Anabaena spiroides	RPAN58	EU599122
66	Nostocaceae	Anabaena spiroides	RPAN20	EU599121
67	Nostocaceae	Anabaena spiroides	RPAN15	EU599120
68	Nostocaceae	Anabaena spiroides	TAC27	AY701562
69	Nostocaceae	Anabaena spiroides	LMECYA	EU078524
70	Nostocaceae	Anabaena spiroides	strain PMC9702	AJ293118
71	Nostocaceae	Anabaena spiroides	strain PMC9403	AJ293116
72	Nostocaceae	Nostoc commune	strain: KU008	LC013478
73	Nostocaceae	Nostoc commune	strain: KU007	AB986249
74	Nostocaceae	Nostoc commune	strain:KU002	AB088375
75	Nostocaceae	Nostoc commune	strain: KU006	AB933330
76	Nostocaceae	Nostoc commune	-	AB933329
77	Nostocaceae	Nostoc commune	-	AB251862
78	Nostocaceae	Nostoc commune	-	AB251859
79	Nostocaceae	Nostoc commune	strain:SO-42	AB098071
80	Nostocaceae	Nostoc commune	gene strain: M-13	AB088405
81	Nostocaceae	Nostoc muscorum	Ind33	HM573462
82	Nostocaceae	Nostoc muscorum	strain Lukesova 2/91	AM711524
83	Nostocaceae	Nostoc muscorum	strain Lukesova 1/87	AM711523
84	Nostocaceae	Nostoc muscorum	CCAP 1453/22	HF678509
85	Nostocaceae	Nostoc muscorum	CCAP 1453/8	HF678508
86	Nostocaceae	Nostoc muscorum	CCAP 1453/20	HF678506
87	Rivulariaceae	Gloeotrichia echinulata	URA3	AM230705
88	Rivulariaceae	Gloeotrichia echinulata	PHY6	AM230703
89	Rivulariaceae	Gloeotrichia echinulata	PHY14	AM230704
90	Rivulariaceae	Gloeotrichia echinulata	-	AF527469
91	Rivulariaceae	Gloeotrichia echinulata	FACHB-419	JX872525
92	Rivulariaceae	Rivularia atra	BIR MGR1	AM230675
93	Rivulariaceae	Rivularia atra	BIR KRIV1	AM230674
94	Rivulariaceae	Rivularia sp.	E7 UAM-313	EU009150
95	Rivulariaceae	Rivularia sp.	MU24 UAM-305	EU009149
96	Rivulariaceae	Rivularia sp.	MU15 UAM-369	EU009148
97	Rivulariaceae	Rivularia sp.	E1 UAM-302	EU009147
98	Rivulariaceae	Rivularia sp.	MA8 UAM-355	EU009145
99	Rivulariaceae	Rivularia sp.	HKAR-4	FJ939128
100	Rivulariaceae	Rivularia sp.	IAM M-261	AB325536
101	Scytonemataceae	Scytonema sp.	HA4185-MV1 clone	HQ847565
102	Scytonemataceae	Scytonema sp.	HAF2-B2-c1 clone	JQ083659
103	Scytonemataceae	Scytonema sp.	HAF2-B2-c1 clone	HQ847553
104	Scytonemataceae	Scytonema sp.	HKAR-3	FJ939127

Sl. No.	Family	Sc. Name	Strain	Accession No.
105	Scytonemataceae	Scytonema sp.	CXA108-5-BZ clone	KF934174
106	Scytonemataceae	Scytonema sp.	CMT-1BRIN-NPC32	KF934173
107	Scytonemataceae	Scytonema sp.	WJT9-NPBG6B clone	KF934172
108	Scytonemataceae	Scytonema sp.	WJT9-NPG6A clone	KF934171
109	Scytonemataceae	Scytonema sp.	ATA-SAL-RM1 clone	KF934169
110	Scytonemataceae	Scytonema sp.	WJT4-NPBG1 clone	KF934162
111	Scytonemataceae	Scytonema sp.	CMT-1SWIN-NPC17	KF934160
112	Scytonemataceae	Tolypothrix sp.	HA4266-MV1 clone	JN385291
113	Scytonemataceae	Tolypothrix sp.	CNP3-B1-C1 clone	JQ083658
114	Scytonemataceae	Tolypothrix sp.	CNP3-B1-C1 clone	JQ083657.1
115	Scytonemataceae	Tolypothrix sp.	CXA109-3-BZ clone	KF934130.1
116	Scytonemataceae	Tolypothrix sp.	PCC 7601 = UTEX B	JX827161.1
117	Scytonemataceae	Tolypothrix sp.	SN439	KR709127.1
118	Scytonemataceae	Tolypothrix sp.	UAM 335	HM751850.1
119	Scytonemataceae	Tolypothrix sp.	UAM 333	HM751848.1
120	Scytonemataceae	Tolypothrix sp.	UAM 357	HM751846.1
121	Scytonemataceae	Tolypothrix sp.	PCC 7504	FJ661002.1

Table-2
List of Cyanobacteria containing <i>rbcL</i> gene sequences

Sl. No.	Family	Sc. Name	Strain	Accession No.
1	Chroococcaceae	Gloeocapsa sp.	PCC 7428	NC_019745
2	Chroococcaceae	Microcystis sp	KLL-C019 clone a	KP698050
3	Chroococcaceae	Microcystis sp.	KLL-C019 clone b	KP698049
4	Chroococcaceae	Microcystis sp	KLL-C005 clone c	KP698048
5	Chroococcaceae	Microcystis sp.	KLL-C005 clone b	KP698047
6	Chroococcaceae	Microcystis sp.	KLL-C005 clone a	KP698046
7	Chroococcaceae	Microcystis sp.	KLL-C018 clone b	KP698045
8	Chroococcaceae	Microcystis sp.	KLL-C018 clone a	KP698044
9	Chroococcaceae	Microcystis botrys	KLL- C017 clone a	KP698054
10	Chroococcaceae	Microcystisaeruginosa	KLL-C004 clone b	KP698052
11	Chroococcaceae	Microcystis aeruginosa	KLL-C004 clone a	KP698051
12	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA 143	Z94909
13	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA	Z94908
14	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA 57	Z94907
15	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA 43	Z94906
16	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA 166	Z94896
17	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA	Z94895
18	Chroococcaceae	Microcystis aeruginosa	strain NIVA-CYA 31	Z94894
19	Chroococcaceae	Microcystis sp.	strain NIVA-CYA	Z94905
20	Oscillatoriaceae	Lyngbya aestuarii	PCC 7419	AB075915
21	Oscillatoriaceae	Lyngbya sp.	PCC8106	NZ_AAVU0100002

Sl. No.	Family	Sc. Name	Strain	Accession No.
22	Oscillatoriaceae	Oscillatoria tenuis	strain PCC 9107	FN813332
23	Oscillatoriaceae	Oscillatoria sp.	KNUA009	HQ201394
24	Oscillatoriaceae	Oscillatoria sancta	PCC 7515	FN813331
25	Oscillatoriaceae	Oscillatoria sp.	PCC 6506	NZ_CACA0100025
26	Nostocaceae	Anabaena circinalis	CENA193	FJ830544
27	Nostocaceae	Anabaena circinalis	CENA191	FJ830543
28	Nostocaceae	Anabaena circinalis	CENA190	FJ830542
29	Nostocaceae	Anabaena circinalis	strain 123	AJ293135
30	Nostocaceae	Anabaena circinalis	strain 86	AJ293134
31	Nostocaceae	Anabaena circinalis	1tu34s5	AJ632049
32	Nostocaceae	Anabaena circinalis	strain 90	AJ293137
33	Nostocaceae	Anabaena macrospora	strain PMC9301	AJ293161
34	Nostocaceae	Anabaena spiroides	strain PMC9702	AJ293164
35	Nostocaceae	Anabaena spiroides	strain PMC9403	AJ293162
36	Nostocaceae	Anabaena spiroides	1tu39s17	AJ632055
37	Nostocaceae	Cylindrospermum sp.	Ind14	JX993233
38	Nostocaceae	Nostoc commune	0'Brien 02011101	DQ18528
39	Nostocaceae	N.flagelliforme	-	Z94893
40	Nostocaceae	N.commune	-	Z94892
41	Nostocaceae	Nostoc muscorum	Ind34	JX993243
42	Nostocaceae	Nostoc muscorum	Ind33	JQ918774
43	Nostocaceae	Nostoc muscorum	SAG 57.79	DQ185313
44	Scytonemataceae	Scytonema sp.	BK4	KT873797
45	Scytonemataceae	Tolypothrix sp.	CCMP1185	AB075923

Results and Discussion

The phylogenetic analysis of 122 cyanobacterial 16S rRNA shows many cluster²⁷. The phylogenetic tree is indicates that Thermus caliditerrae is the out group of this tree which helps to separate all the species properly. This species is very close to cyanobacteria. Among the cyanobacteria the phylogenetic tree indicates that Anabaena cycadae is the most primitive species which is most similar with Oscillatoria acuminata of family Oscillatoriaceae. Then different species are evolved. Than the Oscillatoria acuminate having closer similarity with some species of family Chroococcaceae. The boot strap value indicates the time of species clustering with each other. Then the species of Nostocaceae like Anabaena spiroides and Chroococcus turgidus of Chroococaceae are evolved which are slightly divergent from their own family. After that Lyngbya sp. of family ocillatoriaceae are evolved which is present in between of Chroococaceae family. This species have closely similarity with some species like Oscilatoria acuminate strain PCC 6304, Lyngbya sp JW2010b. Then these species are 86times clusters with the family Oscillatoriaceae. From the

Oscillatoriaceae family Scytonemataceae family is evolved and from Scytonemataceae family Rivulariaceae and Nostocaceae family is evolved. Here also some divergent is seen that some species of Scytonemataceae like *Tolypothrix sp.* are evolved.

After that again some species of Nostocaceae like Anabaena sphaerica, Anabaena spiriodes, Anabaena macrospora, Anabaena circinalis are evolved. So form this phylogenetic tree of 16S rRNA it is concluded that Nostocaceae is the most advanced family and Anabaena circinalis is the most advance species among all the species where as Anabaena cyacadae PKGSAK6 (JQ964322).

The phylogenetic analysis of *rbcL* (Ribulose-biphosphate carboxylase / oygenase) containing 46 species indicates that Oscillatoriaceae is the most primitive family through which all family and species are evolved. So this is known as the most primitive family in the process of evolution. From this family *Tolypothrix sp.* CCMP1185 and *Gloeocapsa sp.* PCC 7428 are evolved. Than the *Microcystis sp.* of family Chroococcaceae having different different strains are evolved. This family has

close similarity with *Scytonema sp.* BK4 and this shows similarity with some *Anabaena circinalis* having different different strains. After that also some members of Chroococcaceae are evolved. Than the Members of Nostocacea are evolved known as the most advanced family among all the families. After that some species of Oscillatoriaceae are also evolved.

According to our study both phylogenetic tree of 16S gene sequence and *rbcL* gives the information that Oscillatoriaceae is the most primitive family among all the family of cyanobacteria having less divergence, through which all species are evolved and *Anabaena circinalis* of family Nostocaceae is most advanced species which is more divergence now a days. Even all species are morphologically different, they are genetically quite similar. They are evolved by the process of evolution⁸.



Figure-1 Diagram showing the phylogenetic tree of 16S rRNA gene sequences



Figure-2 Phylogenetic tree of rbcL

Conclusion

Phylogenetic analysis and alignment of sequences are closely related. Construction and interpretation of phylogenetic tree helps to classify evolutionary relationships between homologous genes represented in the genomes of divergent species. The sequences are qualitatively related to one another which are evolutionary distantly present.

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