

Studies on Phylogeny and Evolution of Cyanobacteria Based on 16S rRNA and Other Gene Sequences

Akira Yokota

Institute of Molecular and Cellular Biosciences
The University of Tokyo, Japan

Cyanobacteria are morphologically diverse bacteria that perform oxygenic photosynthesis. Geochemical and fossil evidence indicates that in Precambrian Era they caused a transition in the Earth's atmosphere from its primordial, anaerobic state to its current, aerobic condition. Cyanobacteria are classified into five sections at present, based on their morphology: Subsections I, II, III, IV and V. Cyanobacterial classification based on morphological characteristics has been still controversial and does not reflect true phylogenetic relationships.

In this study, in addition to the small subunit ribosomal RNA (16S rRNA) gene sequence analysis, the phylogenetic analyses using the subunit B protein of DNA gyrase (topoisomerase type II) (*gyrB*), γ subunit of DNA-dependent RNA polymerase (*rpoC1*) and the principal sigma factor of DNA-dependent RNA polymerase (*rpoD1*) gene sequences, which were expected to provide higher resolution than that using 16S rRNA sequences, were carried out. The phylogenetic relationships and evolution of cyanobacterial strains were elucidated, based on the sequences of 16S rRNA, *gyrB*, *rpoC1* and *rpoD1* genes.

Constructed phylogenetic trees, the neighbor-joining (NJ), the maximum likelihood (ML), and maximum parsimony (MP) trees, revealed that all the numbers of cyanobacterial Subsection I, II, III were shown to be polyphyletic, but those of Subsection V were monophyletic. Unicellular cyanobacterial strains of Subsection I and II, and filamentous cyanobacterial strains of Subsection III were shown to be polyphyletic, and heterocystous strains of Subsection IV and V were shown to be monophyletic. However, our phylogenetic analyses indicated that Subsection IV were not monophyletic. The cluster of the genus *Scytonema*, which belongs to Subsection IV, was placed external to those of Subsection IV and V, forming a well-supported cluster with high bootstrap. Thus, all the member of cyanobacteria excepting Subsection V were revealed to be polyphyletic.

Subsection III was clearly divided into at least five clusters. It is interesting that the *Synechocystis* sp. PCC 6803 (Subsection I) and *Oscillatoria rosea* IAM M-220 (Subsection III) probably diverged recently. Also, *Leptolyngbya* sp. PCC 7375 (Subsection III) was clustered with strains of Subsection I. Thus, unicellular and filamentous strains are close, and it may help in the determination of morphological change from unicellular to filamentous strains or filamentous to unicellular strain.

Subsection I also contained phylogenetically different strains. They seem to be the earliest divergence of cyanobacterial evolution in some members. Especially, the genus *Gloeobacter* might appear earlier rather than chloroplasts. The phylogenetic relationship between cyanobacteria and

chloroplasts is controversial, and many reports based on 16S rDNA sequence analysis suggest that chloroplasts might originate relatively lately during the divergence of cyanobacteria.

Phylogenetic trees constructed on the basis of *gyrB* and *rpoD1* showed that all cyanobacterial Subsections IV and V strains were monophyletic, although *Chroococcidiopsis* sp. PCC 7431 (Subsection II) was positioned at outside of Subsection V on GyrB-based trees. Members of Subsection II and III containing *Chroococcidiopsis* sp. PCC 7431 were diverged earlier than any other member of Subsection IV and V, which means the evolution of heterocystous cyanobacteria may be connected directly or indirectly with some members of Subsection II and III. Subsections I, II and III were shown to be polyphyletic. In addition, the analyses of *gyrB*, *rpoC1* and *rpoD1* indicated that some members of Subsection I and III diverged earlier than other subsection of cyanobacteria.

We investigated evolution of cyanobacteria based on the phylogenetic analyses of 16S rRNA, *gyrB*, *rpoC1* and *rpoD1* genes. It revealed slightly different patterns in evolution of cyanobacteria with those of previous studies. Cyanobacterial evolution might be able to explain that an ancestor of cyanobacteria was diverged to cyanobacteria of Subsection I and Subsection III in early evolution. Subsection IV and V may be diverged relatively recently. The phylogenetic analyses of 16S rRNA, *rpoC1* and *rpoD1* genes exhibited that the Subsection V may result in some strains of Subsection IV. The results based on the phylogenetic analyses of 16S rRNA, *gyrB*, *rpoC1* and *rpoD1* genes strongly support the possibilities.

Thus, all the morphological characteristics at the genus level were not always significant. Namely, a taxonomic system of cyanobacteria reflecting phylogenetic relationships should be reconstructed.

References

1. Ishida et al., Phylogenetic relationships of filamentous cyanobacteria taxa inferred from 16S rRNA sequence divergence. *J. Gen. Appl. Microbiol.*, **43**: 237-241, 1997
2. Hond et al., Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. *J. Mol. Evol.*, **48**:723-739, 1999
3. Ishida et al., Evidence for polyphyletic origin of the members of the orders of Oscillatoriales and Pleurocapsales as determined by 16S rDNA analysis. *FEMS Microbiol. Lett.*, **201**: 79-82, 2001
4. Seo et al., Phylogenetic analysis of cyanobacteria based on 16S rRNA, *gyrB*, *rpoC1* and *rpoD* gene sequences. *J. Gen. Appl. Microbiol.*, **49**: 2003 (in press)

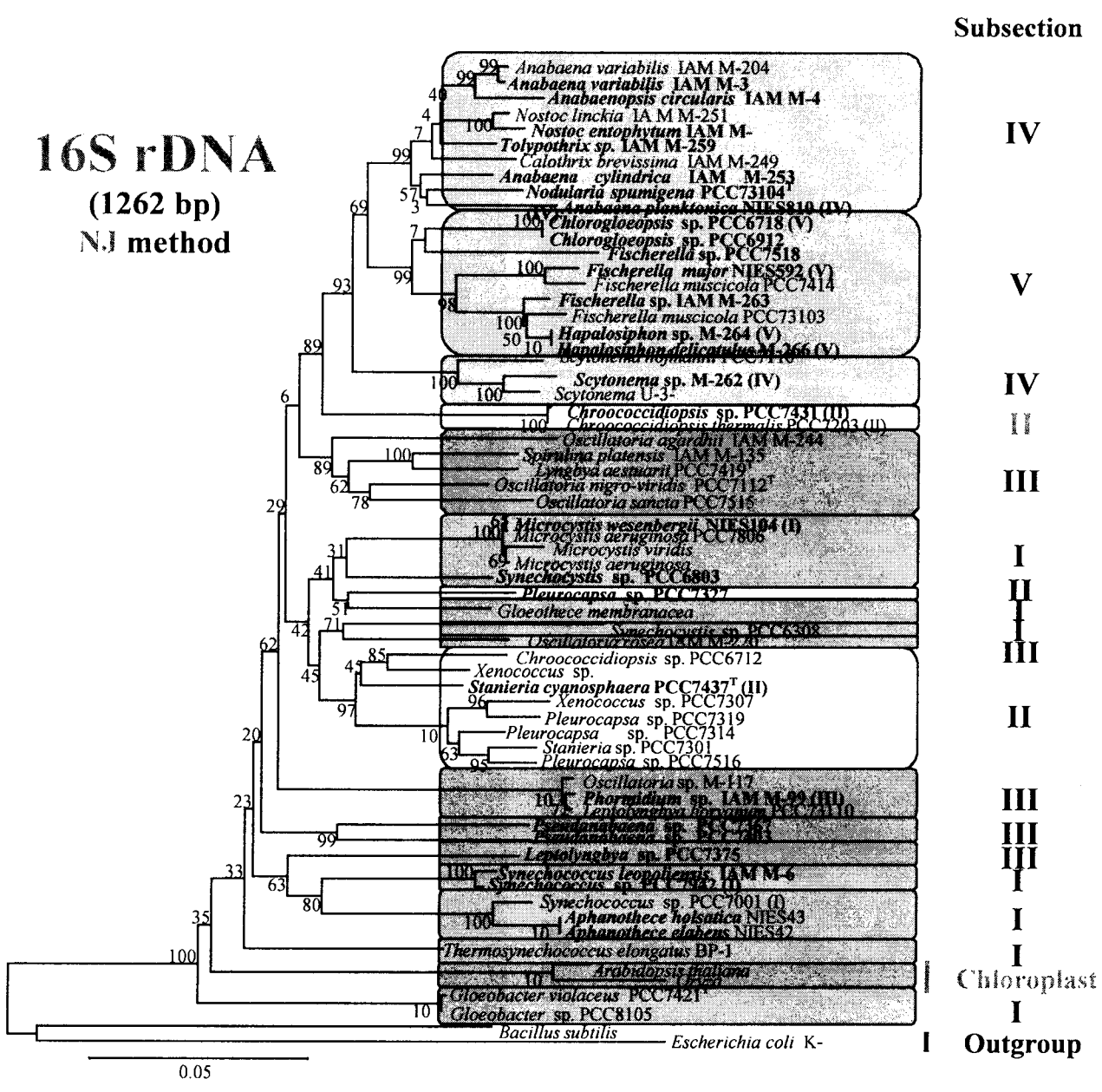


Fig. 1 Phylogenetic tree of cyanobacteria based on 16S rDNA sequences