Genome-wide comparison of cyanobacterial transposable elements, potential genetic diversity indicators

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Abstract:

Background:

Transposable elements are widely distributed in archaea, bacteria and eukarya domains. Considerable discrepancies of transposable elements in eukaryotes have been reported; however, the studies focusing on the diversity of transposable systems in prokaryotes were scarce. Understanding the transposable element system in cyanobacteria by the genome-wide analysis will greatly improve the knowledge of cyanobacterial diversity.

Results:

In this study, the transposable elements of seventeen cyanobacterial genomes were analyzed. The abundance of insertion sequence (IS) elements differs significantly among the cyanobacterial genomes examined. In particular, water bloom forming *Microcystis aeruginosa* NIES843 was shown to have the highest abundance of IS elements reaching 10.95% of the genome. IS family is a widely acceptable IS classification unit, and IS subfamily, based on probe sequences, was firstly proposed as the basic classification unit for IS element system. Both of IS family and IS subfamily were set as the two hierarchical units for evaluating the IS element system diversity. Totally, 1982 predicted IS elements, within 21 IS families and 133 subfamilies were identified in the examined cyanobacterial genomes. Families IS4, IS5, IS630 and IS200-605 are widely distributed, and therefore supposed to be the ancestral IS families. Analysis on the intactness of IS elements showed that the percentage of the intact IS differs largely among these cyanobacterial strains. Higher percentage of the intact IS detected in the two hot spring cyanobacterial strains implied that the intactness of IS elements may be related to the genomic stabilization of cyanobacteria inhabiting in the extreme environments. The frequencies between IS elements and miniature inverted-repeat transposable elements **(**MITEs) were shown to have a linear positive correlation.

Conclusions:

The transposable element system in cyanobacterial genomes is of hypervariabilty. With characterization of easy definition and stability, IS subfamily is considered as a reliable classification unit in IS element system. The abundance of intact IS, the composition of IS families and subfamilies, the sequence diversity of IS element nucleotide and transposase amino acid are informative and suitable as the indicators for studies on cyanobacterial diversity. Practically, the transposable system may provide us a new perspective to realize the diversity and evolution of populations of water bloom forming cyanobacterial species.

Keywords: Transposable element; Insert sequence; MITE element; IS intactness; IS diversity; Cyanobacterial genomes; IS family; IS subfamily

Background

Transposable elements (also called mobile element or jumping genes) are widely distributed in a variety of organisms including prokaryotes and eukaryotes [1]. A large amount of transposable elements enhanced the potential for their hosts' adaptation to different environments and created considerable interspersed repeats within genomes by transposition events accumulating over evolutionary time [2, 3]. Transposable element system has been proven to be a powerful marker for divergent populations in different groups of organisms [1, 4, 5, 6]. In eukaryotic organisms, much is known about the transposable element system, including the element structure, transposition mechanisms, copy number variance (CNVs) and evolutionary history of transposable elements [7, 8]. In bacteria, insert sequences (IS) and miniature inverted-repeat transposable elements (MITEs) are two principal types of transposable elements, which can move from place to place via a DNA intermediate by a cut and paste mechanism (class II element) [9] or spread to other organisms by horizontal gene transfer [10, 11]. Insertion sequences in prokaryotes were assumed to be an important driving force for novel genotypic and phenotypic variants. An investigation on the IS diversity of *Enterococcus faecium* confirmed that divergent IS could be used to distinguish subspecies from different environments and evaluated their evolutionary relationship [11]. Studies on the *Rhizobium meliloti* populations indicated IS-fingerprinting approach was a fine resolution for differing close species (strains) and would be suitable for ecological studies of individual strains in some complex ecosystem [12, 13]. In addition, the evolutionary dynamics of insertion sequences in *Rhizobium etli* populations were shown to be related to the evolutionary histories of the chromosome and symbiotic plasmid [14].

The recent release of prokaryotic genomes considerably contributed to the reorganization of a large number of IS families, especially in archaea. A systematical IS element collection and IS family based classification system have been established by some professional database, such as IS Finder [15] and GenBank. Cyanobacteria, considered as

the ancestor of photosynthetic organisms on the earth, consist of large groups of organisms from unicellular to filamentous forms [16]. However, less is known about the transposable elements in cyanobacteria. IS elements have been briefly described in several cyanobacterial genomes [17, 18, 19, 20, 21], and MITE was firstly analyzed in the recently released *Microcystis aeruginosa* NIES 843 genome. Zhou et al (2008) reported the genetic map of recently active IS elements in cyanobacterial genomes, and they presented a heavy dependence of the activities of IS elements on the environments, and the close linkage between the abundance of recently active IS elements with genome size [22]. However, recently released cyanobacterial genomes were not included in the above study, especially lacking high IS containing cyanobacerial genomes, which did not demonstrate and provide the general knowledge of IS diversity in cyanobacteria. Building a refine hierarchy for IS classification system is one goal of this study. IS family has been widely used in previous studies [19, 23, 24] and therefore recognized as an approved classification unit. However, the lower unit below IS family is obscure. IS group, a lower unit, was proposed and partly applied in the IS Finder database and in the comparative analyses on archaeal genomes by Chandler et al. [15, 23], but It is not easy to practically apply this IS group system because of its vague classification criterion, and incomplete database group annotation. Due to an extremely high diversity of IS nucleotide/ transposase existing in prokaryotes, establishing a lower IS classification unit is highly expected. Therefore, IS subfamily, a new classification unit was suggested in this study. By definition, all the nucleotide sequences fished by the same nucleotide probe were classified into one subfamily.

In the present study, we analyzed and compared the general characters of transposable element systems in seventeen cyanobacterial genomes, including their abundance, distribution and family/subfamily compositions. Analyses on parsimonious evolutionary scenario, IS copy number variance, element intactness and the nucleotide and transposase

amino acid sequences of these cyanobacterial transposable element systems, were performed as well. The framework for selecting the interspersed repeats encoding transposase was developed, and several complete cyanobacterial genomes released recently, including those from water bloom forming species such as *Microcystis aeruginosa* NIES 843, *Microcystis aeruginosa* PCC7806 and *Trichodemium erythraeum* ISM101 were included in this study. This combination is expected to achieve a comprehensive evaluation on the genetic diversity of cyanobacterial transposable system in more details and shed light on the feasibility of using the transposable element diversity information for the studies on cyanobacterial population diversity and evolutionary history.

Methods

Genomes of cyanobacterial strains

Seventeen cyanobacterial chromosome genomes and plasmid sequences were used in this study, and these strains cover twelve genera with chromosome size from 1.68 Mbp to 8.23 Mbp. Besides the well sequenced and spliced ring shape genomes, some genomes are assemblages of contigs. The contig numbers of the genomes of *Microcystis aeruginosa* PCC7806, *Crocosphaera watsonii* WH 8501, *Raphidiopsis brookii* D9 and *Cylindrospermopsis raciborskii* CS-505 are 116, 323, 47 and 93 respectively. The cyanobacterial strains used in this study can be morphologically divided into unicellular and filamentous, and have diverse inhabits including terrestrial, freshwater, marine water and hot spring (Table 1).

Construction of the nucleotide and transposase amino acid probe libraries

Two sets of IS sequence probe libraries were generated in this research. One set aims at rough nucleotide sequence mining, and the other was the transposase amino acid probe library corresponding to each nucleotide probes aiming at reexamination of nucleotide

candidate sequences reexamination and intactness judgment. The procedure for nucleotide probe library construction was as follows: all the repeat elements longer than 500 bp were collected using the Vmatch program package [25]. Sequence consensus was executed by Cap3 program [26], and all the consensus sequences were examined by reiterative BLAST analysis setting the parameters of e value cutoff of 10^{-20} and key word of 'Transposase'. The positive hits of nucleotide sequences were selected as IS nucleotide probes. For transposase amino acid probes, the open reading frames (ORFs) of transposable element corresponding to each IS nucleotide probes were recognized by getorf program from the EMBOSS package. Transposase with longer transposase, as the best representation of the intact transposase subfamily, were collected as IS transposase amino acid probes. The strategy used to define the ORFs in this study is searching the region that is free of STOP codons. IS family was identified by the homologous search mainly according to IS Finder and GenBank.

IS element mining

To identify possible IS elements in cyanobacterial genomes, each of genome sequences was screened with RepeatMasker 3.2.9 [27]. This program is able to identify copies of IS element candidates by pairwise sequence comparisons with a self- constructive IS nucleotide probe library described above. The following arguments were used for this search: ‘cross_match’ as the search engine; ‘slow’ to obtain a search 0–5% more sensitive than default; ‘nolow’ to not mask low complexity DNA or simple repeats. All the putative ORFs recognized by EMBOSS: getorf were judged by the blastp and the hits with lower e values (1e-50) were picked out and recognized as the predicted IS elements. The reliability of this method is verified to be credible (Additional File 1).

Corresponding to the above two sets of probe libraries, two types of intact IS elements were defined (Figure 1). N-intact elements represent ISs which cover at least 95%

nucleotide sequence corresponding to the nucleotide probe. The ISs, which cover at least 99% amino acid sequence with correspondence to transposase amino acid probe, are defined as P-intact elements.

MITE element mining

The strategy for the MITE search is an integration of repeated elements and TIR/DR border identification. All the repeated elements longer than 100 bp were collected by the Vmatch package, and 15 bp left/ right flanking wings were added to ensure the potential intactness of TIR/ DR border. The candidates containing the TIR/ DR structure and shorter than 499bp by MUST [28] were defined as MITE. The genomes were scanned using RepeatMasker with the same argument setting to IS mining, and all the sequences homologous to the nucleotide probes were defined as type I, and the remains were type II.

Phylogenetic analysis

Nucleotide and amino acid sequences were aligned using either CLUSTAL W, version 2.0 [29] or MUSCLE [30]. Genetic distances were calculated using the method of Kimura's two-parameter (K2P) for DNA sequences and Poisson correction for protein sequences. The phylogenetic trees were constructed from the multiple-aligned data using the neighbor-joining (NJ) algorithmic. Kimura's two-parameter was implemented within the MEGA4 program package [31].

Result

Abundance and basic properties of cyanobacterial IS

Totally 1982 predicted IS elements including intact and fragmentary ones, were detected in these cyanobacterial genomes, and the abundance of the predicted ISs in different strains varies considerably. *M. aeruginosa* NIES 843, a unicellular water-bloom forming strain with the genome size as 5.8 Mbp, showed to contain the highest IS abundance in the examined strains as 532 IS elements, covering 10.95% of the genome (Figure 1,

Figure 2 and Table 2). While another *M. aeruginosa* PCC 7806 strain was revealed to have 359 pieces of IS elements with 8.98% coverage of the genome. Strains *Acaryochloris marina* MBIC11017 and *Thermosynechococcus elongates* BP-1 were presented to have the IS coverage over 3%. Surprisingly, none of IS elements were detected in two marine strains *Prochlorococcus* sp. MIT 9211 and *Prochlorococcus* sp MIT 9215. The length of the predicted IS elements ranged from 199 bp to 6495 bp, with the majority within the range of 500-2750 bp (Additional File 2). A small amount of IS elements longer than 3 kb were also detected, including the elements from *M. aeruginosa* PCC7806, and Tn elements longer than 4 kb from *Acaryochloris marina* MBIC11017, *Nostoc punctiforme* PCC 73102 and *Anabaena variabilis* ATCC 29413. One IS element could be detected as roughly 45 kb size within the cyanobacterial genome. *Trichodesmium erythraeum* IMS 101 was shown to contain the lowest GC content of IS elements, contrasting to the two hot spring strains *Synechococcus* sp. JA-3-3Ab and *Thermosynechococcus elongatus* BP-1 with GC contents of ISs reaching 60% and 53% respectively.

Subfamily- a lower classification unit of IS elements

According to the IS subfamily definition described above, 133 IS subfamilies were identified in the cyanobacterial genomes in the present study. Among them, ten subfamilies containing the ORF coding region with high homologous to transposase annotated in GenBank can not match any homologies in the IS Finder, and thus are marked as 'Undefined' (Additional File 2). The copy number of the IS elements in one subfamily ranged from two to ninety-seven (048M843 subfamily). One subfamily was found to be mostly shared by only six strains within the 17 examined strains, indicating that universe subfamilies hardly exist. The phylogeny based on either the IS nucleotide

sequencea or transposase amino acid sequences within a subfamily were not well consistent to the 16S rDNA based phylogeny (Figure 4).

Fifty-five subfamilies were found in the genomes of the two *Microcystis* strains, and thirty of them were shared by both strains, while the remaining sixteen and nine subfamilies were present individually. The thirty shared subfamilies including 361 IS elements in *M. aeruginosa* NIES843 and 259 IS elements in *M. aeruginosa* PCC7806, respectively. The filamentous heterocystous strains *Anabaena* sp. PCC7120 and *Anabaena variabilis* ATCC 29413 contain thirty-three subfamilies, seven of which were shared by both strains. Twenty-one IS elements from *Anabaena* sp. PCC7120 were shown to have homologous IS elements in *A. variabilis* ATCC29413 genome, and the percentage of homologous elements in two strains is higher than 24%. Compared to the seventy-one of IS elements contained in the hot-spring strain of *Synechococcus* sp. JA-3-3Ab, only one IS was found in the plasmid of the freshwater strain *Synechococcus* sp PCC7002. It is seemingly shown that the cyanobacterial strains isolated from hot spring have less IS subfamilies, since only six and four were respectively found in *Synechococcus* sp. JA-3-3Ab and *Thermosynechococcus elongatus* BP-1.

IS family composition in cyanobacterial genomes

94% of the predicted IS elements could be classified into twenty-one bacterial IS families (Figure 1). Compared with the IS elements in archaea, six IS families including IS3, IS1380, IS701, ISAs1, ISNCY and Tn, were only found in cyanobacteria, while ISA1214, ISM1, IS1595, ISBst12, IS1182, ISH6 and ISC1217 were not found with any homologues in cyanobacteria. IS4, IS5, IS630 and IS200-605 were four dominant and widely distributed IS families in these cyanobacterial genomes. *M. aeruginosa* NIES843 and *Acaryochloris marina* MBIC11017 contained thirteen IS families, while the two hot spring strains were shown to have only three IS families. It is apparently shown that IS

discrepancies exist among the morphologically similar strains. For instance, IS families including IS701, IS30, IS110 and IS1380 detected in *M. aeruginosa* NIES843 were not found any homologous ones in *M. aeruginosa* PCC7806, while nine of fourteen IS families were shared by the both *M. aeruginosa* strains.

Estimated ancestral IS families

a. IS4 family

333 IS elements distributing in eight cyanobacterial strains were included in IS4 family. And these IS elements could be further classified into twenty-two IS subfamilies. The phylogenetic relationship among the twenty-two subfamilies was constructed in this study. Nineteen of the subfamilies were shown to be significantly divided into four dominant clusters, while the other three formed dispersed linkages (Figure 3). Most of IS elements within the same IS groups defined by IS Finder could be included in a cluster, such as IS elements from group 10, group 50 and group IS4 Sa. However, two IS elements of group 1634 in IS Finder were separated into Cluster Ⅲ and cluster Ⅳ, though these two clusters were closely related in the phylogenetic tree (Figure 3).

b. IS5 Family

IS5 family contained 223 IS elements from eight cyanobacterial strains, and all these elements could be further classified into sixteen IS subfamilies. The phylogenetic relationship among these sixteen subfamilies in IS5 family showed that fourteen of the subfamilies could be divided into four dominant clusters. Eleven IS elements within the IS groups defined by ISFinder were included. The IS elements from group ISL2, group IS5 and group 930 were mixed in to cluster Ⅰ, cluster Ⅱ and cluster Ⅲ respectively. Two sequences of group 1031 and one sequences of group 427 were mixed into cluster Ⅳ(Figure. 3).

c. IS630 Family

The IS elements identified as IS630 family could be found in eleven cyanobacterial strains. 430 IS elements belonging to thirty-one IS subfamilies showed an extremely high level of internal divergences in this family. The phylogenetic relationship among the thirty-one IS subfamilies in IS630 family was constructed in this study. Eighteen of IS subfamilies were divided into three dominant clusters, while the others formed dispersed lineage.

d. IS200-605 Family

In IS200-605 family, 217 IS elements from ten cyanobacterial strains were included and were further classified into eleven IS subfamilies. The phylogenetic relationship among the eleven IS subfamilies in IS200-605 family showed that all of these subfamilies could be divided into three dominant clusters. Four closely related IS elements of group 1341 had different phylogenetic locations of which three were gathered in cluster I and cluster Ⅱ, and one formed a unique linkage close to cluster I and cluster Ⅱ.

The IS intactness diversity

The intactness of transposase ORF is the most important factor determining the autonomous transposable action. Segment loss, nucleotide mutations, insertions, and deletions caused by reading frame interrupted or shift are the principal mechanisms for interrupting the intactness. The number of P-intact IS elements in the examined cyanobacterial genomes was 1234, accounting for 62.3% of all the predicted IS elements. 74.6% of these ORF-intact sequences were further found to have more than 99% similarities with the probe sequences. The IS elements shorter than 500 bp were mostly considered to be non-P-intact. The percentages of the ORF-intactness in different IS families were different, from 46.7% (Tn family) to 100% (IS982 family). *M. aeruginosa*

NIES 843 was found to contain 10% higher abundance of the ORF-intact IS elements than *M. aeruginosa* PCC7806. Subfamily 048M843 contained the highest abundance of IS element copy. Sixty-three IS elements in this subfamily detected in the genomes of *M. aeruginosa* NIES843 and *M. aeruginosa* PCC7806 were P-intact ones, while four pieces of IS elements in *M. aeruginosa* NIES 843 and one in *M. aeruginosa* PCC7806 sharing the same nucleotide substitution were ORF fractured ones.

N-intact IS elements were shown to be partly different from the P-intact ones. More than 99% of the P-intact IS elements were simultaneously defined as N-intact IS elements, and 82.78% N-intact IS elements are composed by the P-intact IS elements. The average percentage of the N-intact IS elements is 74.8%, ranging from 62.1%- 100%. The percentage of the N-intact IS in the genomes of the two hot spring strains was high, reaching 94.8% and 95.7%, respectively. Neither N-intact nor P-intact IS could be detected in the genome of *Gloeobacter violaceus* PCC7421.

Nucleotide and protein sequence diversity in IS elements

The phylogenetic analysis based on the all the IS nucleotide sequences within subfamilies 113P7120, 128M7806 and 048M843, which are representatives of the most extensive strain resources, highest subfamily divergence and most copy number, was executed respectively. In subfamily 048M843, the nucleotide sequence divergence of the IS elements from *M. aeruginosa* PCC 7806 was much higher than that from *M. aeruginosa* NIES843 (Figure 4). The IS elements from *M. aeruginosa* NIES843 were mostly gathered in one lineage, further reflecting that the ORF fractured segments were mixed with the intact ones. The only one ORF-fractured IS element from *M. aeruginosa* NIES843 was clustered together with the IS elements from *M. aeruginosa* PCC7806. In subfamily 128M7806, *M. aeruginosa* PCC 7806 and *M. aeruginosa* NIES 843 are distantly separated from two *Anabaena* strains. In subfamily 113P7120, the IS elements

were mainly from two *Microcystis* strains and two *Anabaena* strains. The phylogeny based on the IS nucleotide sequences showed that the IS elements from *Microcystis* form four clusters, while the IS elements from *Anabaena* were grouped as two clusters. It is shown that one genome may contain many IS elements of one subfamily from extensive resources. The IS elements from *Cyanothece* sp. PCC 7425 and *Synechococcus* sp. JA-3-3Ab form a single cluster away from others.

Diversity index of both nucleotide and transposase amino acid sequences from the P-intact IS elements of the 133 subfamilies were calculated (Additional File 2). The highest nucleotide and amino acid divergences were found in the subfamily 128M7806, with the index values as 0.21656 and 0.9289 respectively. High conservation of transposase amino acid sequences in 42 IS subfamilies was also shown, with their protein diversity indices as 0. Twelve subfamilies with high conservation of protein sequence correspond to vary of nucleotide sequences.

MITE in cyanobacterial genomes

Totally 7763 MITEs were identified in these cyanobacterial genomes, and 3249 pieces of them can be classified as type I. All the type I MITEs detected in this study have been found to be IS originated. The remaining 4514 MITE elements were classified as type-II. The length of most MITEs ranged from 100bp to 499bp (Addition File 2). The abundance is inversely correlated to the length of MITEs, and 60% of MITEs were in the length ranging between 120-260bp. The frequency of the MITEs in cyanobacterial genomes analyzed in this study varied from 0 to 2466 pieces, taking the percentages from 0 to 8.76%. The highly linear correlation between the IS and MITE elements was found in this study. The correction coefficients for the frequency of IS vs type I MITE, IS vs type II MITE and IS vs all MITE reach 92.3%, 81.8% and 87.5% respectively. The frequency of type II MITEs was one to three times higher than that of type I ones, with the exception

for the genomes of *Synechocystis* sp. PCC6803 and two plasmids from the strains PCC 7120 and PCC7425. Unexpectedly, the TIR border couldn't be detected in the genome of *Trichodesmium erythraeum* IMS101. Similar to IS elements, MITEs have no AT or GC bias. The lowest GC content of IS elements was 36.2% in *M. aeruginosa* PCC 7806 genome and the higher ones were found in *Synechococcus* sp. JA-3-3Ab and *Thermosynechococcus elongatus* BP-1 inhabiting in hot spring, the percentage of which were 60% and 53% respectively.

Discussion

Cyanobacteria have been considered to originate about 2.7 billion years ago [33], and went through the similar evolutionary course with archaea. Regarding the transposable element system, both cyanoabcteria and archaea share highly similar IS family composition and abundance. This study presented an extremely high diversity of transposable element system in cyanobacterial genomes.

The big difference in the abundance of transposable element system was found among cyanobacterial genomes. Zhou et al. (2008) assumed that the frequency of recently active IS elements, which are similar to the defined P-intact elements in this study, positively correlate with genome size [22]. However, the analysis on the transposable element system from recently released cyanobacterial genomes revealed that the frequencies of IS, P-intact and N-intact IS elements have no significant relationship with the genome size. The highest abundance of transposable elements was found in the unicellular *Microcystis aeruginosa* strains with the medium size of genome, while the filamentous *Anabaena variabilis* ATCC29413 and *Nostoc punctiforme* PCC 73102 strains with genome size larger than 6 Mbp were revealed to have smaller and simpler transposable element systems. Genome plasticity in prokaryotes is often considered to be an adaptive strategy allowing microorganisms to promote diversification in the way similar to sexual

reproduction in eukaryotic organisms [23]. Frangeul et al. (2008) pointed that a high frequency of transposable elements inhabiting in genomes would facilitate this adaptive strategy [34]. High abundance of transposable elements found in the *M. aeruginosa* strains examined here demonstrate that their genomes may be rearranged to cause positive mutations accelerating adaptations to various freshwater ecosystems, and this high genome plasticity caused by genomic rearrangement might be an explanation to the fact that *Microcystis* is the most successful organism to compete over others. *Microcystis* species have been globally found as the dominant species, to largely grow in eutrophic freshwaters. *M. aeruginosa* NIES843 and *M. aeruginosa* PCC7806 strains were respectively isolated from Lake Kasumigaura of Japan in 1997 and from Braakman reservoir of Netherlands in 1972, and the difference of IS composition and abundance between the two strains may be caused by the different habitant environment and strain maintenance periods.

IS family and subfamily are two hierarchical classification levels for cyanobacterial transposable element systems. IS subfamily as the basic classification unit in transposable element system is firstly proposed in this study. IS group, as the lower classification unit of IS elements, was used in IS Finder database [15]. However, many IS elements have not been classified as any IS groups. Even some IS sequences within IS group defined by IS Finder, were disorderly clustered in the present study (Figure 3). Based on the stability of IS probes, IS subfamily was proven to be an easy-defined and reliable unit in IS element system classification. The divergence of both IS family and subfamily composition and their nucleotide and transposase amino acid sequences shown in this study also reflected the hypervariabilty of the transposable elements in cyanobacterial genomes. 21 IS families and 133 subfamilies were identified in cyanobacteria genomes examined here. Based on the widely confirmed 16S rRNA phylogeny and the IS family composition for each strains, we dedicate the most parsimonious evolutionary scenario of IS acquisition

for each family (Figure 1). Santiago et al. (2002) indicated that in *Arapdopsis*, the more variable a transposable element family (subfamily) is, the more ancient the amplification burst that has generated it should be [35]. Similarly, four IS families in this study, IS4, IS5, IS 605 and IS630, which were found to exhibit a wide distribution and diversity in cyanobacterial genomes, could be considered as cyanobacterial ancestral IS families. The phylogeny based on the nucleotide sequences of the widely distributed IS subfamilies revealed that the IS elements from one genomes commonly gathered together and the IS elements from close related species have high similarity of nucleotide sequences than that between distantly related species (Figure 4). Such a result implied that the most likely exchange and replication of the transposable elements in cyanobacteria may occur within a genome, followed by close related species. Furthermore, more resources of IS elements belonging to one IS family were also found in one genome, which may provide valuable information to analyze the population relationship and species evolution in the future.

In eukaryotes, recent transposable element insertions have been used in population genetics studies and regarded as identical-by-descent genetic markers for the evolution, forensics and population history studies [14, 36, 37, 38)]. A transposable element family/ subfamily insertion with lower nucleotide divergence (<1% or lower) has been considered as a recent insertion [14, 38]. Among all the IS subfamilies examined in the cyanobacterial genomes, many of them were shown to have a lower nucleotide diversity (Additional File), and thirty IS subfamilies even having the nucleotide diversity index as zero. Therefore, these IS subfamilies with lower diversity index were considered as the putative recent IS subfamily insertions, which have the potential used for the analyses of cyanobacterial population relationship in the future.

In the most cyanobacterial genomes examined, the intact IS elements showed to contain more copies and higher sequence diversity than the fractured ones. Surprisingly, *Gloeobacter violaceus* PCC7421 was the only strain without the intact IS elements, which

441 can not be explained so far. Many ORF-fractured transposase still showed to have the
442 basic structure of the N-intact elements, but the fracture of these transposases may
443 attribute to the fact that their coding frames are interrupted by slipped strand mispairing
444 during DNA replication on a single DNA strand, as described by Bichara et al.(2006)
445 [39].

446 Previous studies indicated that unique morphological, physiological and genetic
447 characters were always found in organisms from the extreme environments [40, 41]. Zhou
448 et al (2008) concluded that hot spring seems to be one of the favorite living environments
449 for organisms with active IS elements [22]. In the present study, a medium content of IS
450 elements contained in *Synechococcus* sp. JA-3-3Ab and *Thermosynechococcus elongatus*
451 BP-1 inhabiting in hot spring environments are revealed to have higher intactness of IS
452 family and subfamily compositions. Such results suggest that a high percentage of intact
453 IS might play a partial role in maintaining the genome stability in the extreme
454 environments.

455 Although MITE element system was described in the genome of *M. aeruginosa* NIES
456 843 [19], the information about MITE in prokaryotes is still scarce. In this study, higher
457 abundance of MITEs and two types of MITEs revealed in cyanobacterial genomes
458 provided a basic overview for the knowledge of MITEs in cyanobacteria. Actually, Type I
459 MITE was assumed to be a result of a deletion within an IS element and called as
460 'parasites of parasites' as well [24, 42], thus many of non intact IS elements are belonged
461 to the type I MITE. However, it is still hard to implicate cyanobacterial MITEs as the
462 diversity indicator since they are too short and irregular.

463 Conclusively, the analyses on the transposable system of cyanobacterial genomes will
464 help to improve understanding the knowledge for the diversity of cyanobacteria. The
465 features of the transposable elements in cyanobacteria, including the abundance of intact
466 IS, the composition of IS families and subfamilies, the sequence diversity of IS element

nucleotide and transposase amino acid, have shown to be valuable indicators for studies on cyanobacterial diversity. It is specially noted here that the *Microcystis* strains contain a high abundance of IS elements, which allows us to use the transposable element system as a new perspective to further explore the diversity and population relationship of water bloom forming cyanobacterial species.

Author's contributions

SL, RL and SH designed this study. SL and PX performed the data mining and analysis. TZ and SH made important and meaningful comments; SL and RL wrote this manuscript. MV provided this program a powerful platform. All authors read and approved the final manuscript.

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Table 1. Cyanobacterial strains used in this study and their genome information

Species	GenBank No.	Habitat	Morphology	Length (nt)	GC%	Topology	Sequencing center	Completed date
Microcystis aeruginosa NIES-843	AP009552	Freshwater Lake	unicellular	5,842,795	42	circular	Kazusa, Japan	2008-1-31
Microcystis aeruginosa PCC 7806	AM778843-AM778958	Freshwater Lake	unicellular	5,172,804	42	contigs	Institut Pasteur, France	2007-11-1
Synechocystis sp. PCC 6803	BA000022	Freshwater Lake	unicellular	3,573,470	47	circular	Kazusa, Japan	2001-10-23
Synechococcus sp. JA-3-3Ab	CP000239	Hot spring	unicellular	2,932,766	60	circular	CAG, US	2006-2-7
Synechococcus elongatus PCC 7002	CP000951	Freshwater Lake	unicellular	3,008,047	49	circular	Beijing Genomic Institute, China	2008-3-17
Trichodesmium erythraeum IMS101	CP000393	Marine	filamentous, non-heterocystous	7,750,108	34	circular	DOE	2006-8-30
Nostoc punctiforme PCC 73102	CP001037	Terrestrial	filamentous, heterocystous	8,234,322	41	circular	DOE	2008-4-25
Anabaena variabilis ATCC 29413	CP000117	Terrestrial	filamentous, heterocystous	6,365,727	41	circular	DOE	2005-9-20
Anabaena sp. PCC 7120	BA000019	Terrestrial	filamentous, heterocystous	6,413,771	41	circular	Kazusa, Japan	2001-11-28
Acaryochloris marina MBIC11017	CP000828	Marine	unicellular	6,503,724	47	circular	TGen Sequencing Center, US	2007-10-17
Cyanothece sp. PCC 7425	CP001344	Marine	unicellular	5,374,574	50	circular	DOE	2009-1-15
Prochlorococcus marinus str. MIT 9211	CP000878	Marine	unicellular	1,688,963	38	circular	MOORE	2007-11-13

Prochlorococcus marinus str. MIT 9215	CP000825	Marine	unicellular	1,738,790	31	circular	DOE	2007-9-21
Thermosynechococcus elongatus BP-1	BA000039	Hot spring	unicellular	2,593,857	53	circular	Kazusa, Japan	2002-8-19
Gloeobacter violaceus PCC 7421	BA000045	Terrestrial	unicellular	4659019	61	circular	Kazusa, Japan	2003-10-6
Cylindrospermopsis raciborskii CS-505	ACYA00000000	Freshwater Lake	filamentous, heterocystous	3879030	40	contigs	Germany	2010-1-4
Raphidiopsis brookii D9	ACYB00000000	Freshwater Lake	filamentous, non-heterocystous	3186511	40	contigs	Germany	2010-1-4

* DOE means DOE Joint Genome Institute, US; MOORE means The Gordon and Betty Moore Foundation Marine Microbiology Initiative, US; NARA means Nara Institute of Science and Technology, Japan; CAG means Center for the Advancement of Genomics, US

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Table 2. The IS and MITE elements distributing in the cyanobacterial genomes

		IS									MITE						
Cyanobacteria strains	Genome Size	IS Frequency	All IS/ Genome size %	P-Intact IS	P-Intact IS/ Genome size %	N-Intact IS	N-Intact IS/ Genome size %	Average Length	Min Length	Max Length	Number of Subfamilies included	Type I MITEs	Type II MITEs	MITEs all	Percentage	MITE GC%	IS GC%	Genome GC%
Microcystis aeruginosa NIES-843	5,842,795	534	10.85	348	7.02	375	8.66	1187	188	2451	47	1110	1356	2466	8.76	39.2	38.6	42.0
Microcystis aeruginosa PCC 7806	5,172,804	359	9	186	5.34	240	6.93	1293	285	3696	39	890	1133	2023	8.16	36.2	36.4	42.0
Synechocystis sp. PCC 6803	3,573,470	58	1.41	24	0.70	36	0.98	878	350	1175	8	113	98	211	1.29	39.7	37.2	47.0

Anabaena sp. PCC 7120	6,413,771	56	0.98					1121	492	1525	15	47	133	180	0.65	43.8	41.1	41.0
Plasmid 7120alpha	408,101	23	6.67					1182	643	1677	9	24	3	27	1.72	36.6	38.6	40.5
Plasmid 7120beta	18,614	3	14.12					876	553	1049	3	0	0	0	0	0	41.1	40.2
Plasmid 7120gamma	101,965	4	4.35	54	1.41	64	1.11	1108	670	1364	4	0	3	3	0.75	34.3	42.9	41.0
Plasmid 7120zeta	5,584	0	0					0	0	0	0	0	0	0	0	0	0	44.2
Plasmid 7120delta	55,414	0	0					0	0	0	0	0	0	0	0	0	0	41.6
Plasmid 7120epsilon	40,340	0	0					0	0	0	0	0	0	0	0	0	0	40.9
Gloeobacter violaceus PCC 7421	4,659,019	16	3.05	0	0	0	0	889	587	1089	6	4	38	42	0.18	59.7	52.1	61.0
Acaryochloris marina MBIC11017	6,503,724	188	3.47					1200	315	4584	30	214	274	488	1.75	49.1	0	47.0
Plasmid AcarypREB1	374,161	6	3.65					2278	1009	4584	5	0	0	0	0	0	0	47.3
Plasmid AcarypREB2	356,087	17	7.8					1632	580	4584	12	0	6	6	0.61	45.8	0	45.3
Plasmid AcarypREB3	273,121	17	6.46					1038	493	2670	13	0	0	0	0	0	0	45.2
Plasmid AcarypREB4	226,680	5	3.52	191	3.29	214	3.52	1597	1060	2669	5	0	0	0	0	0	0	45.9
Plasmid AcarypREB5	177,162	6	4.86					1435	1060	2297	6	0	0	0	0	0	0	44.7
Plasmid AcarypREB6	172,728	7	4.99					2642	481	4603	5	0	12	12	1.96	47.4	0	47.1
Plasmid AcarypREB7	155,110	3	3.38					1749	1183	2669	2	0	0	0	0	0	0	45.6
Plasmid AcarypREB8	120,693	5	7.12					1719	864	2669	4	0	3	3	0.3	64.2	0	45.4
Plasmid AcarypREB9	2,133	0	0					0	0	0	0	0	0	0	0	0	0	42.5
Anabaena variabilis ATCC29413	6,365,727	53	0.98					1648	456	6495	11	83	117	200	0.74	44.5	42.0	41.0
Plasmid AnabA	366354	10	4.5	54	1.41	60	1.54	1648	595	6495	6	18	0	18	1.11	45.1	42.5	40.5
Plasmid AnabB	35762	0	0					0	0	0	0	0	0	0	0	0		38.5
Plasmid AnabC	300,758	7	4.28					1838	500	6495	6	0	0	0	0	0	40.7	42.0
Nostoc punctiforme PCC 73102	8,234,322	146	2.03	114	1.62	138	2.00	1142	419	4826	27	258	305	563	1.45	39.8	38.9	41.0
Plasmid pNUN01	354,564	14	5.02					1271	548	4826	9	3	0	3	0.22	36.6	37.8	40.5
Plasmid pNUN02	254,918	14	7.72					1404	681	4824	11	0	0	0	0	0	36.6	40.7
Plasmid pNUN03	123,028	4	9.78					3008	1002	5031	3	0	0	0	0	0	40.5	40.9

Plasmid pNUN04	65,940	2	8.7					2868	908	4828	2	0	0	0	0	0	39.4	41.5
Plasmid pNUN05	26,419	0	0					0	0	0	0	0	0	0	0	0	0.0	42.3
Synechococcus sp. PCC 7002	3,008,047	0	0					0	0	0	0	0	0	0	0	0	0.0	49.0
Plasmid 7002pAQ1	4,809	0	0					0	0	0	0	0	0	0	0	0	0.0	49.0
Plasmid 7002pAQ2	16,103	0	0					0	0	0	0	0	0	0	0	0	0.0	45.9
Plasmid 7002pAQ4	31,972	1	1.82	2	0	0	0	582	582	582	1	0	0	0	0	0	50.0	44.1
Plasmid 7002pAQ5	38,515	0	0					0	0	0	0	0	0	0	0	0	0.0	42.6
Plasmid 7002pAQ6	124,030	0	0					0	0	0	0	0	0	0	0	0	0.0	45.1
Plasmid 7002pAQ7	18,459	1	3.15					582	582	582	1	0	0	0	0	0	50.0	47.3
Cyanothece sp. PCC 7425	5,374,574	91	2.09					1233	382	2666	17	95	101	196	1.01	52.9	52.2	50.0
Plasmid 742501	196,837	6	4.57	85	2.13	89	2.24	1500	802	2665	5	3	0	3	0.47	53.2	53.1	48.9
Plasmid 742502	179,973	23	16.2					1268	456	2664	11	14	9	23	3.72	52.9	52.8	49.1
Plasmid 742503	34,726	0	0					0	0	0	0	0	0	0	0			47.1
Synechococcus sp. JA-3-3Ab	4,659,019	71	1.49	47	0.68	68	1.10	747	417	1054	6	85	147	232	1.55	54.3	52.1	60.0
Thermosynechococcus elongatus BP-1	2,593,857	58	2.53	52	2.31	55	2.47	1130	348	1473	4	100	138	238	1.93	51.8	49.9	53.0
Trichodesmium erythraeum IMS101	7,750,108	106	1.53	83	1.37	93	1.66	1130	353	1386	12	0	0	0	0	0	34.0	34.0
Cylindrospermopsis raciborskii cs-505	3,879,030	58	0.89	31	1.28	36	1.34	1227	281	2202	4	185	631	816	3.86	39.5	32.9	40.2
Raphidiopsis brookii D9	3,186,511	10	0.29	6	0.20	7	0.24	927	504	1105	4	3	7	10	0.093	36.9	42.6	40.1

Legends of Figures

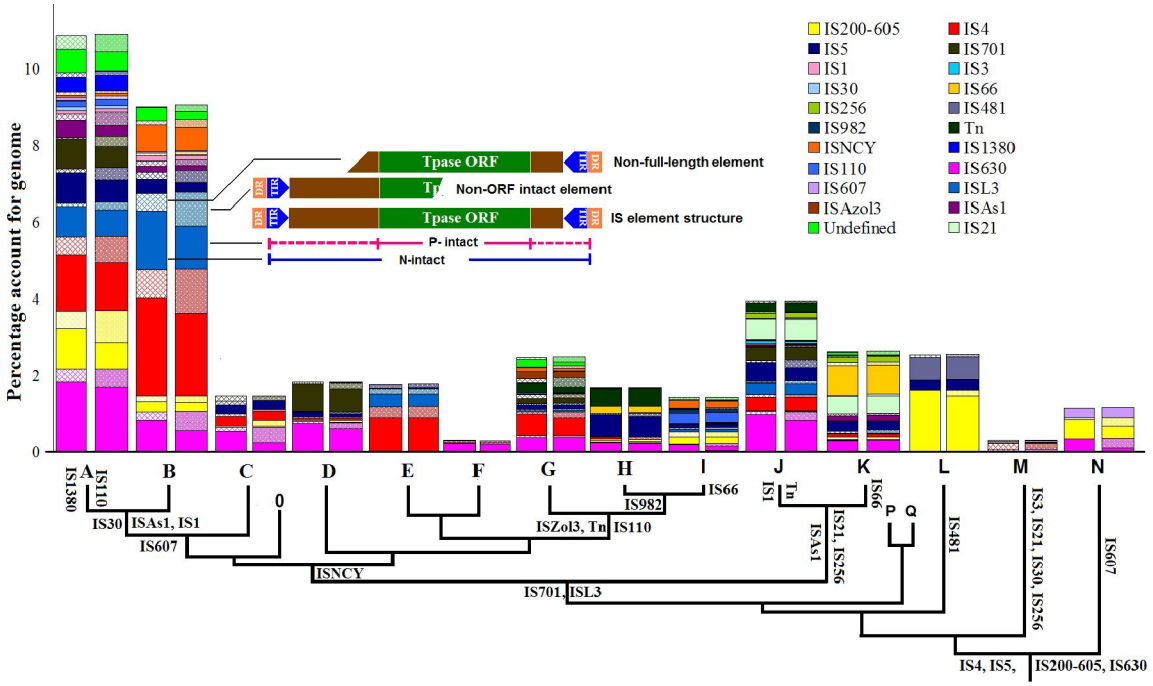

Figure 1. The IS family composition of seventeen cyanobacterial genomes. For each strain, the left and right columns represent the N-intact and P-intact IS distributions respectively. Grid columns represent non-intact elements. The numbers marked above each column are the abundances of N- or P-intact elements. **A.** *Microcystis aeruginosa* NIES-843, **B**. *Microcystis aeruginosa* PCC 7806, **C**. *Synechocystis* sp. PCC 6803, **D.** *Trichodesmium erythraeum* IMS101, **E.** *Cylindrospermopsis raciborskii* CS-505. **F.** *Raphidiopsis brookii* D9, **G.** *Nostoc punctiforme* PCC 73102, **H.** *Anabaena variabilis ATCC 29413*, **I.** *Anabaena* sp. PCC 7120, **J.** *Acaryochloris marina* MBIC11017, **K.** *Cyanothece sp.* PCC 7425, **L.** *Thermosynechococcus elongatus* BP-1, **M.** *Gloeobacter violaceus* PCC 7421, **N**. *Synechococcus* sp. JA-3-3Ab, **O.** *Synechococcus* sp. PCC7002, **P.** *Prochlorococcus* sp. MIT 9211, **Q.** *Prochlorococcus* sp. MIT 9215 (The last three strains weren't shown due to no IS elements identified in chromosome genomes). The lower figure is the 16S rDNA sequences based phylogeny of the strains investigated. For each IS family we highlight the most parsimonious scenario of IS families gained by mapping

642 acquisition of elements at each node. The distribution of IS families were also indicated
643 for each strains.
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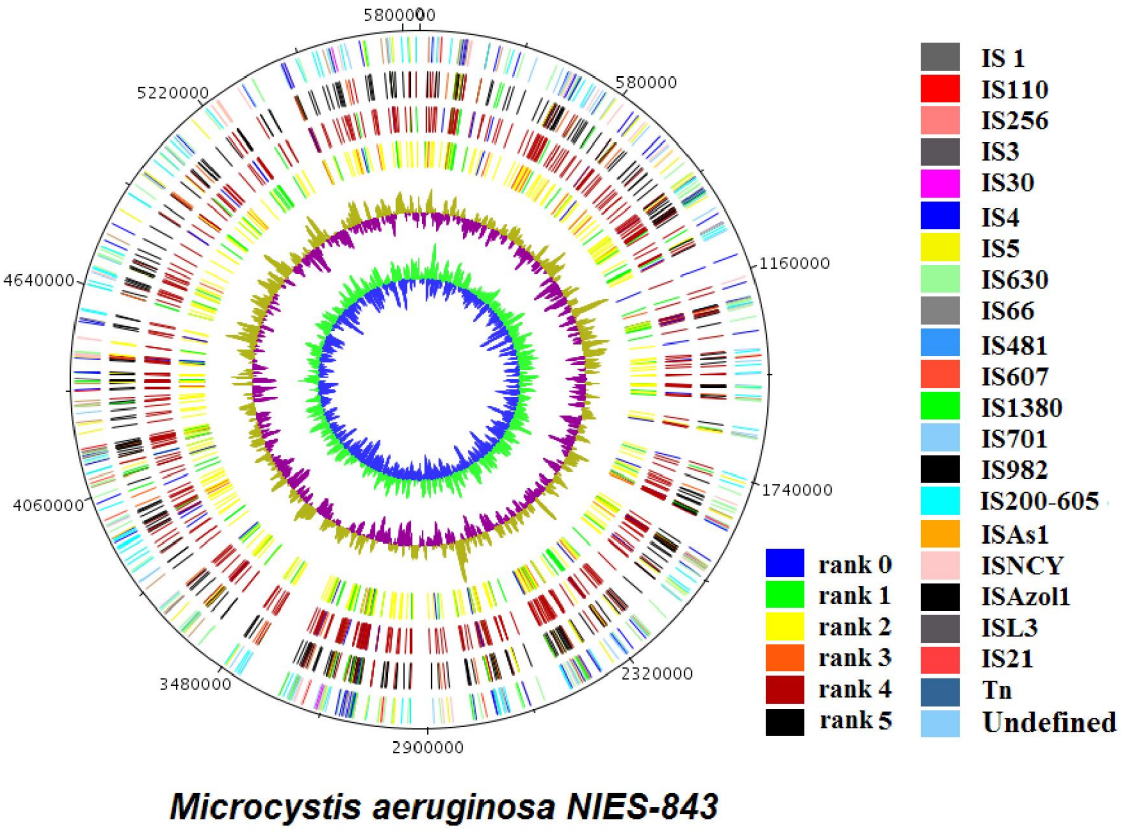

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646 Figure 2. The insert element map portrayed in the circular chromosome of *Microcystis*
647 *aeruginosa* NIES 843 genomes. The scale indicates location in bp. The bars marked from
648 outmost circle to the inner ones with colorful marks corresponding to the different IS
649 families, the coverage rank, the similarity rank and the length rank, the GC plot and GC
650 skew respectively. The rank setting for coverage: rank5: 99%-100%; rank4: 80%-99%;
651 rank3: 60%-80%; rank2: 40-60%; rank1: 20%-40% and rank0: <20%. The rank setting
652 for similarity: rank4: 0.9-1; rank3: 0.8-0.9; rank2: 0.7-0.8; rank1: 0.6-0.7 and rank0: <0.7.
653 The rank setting for length: rank4: >3000bp; rank3: 2000-3000bp; rank2: 1000-2000bp;
654 rank1: 500-1000bp and rank0: <500bp.
655

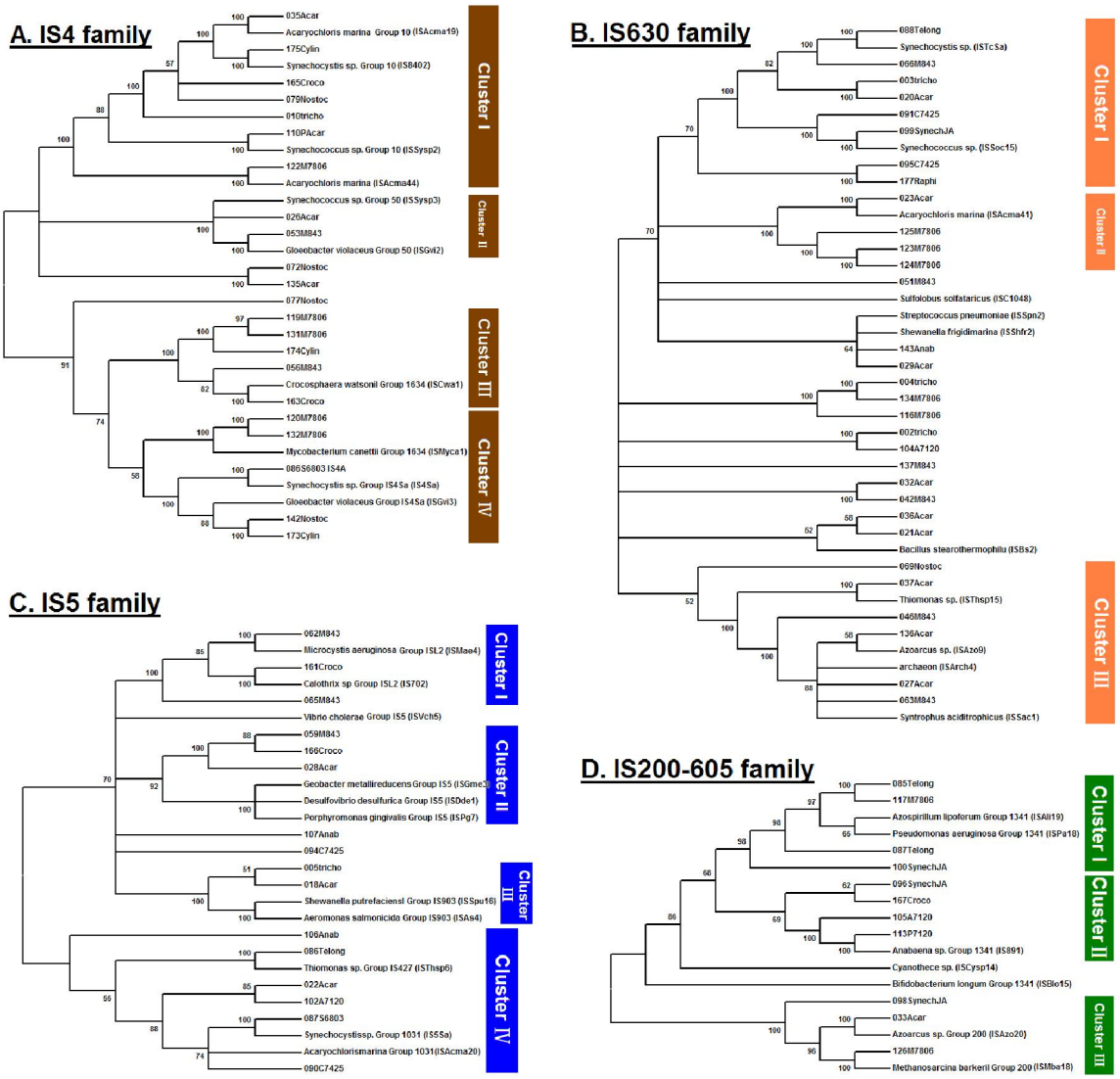


Figure 3. Phylogenies based on transposase amino acid sequences of the putative ancestral IS families in cyanobacteria. Bootstrap values greater than 50% with neighbor-joining methods are indicated on the trees. The records with brackets were from ISFinder database.

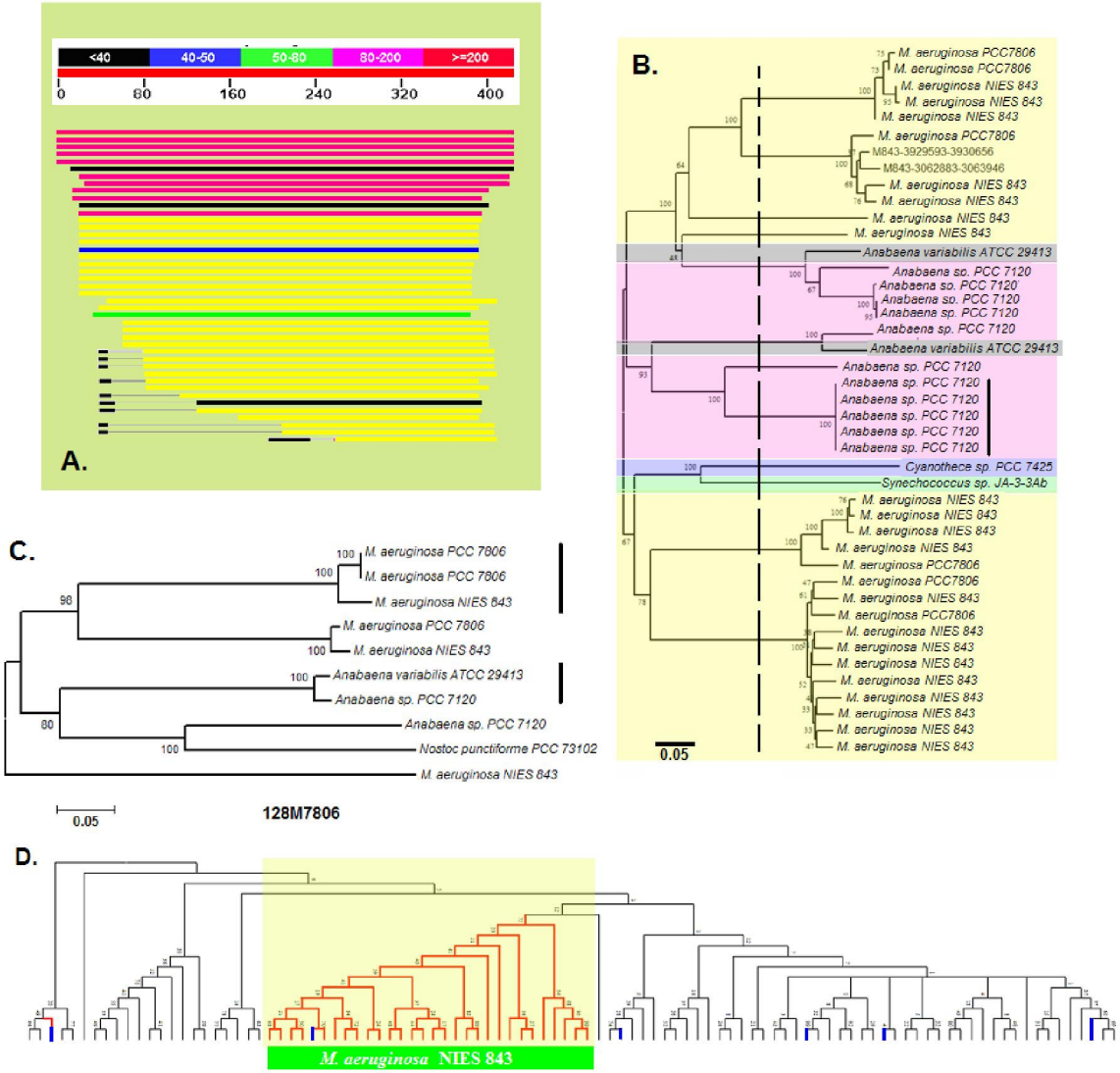


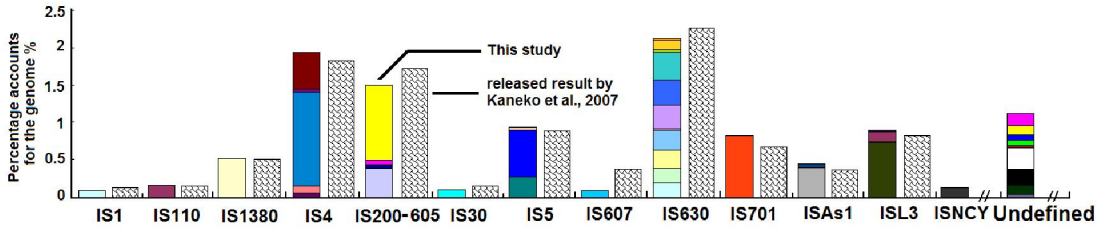
Figure 4. The phylogenies based on the all the IS nucleotide/ transposase amino acid sequences of subfamilies 113P7120, 128M7806 and 048M843. 4A. the alignment of all the transposase amino-acid sequences of the IS subfamily 113P7120; 4B. The phylogeny based on the nucleotide sequences from IS subfamilies 113P7120, the bars in pink, black, yellow, blue and green represent the sequences from *Anabaena* sp 7120, *Anabaena variabilis* ATCC29413, two *Microcystis aeruginosa* strains of NIES843 and PCC7806, *Cyanothece* sp. 7425 and *Synechococcus* sp. JA-3-3Ab respectively; 4C. the phylogeny based on the nucleotide sequences of the IS subfamily 128M7806; 4D, the phylogeny based on the nucleotide sequences of the IS subfamily 048M843. All the clades in

black represent the clades of ISs from the strain of PCC7806. The clade lines in red represent the clades of ISs from the strain of NIES843 and the clade lines in blue represent the clades of ORF fractured IS elements.

Supplemental File -1
by Lin et al.,

Evaluating the reliability of the repeat elements based IS element mining method

Microcystis aeruginosa NIES 843 was shown to contain the highest abundance of IS element system. To evaluate the reliability of the repeat elements based IS element mining, a comparison of the result on the IS element abundance and composition of genome in this study with that reported by Kaneko et al. (2007), was performed (Supplemental Figure 1). As shown in supplemental figure 1, 534 pieces of IS elements were collected and defined in this study, while 452 pieces were reported by Kaneko et al. (2007). 98% of IS elements reported by Kaneko et al. (2007) was covered in this study. Pair-samples test result illustrated that no significant difference was reflected by these two sets of results (p value=0.444 >>0.05). The MITE element was predicted to cover 91.9% of the previously reported MITE elements (Kaneko et al., 2007), however the frequency of MITEs mined in this study was four folds more than that reported by Kaneko et al. (2007).



Supplemental figure 1. The comparison of the IS element abundance and composition of *M. aeruginosa* NIES 843 genome between this study and the previous report by Kaneko et al. (2007). The elements belonging to different IS subfamilies were marked in different colors. In each IS family, two columns represent the IS element contents shown in this study (Left) and in the study by Kaneko et al. (2007) (Right) respectively.