RESEARCH ARTICLE

Role of habitat and great oxidation event on the occurrence of three multisubunit inorganic carbon-uptake systems in cyanobacteria

VANDANA TOMAR, GURPREET KAUR SIDHU, PANCHSHEELA NOGIA, RAJESH MEHROTRA and SANDHYA MEHROTRA*

Department of Biological Sciences, Birla Institute of Technology and Science, Pilani 333 031, India

Abstract

The oxygenase reaction catalyzed by RuBisCO became an issue only after the evolution of the oxygenic photosynthesis in cyanobacteria. Several strategies were developed by autotrophic organisms as an evolutionary response to increase oxygen levels to help RuBisCO maximize its net carboxylation rate. One of the crucial advancements in this context was the development of more efficient inorganic carbon transporters which could help in increasing the influx of inorganic carbon (Ci) at the site of CO_2 fixation. We conducted a survey to find out the genes coding for cyanobacterial Ci transporters in 40 cyanobacterial phyla with respect to transporters present in *Gloeobacter violaceous* PCC 7421, an early-diverging cyanobacterium. An attempt was also made to correlate the prevalence of the kind of transporter present in the species with its habitat. Basically, two types of cyanobacterial inorganic carbon transporters exist, i.e. bicarbonate transporters and CO_2-uptake systems. The transporters also show variation in context to their structure as some exist as single subunit proteins (BicA and SbtA), while others exist as multisubunit proteins (namely BCT1, $NdhI_3$ and $NdhI_4$). The phylogeny and distribution of the former have been extensively studied and the present analysis provides an insight into the latter ones. The *in silico* analysis of the genes under study revealed that their distribution was greatly influenced by the habitat and major environmental changes such as the great oxidation event (GOE) in the course of their evolution.

[Tomar V., Sidhu G. K., Nogia P., Mehrotra R. and Mehrotra S. 2016 Role of habitat and great oxidation event on the occurrence of three multisubunit inorganic carbon-uptake systems in cyanobacteria. *J. Genet.* **95**, 109–118]

Introduction

Cyanobacteria or blue green algae are one of the most diverse groups of organisms which colonize various niches including freshwater (rivers, ponds and lakes), polar caps, hot springs, alkaline, estuarine, open as well as saline oceans. During the ancient environmental adversities, these organisms along with certain algal species developed active systems known as carbon-concentrating mechanisms (CCM) to concentrate CO_2 at the site of RuBisCO activity which in turn led to a positive impact on photosynthetic ability of these organisms (Caemmerer and Evans 2010). This strategy was also adopted by the land plants and they developed certain anatomical features (Kranz anatomy) to increase the carboxylation activity of RuBisCO, thus reducing the resultant photorespiratory losses (Raven *et al.* 2008).

The CCM present in cyanobacteria are highly efficient and can accumulate CO_2 around RuBisCO by a factor of 1000-fold above ambient levels (Price *et al.* 2011). CCMs in cyanobacteria and proteobacteria as a whole include (i) RuBisCO and carbonic anhydrases (CA) enclosed in microcompartments known as carboxysomes and (ii) Ci transporters, which regulate the CO_2/HCO_3^- influx and efflux at the site of RuBisCO activity and hence lead to a marked elevation in the CO_2 concentration in the vicinity of RuBisCO.

Cyanobacteria are dependent on active accumulation of Ci to achieve a satisfactory rate of CO_2 fixation and growth (Badger *et al.* 2002). The efficacy of any CCM relies on the ability to minimize the loss of CO_2 from the CO_2 elevation zone or the dissolved Ci accumulation zone (Price *et al.* 2007). It can be accomplished in four ways. First, the accumulation of bicarbonate instead of CO_2 reduces the chances of Ci leakage, since the former is less permeable through the plasma membrane as compared to the latter. Secondly, the absence of CA activity in the cytosol minimizes leakage due to wasteful conversion to CO_2 and subsequent diffusion back to the external medium. Thirdly, the carboxysome protein shell is proposed to have specifically

*For correspondence. E-mail: sandhyamehrotrabits@gmail.com.

Keywords. inorganic carbon transporters; *Gloeobacter violaceous* PCC 7421; great oxidation event; cyanobacteria; evolution; carbon-concentrating mechanism.

charged pores which allow entry of only specific polar moieties like HCO_3^-, RuBP etc., while retarding the entry/exit of nonpolar molecules like CO_2 and O_2 which helps to reduce photorespiration and increase efficient CO_2 fixation. Finally, thylakoid located CO_2 pumps (e.g., $NdhI_3$ in *Synechococcus* sp.) play a key role in recycling CO_2 that leaks from the carboxysome back into the HCO_3^- pool/cytosol.

The type of Ci transporters present in cyanobacteria is proposed to be governed by the habitat in which they dwell (Badger *et al.* 2006). Basically, they are of five different types which can be broadly categorized into two types namely, (i) bicarbonate transporters (substrate - HCO_3^-) and (ii) CO_2-uptake complexes (substrate - CO_2). Bicarbonate transporters include BCT1, BicA and SbtA. BCT1 is an inducible, high-affinity bicarbonate transporter, encoded by *cmpABCD* operon and comes under traffic ATPase family. BicA and SbtA are sodium-dependent and probably act as Na^+/HCO_3^- symporters (Price and Howitt 2011). CO_2-uptake systems include the $NdhI_3$ ($ndhF_3D_3chpY$) and $NdhI_4$ ($ndhF_4D_4chpX$) complexes, which are based on specialized NADPH dehydrogenase complex.

Higher-affinity Ci transporters are switched on only when the Ci concentration in the environment becomes limited. There is less variety in terms of transporters existing in marine species like *Prochlorococcus* sp. which possess a limited range of HCO_3^- transporters and no CO_2-uptake systems but at the same time certain marine species exhibit enormous variations in Ci transporter complement. This fluctuation is observed due to varying Ci levels and temperature in different habitats. Freshwater species also show a range of Ci transporters. Species which occupy lake environments and peak in their abundance during summer contain the most complete complement of transporters, being correlated with a great deal of environmental fluctuations in Ci levels, temperature, oxygen and nutrients. Species with reduced sets of transporters are correlated with growth in symbiotic environments, thermal hotsprings and calcareous rocks, where environmental fluctuations are expected to be less extreme.

BicA, a sodium-dependent bicarbonate transporter is present in almost all cyanobacterial species but is absent in *Gloeobacter* sp. This cyanobacterial species is slow-growing in nature and is found in an environment which is rich in both CO_2 and HCO_3^- resources which makes it a suitable candidate to possess low-affinity rather than high-affinity bicarbonate transporters (Price *et al.* 2011). BCT1 and SbtA are abundant in β-cyanobacteria but lack presence in α-cyanobacteria. Among the CO_2-uptake systems, $NdhI_4$ complex has credited its presence in all cyanobacterial species, whereas $NdhI_3$ is thought to exist only in certain fresh water species (table 1).

The transporter complexes for both CO_2 and HCO_3^- uptake are located on the plasma membrane of the cyanobacteria. The only exception to it is the $NdhI_3$-uptake system for CO_2 which is present on the thylakoids. In *Gloeobacter* sp., this complex is situated at the plasma membrane itself as it lacks thylakoids. The fresh water species (abundantly the β-cyanobacteria) possess a wide array of transporters, whereas the α-cyanobacteria have few uptake systems. Among α-cyanobacterial HCO_3^--uptake systems, BCT1 and SbtA are absent, whereas BicA is widely distributed. The thylakoid located high-affinity transporter ($NdhI_3$) is a less abundant CO_2 transport system in α-cyanobacteria as compared to its low-affinity counterpart ($NdhI_4$).

There have been several speculations regarding the first evolutionary steps towards developing a cyanobacterial CCM (Badger *et al.* 2002). The initial step for the same, during the stages of CO_2 decline would have been the evolution of a carboxysome structure for RuBisCO to accumulate CO_2 near RuBisCO and thereby increase the efficiency of CO_2 fixation (Parry *et al.* 2003). This structure is essential for concentration of CO_2 and hence Ci transporters are ineffectual without it. A carboxysomal CA would probably also have been acquired at this stage as the rate of a non-catalyzed chemical conversion of HCO_3^- to CO_2 would have been too slow to support photosynthetic CO_2 supply. As CO_2 limitation became more severe, the CCM would probably have been improved by the development of a diverse array of both CO_2 and HCO_3^- uptake systems of varying affinities to acquire Ci actively from the surrounding environments.

The evolutionary studies based on 16s rRNA suggest that *Gloeobacter* and *Thermosynechococcus* sp. are amongst the most primitive cyanobacteria and possibly the ancestors of all cyanobacteria (Nelissen *et al.* 1995). *G. violaceus* diverged very early during the cyanobacterial radiation, in an ancient lineage preceding the cyanobacterial chloroplast ancestors. It possesses a unique molecular structure of photosystems I and II and an unusual morphology of its phycobilisomes (PBS), which enables it to harvest light and transfer energy in a manner which is different from other photosynthetic organisms (Mimuro *et al.* 2010). *Gloeobacter* sp. carries a single HCO_3^- transporter (BCT1) and two CO_2-uptake systems. Therefore, it can be presumed that a relational study of transporters present in this organism can pave the path for determination of evolutionary pattern of Ci transporters in cyanobacteria. Thus, the present research envisages the study of Ci transport systems present in *Gloeobacter* sp. in relation to other cyanobacteria and makes an effort to comprehend the arrangement of the genes constituting these transport systems and influence of habitat and the GOE on their evolution.

Materials and methods

Cyanobacterial species, gene sequences of transporters

To predict the evolutionary trend of these genes, the gene sequences encoding the operons (*cmpABCD*, *NdhI*$_3$ and *NdhI*$_4$) were searched in 40 cyanobacterial species namely, *G. violaceous* PCC 7421, *Thermosynechococcus elongatus* bp-1, *Synechococcus elongatus* PCC 7942, *Synechocystis* sp. PCC 6803, *Synechococcus elongatus* PCC 6301,

Table 1. List of various transporters present in α-cyanobacteria and β-cyanobacteria.

Strain	CO_2-uptake systems		HCO_3-uptake systems		
	$NdhI_3$	Ndh I_4	BCT1	SbtA	BicA
β-cyanobacteria					
Gloeobacter violaceous PCC 7421	+	+	+	−	−
Thermosynechococcus elongates bp-1	+	+	+	−	−
Synechocystis 6803	+	+	+	+	+
Nostoc punctiforme ATCC 29133	+	+	+	−	+
Anabaena 7120	+	+	+	−	−
Anabaena variabilis 29413	+	+	+	−	−
Synechococcus 6301	+	+	+	+	+
Synechococcus 7942	+	+	+	+	+
Synechococcus sp. PCC 7002	+	+	−	+	+
Arthrospira platensis NIES-39	+	+	+	+	+
Trichodesmium erythraeum IMS101	+/?	+/?	−	−	−
Cyanothece sp. PCC 7424	+	+	+	−	−
Cyanothece sp. PCC 7425	+	+	+	−	−
Acaryochloris marina MBIC 1107	+	+	−	−	−
Cyanothece sp. PCC 8801	+	+	+	−	−
Cyanothece sp. ATCC 51142	+	+/?	+	+	+
Synechococcus sp. RCC 307	−	+	+/?	−	−
Microcystis aeruginosa NIES-843	+	+/?	+	+/?	+/?
Synechococcus sp. JA-3-3Ab	+	+	+	−	−
Synechococcus sp. JA-2-3B′a (2-13)	+	+	+	−	−
α-Cyanobacteria					
Synechococcus 9902	−	+	−	−	−
Synechococcus sp. WH 8102	−	+	−	−	−
Synechococcus sp. WH 7803	−	−	−	−	−
Synechococcus sp.PCC 9605	−	+	−	−	−
Synechococcus sp. CC 9311	−	+	−	−	−
Synechococcus sp. BL107	−	+/?	−	−	−
Prochlorococcus 9313	−	−	−	+	−
Prochlorococcus SS120	−	−	−	+	−
Prochlorococcus 9312	−	−	−	−	+/?
Prochlorococcus MED4	−	−	−	+	−
Prochlorococcus marinus str. NATL1A	−	−	−	−	+/?
Prochlorococcus marinus str. NATL2A	−	−	−	−	+/?
Prochlorococcus marinus str. AS9601	−	−	−	−	+/?
Prochlorococcus marinus str. MIT 9515	−	−	−	−	+/?
Prochlorococcus marinus str. MIT 9303	−	−	−	−	+/?
Prochlorococcus marinus str. MIT 9301	−	−	−	−	+/?
Prochlorococcus marinus str. MIT 9215	−	−	−	−	+/?
Prochlorococcus marinus str. MIT 9211	−	−	−	−	+/?
Prochlorococcus marinus subsp. *marinus* str. CCMP 1375	−	−	−	−	+/?
Prochlorococcus marinus subsp. *pastoris* str. CCMP 1986	−	−	−	−	+/?

Anabaena 7120, *Anabaena variabilis* 29413, *Nostoc punctiforme* ATCC 29133, *Synechococcus* sp. PCC 7002, *Arthrospira platensis* NIES-39, *Synechococcus* sp. WH 8102, *Synechococcus* sp. CC 9605, *Synechococcus* sp. CC 9311, *Cyanothece* sp. PCC 7424, *Cyanothece* sp. ATCC 51142, *Synechococcus* sp. RCC 307, *Synechococcus* sp. CC 9902, *Acaryochloris marina* MBIC 11017, *Cyanothece* sp. PCC 8801, *Microcystis aeruginosa* NIES-843, *Trichodesmium erythraeum* IMS101, *Cyanothece* sp. PCC 7425, *Synechococcus* sp. BL107, *Synechococcus* sp. WH 7803, *Synechococcus* sp. JA-3-3Ab, *Synechococcus* sp. JA-2-3B′ a(2-13), *Prochlorococcus* 9313, *Prochlorococcus* SS120, *Prochlorococcus* 9312, *Prochlorococcus* MED4, *Prochlorococcus marinus* str. NATL1A, *Prochlorococcus marinus* str. NATL2A, *Prochlorococcus marinus* str. AS9601, *Prochlorococcus marinus* str. MIT 9515, *Prochlorococcus marinus* str. MIT 9303, *Prochlorococcus marinus* str. MIT 9301, *Prochlorococcus marinus* str. MIT 9215, *Prochlorococcus marinus* str. MIT 9211, *Prochlorococcus marinus* subsp. marinus str. CCMP 1375 and *Prochlorococcus marinus* subsp. pastoris str. CCMP 1986 from the Kazusa cyanobase (http://genome.microbedb.jp/cyanobase) and NCBI (http://www.ncbi.nlm.nih.gov/cdd/).

Multiple sequence alignment, homology prediction, phylogenetic analysis and conserved domain analysis

Since the genes for these transporters under study are not yet well-characterized in several organisms, the sequences for

the same were identified by searching homologous sequences of these genes by performing multiple sequence alignment using BioEdit ver. 7.2.5 (Hall 1999). The retrieved sequences were then subjected to phylogenetic analysis. Phylogenetic trees were constructed by maximum likelihood using MEGA software ver. 5.1 (Tamura *et al.* 2011) using 1000-bootstrap replicates. The distribution of various transporters among the cyanobacterial species under study was correlated to the evolution of the cyanobacteria which was inferred from 16s rRNA analysis of cyanobacteria by Nelissen *et al.* (1995). Conserved domains were analysed using conserved domain database at NCBI (http://www.ncbi.nlm.nih.gov/cdd/).

Results

The cyanobacterial genes coding for the Ci transportation are present not only as single gene encoded units (e.g., BicA and SbtA) but also exist as a part of transport machinery i.e. in the form of operons (e.g., *BCT1*, *NdhI*$_3$ and *NdhI*$_4$). BCT1 is encoded by *cmpABCD* operon in which *cmpB* forms transmembrane helices and *cmpA*, *cmpC* and *cmpD* aid in bicarbonate entry and exit. The CO_2-uptake systems exist as $NdhI_3$ and $NdhI_4$ complexes which are present as $ndhD_3 - ndhF_3 - cupA$ and $ndhD_4 - ndhF_4 - cupB$ operons respectively. The arrangement of genes encoding the BCT1 transporter namely, *cmp ABCD* operon are depicted in figure 1a and those of *NdhI*$_3$ and *NdhI*$_4$ are represented by figure 1b and figure 1c respectively.

The cyanobacterial evolution according to 16S rRNA has been elucidated by Rudi *et al.* (1997). In accordance with this classification, *G. violaceous* PCC 7421 is the most primitive cyanobacteria and hence the transporters present in *G. violaceous* PCC 7421 can also be considered as the most primitive ones. The arrangement of the genes in the operon is found to be same in all the cyanobacterial strains studied, which implies that possibly all the genes for the transporter evolved as a unit or coevolved in a related pattern. When further investigated, the phylogenetic tree of the each gene brought forth a different picture, showing a different evolutionary pattern for each of the genes of the operon.

Cyanobacteria show diversity in terms of distribution of the inorganic carbon transporters. Among the five most widely known transporters, SbtA and BicA are less widely distributed in comparison to BCT1, $NdhI_3$ and $NdhI_4$ transporters. *Prochlococcus* sp. which is an α-cyanobacteria is devoid of both CO_2 transporters and harbours only one bicarbonate transporter, i.e. either BicA or SbtA. *Prochlorococcus* MED4, *Prochlorococcus* 9313 and *Prochlorococcus*

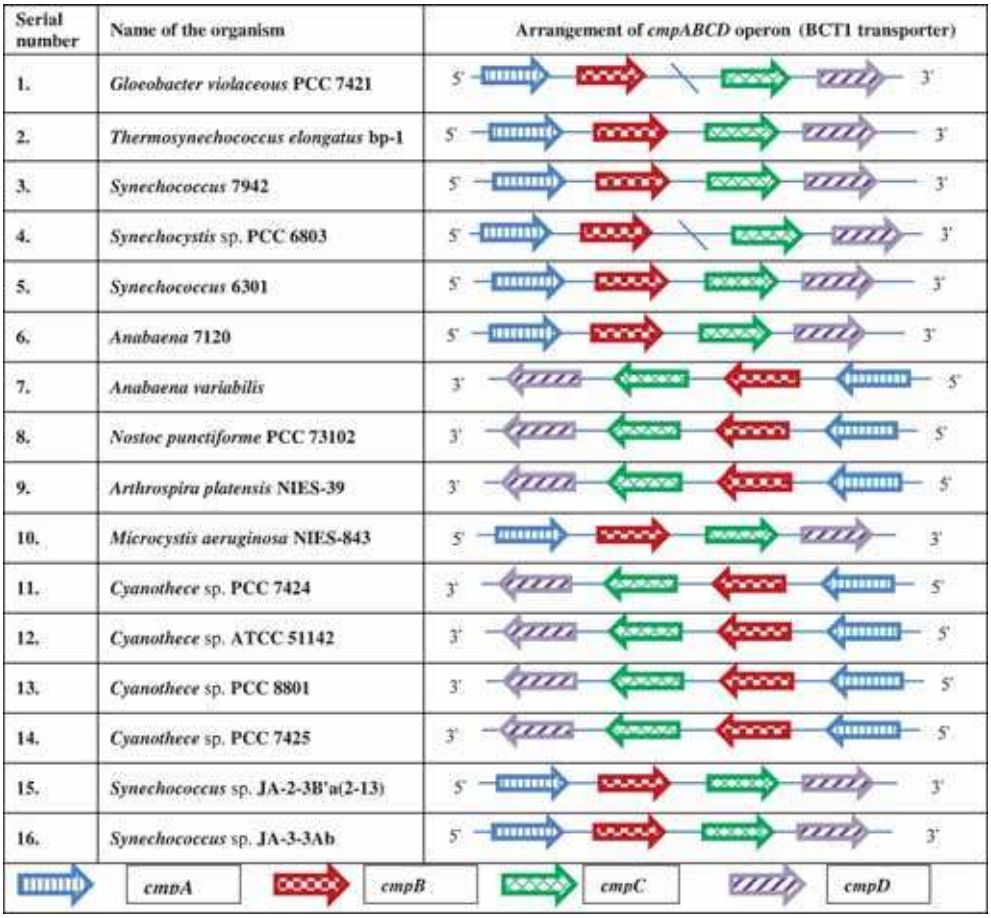


Figure 1a. Arrangement of the genes encoding the cyanobacterial Ci transporters. Schematic representation of the arrangement of genes in BCT1 transporter (*cmp ABCD*).

Serial number	Name of the organism	Arrangement of Ndh-I_3 operon
1.	*Gloeobacter violaceous* PCC 7421	
2.	*Thermosynechococcus elongatus* bp-1	
3.	*Synechococcus* 7942	
4.	*Synechocystis* sp. PCC 6803	
5.	*Synechococcus* 6301	
6.	*Anabaena* 7120	
7.	*Anabaena variabilis*	
8.	*Nostoc punctiforme* PCC 73102	
9.	*Synechococcus* sp. PCC 7002	
10.	*Arthrospira platensis* NIES-39	
11.	*Microcystis aeruginosa* NIES-843	
12.	*Cyanothece* sp. PCC 7424	
13.	*Cyanothece* sp. ATCC 51142	
14.	*Acaryochloris marina* MBIC 11017	
15.	*Cyanothece* sp. PCC 8801	
16.	*Cyanothece* sp. PCC 7425	
17.	*Synechococcus* sp. JA-2-3B'a(2-13)	
18.	*Synechococcus* sp. JA-3-3Ab	

Figure 1b. Arrangement of the genes encoding the Ndh-I_3 uptake system.

SS120 show presence of SbtA, a high-affinity bicarbonate transporter, whereas other *Prochlococcus* sp. show the presence of homologues of BicA, a low-affinity bicarbonate transporter. The β-cyanobacteria have a mixed array of CO_2 as well as bicarbonate transport systems in which mutisubunit transporters were more predominant in comparison to the bicarbonate ones.

The BCT1 operon showed its presence in 16 out of 40 species studied. The phylogenetic tree depicted two major clades. The lower clade mostly consisted of late-diverging cyanobacteria-like *Cyanothece* sp., *Arthrospira platensis* and *Nostoc punctiforme*, while the upper clade comprised mostly the early-diverging ones. The only exception is *Anabaena* 7120 which although being a late-diverging was found in the clade of *G. violaceous* PCC 7421, an early-diverging bacteria. When the protein sequences of CmpA, B, C and D in *S. elongatus* PCC 7942 were further studied and they depicted conserved domains which were characteristic of the ABC type transporters. CmpA has the TauA domain and belongs to periplasmic-binding protein type 2 superfamily. This domain showed about 48–50% similarity to the ABC-type nitrate/sulphonate transport system of several archaea, proteobacteria and plants. The CmpB protein showed 50–60% similarity to the bicarbonate/nitrate ABC transporter permease of various proteobacteria and a few archaea and plants. The CmpC and CmpD have the ntrCD domain which is 55–60% similar to nitrate ABC transporter ATP-binding protein of proteobacteria and archaea.

The $NdhI_3$ operon was present in 18 species out of the 40 investigated. There were two major clades, the lower one primarily consisting of late-diverging cyanbacteria except *S. elongatus* PCC 6301 and *Synechococcus* sp. JA-3-3Ab (both are late-diverging). The upper clade further diverged into two subclades having early- and late-diverging cyanobacteria respectively.

The low affinity CO_2-uptake system namely, $NdhI_4$ is the most widely distributed among the three transporters studied and was found in 21 of all the species analysed. The phylogenetic tree of this transporter reveals two major clades. The upper one mainly consists of early-diverging cyanobacteria, while the lower one is a combination of both. *Synechococcus* sp. JA-3-3Ab, *Thermosynechococcus*

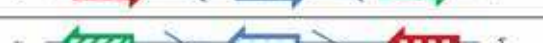
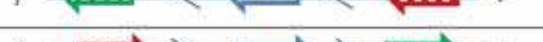
Serial number	Name of the organism	Arrangement of Ndh-I_4 operon
1.	*Gloeobacter violaceous* PCC 7421	
2.	*Thermosynechococcus elongatus* bp-1	
3.	*Synechococcus* 7942	
4.	*Synechocystis* sp. PCC 6803	
5.	*Synechococcus* 6301	
6.	*Anabaena* 7120	
7.	*Anabaena variabilis*	
8.	*Nostoc punctiforme* PCC 73102	
9.	*Synechococcus* sp. PCC 7002	
10.	*Arthrospira platensis* NIES-39	
11.	*Synechococcus* sp. WH 8102	
12.	*Synechococcus* sp. CC 9605	
13.	*Synechococcus* sp. CC 9311	
14.	*Cyanothece* sp. PCC 7424	
15.	*Synechococcus* sp. RCC 307	
16.	*Synechococcus* sp. CC 9902	
17.	*Acaryochloris marina* MBIC 11017	
18.	*Cyanothece* sp. PCC 8801	
19.	*Cyanothece* sp. PCC 7425	
20.	*Synechococcus* sp. JA-2-3B'a(2-13)	
21.	*Synechococcus* sp. JA-3-3Ab	
		

Figure 1c. Arrangement of the genes encoding the Ndh-I_4 uptake system. Ndh-I_4 operon was found to be present in most of the cyanobacterial phyla.

elongatus bp-1 and *S. elongatus* PCC 6301 which are closely related to *G. violaceous* PCC 7421 (an early-diverging cyanobacteria) are present in the upper clade which includes mostly the late-diverging cyanobacteria.

It should be noted that BCT1 of *Cyanothece* sp. PCC 8801, *Cyanothece* sp. PCC 7424 and *Cyanothece* sp. ATCC 51142 is distantly related to *G. violaceous* PCC 7421 which is located in a seperate clade. As far as $NdhI_3$ is concerned, *Cyanothece* sp. PCC 8801 was more closely related to *G. violaceous* PCC 7421 and are located in the same clade. For $NdhI_4$, all the *Cyanothece* sp. under study seem to show diversity in their nucleotide sequences as they appear to be far related to each other.

Another important observation is that *G. violaceous* PCC 7421, *Synechococcus* sp. JA-3-3Ab, *Synechococcus* sp. JA-2-3B′a (2-13) and *S. elongatus* PCC 6301 are closely related to each other when it comes to the BCT1 gene, while in case of $NdhI_3$ and $NdhI_4$, *Synechococcus* sp. JA-3-3Ab is far related from the other three. For the high-affinity transporters (BCT1 and $NdhI_3$) *G. violaceous* PCC 7421 and *Anabaena* sp. PCC 7120 appear to be closely related and fall in the same major clade. In the low-affinity transporter ($NdhI_4$), the early-diverging and late-diverging cyanobacteria are far related with the exception of *Cyanothece* sp. PCC 8801 (a late-diverging cyanobacteria).

In these CO_2-uptake systems CO_2 hydration proteins cupA (chpY) and cupB (chpX), which belong to chpXY superfamily were found to be present only in cyanobacteria, whereas $NdhD_3$/$NdhD_4$ and $NdhF_3$/$NdhF_4$ both had homologues in archea, proteobacteria as well as plants. The detailed phylogenetic study of these transporters showed an unorganized mode of evolution which implies that they might have

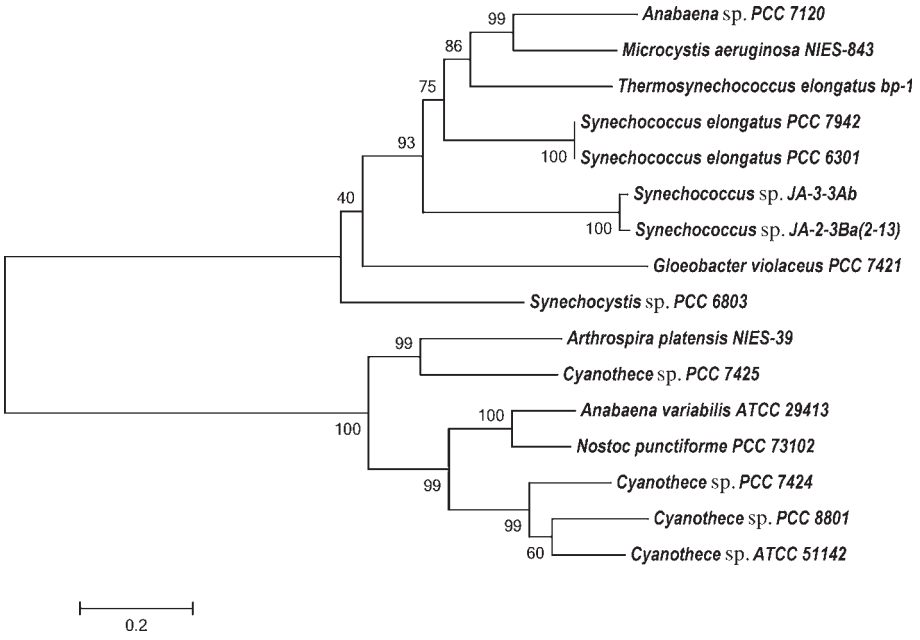


Figure 2. Phylogenetic tree of *cmpABCD* operon (BCT1) in 16 cyanobacterial species. The trees are constructed using maximum-likelihood method. Bootstrap values (1000 replicates) are shown for confidence levels higher than 50%. The scale bars indicate the number of nucleotide substitutions per site.

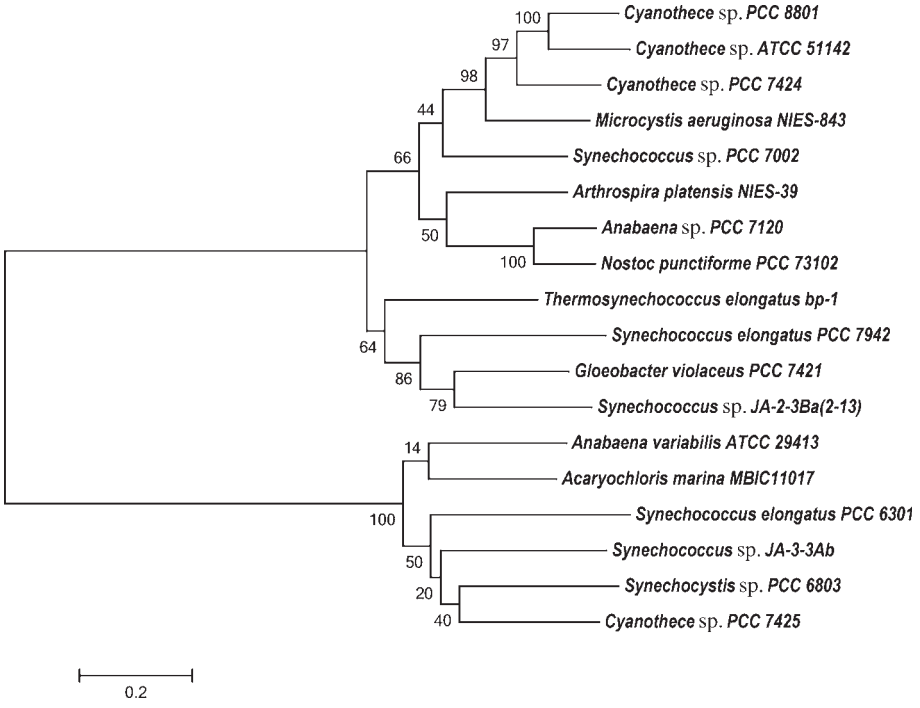


Figure 3. Phylogenetic tree of *Ndh-I*$_3$ operon in 18 cyanobacterial species. The trees are constructed using maximum-likelihood method. Bootstrap values (1000 replicates) are shown for confidence levels higher than 50%. The scale bars indicate the number of nucleotide substitutions per site.

evolved independently as evident from the figures (figures 2–4).

The analysis reveals that the low-affinity CO_2-uptake system ($NdhI_4$) was abundant in most of the cyanobacterial species which reflects that this transporter was possibly present in all the cyanobacteria prior to the GOE as the environment was rich in CO_2. The other two transporters (BCT1 and $NdhI_3$) perhaps evolved on the basis of organisms, need

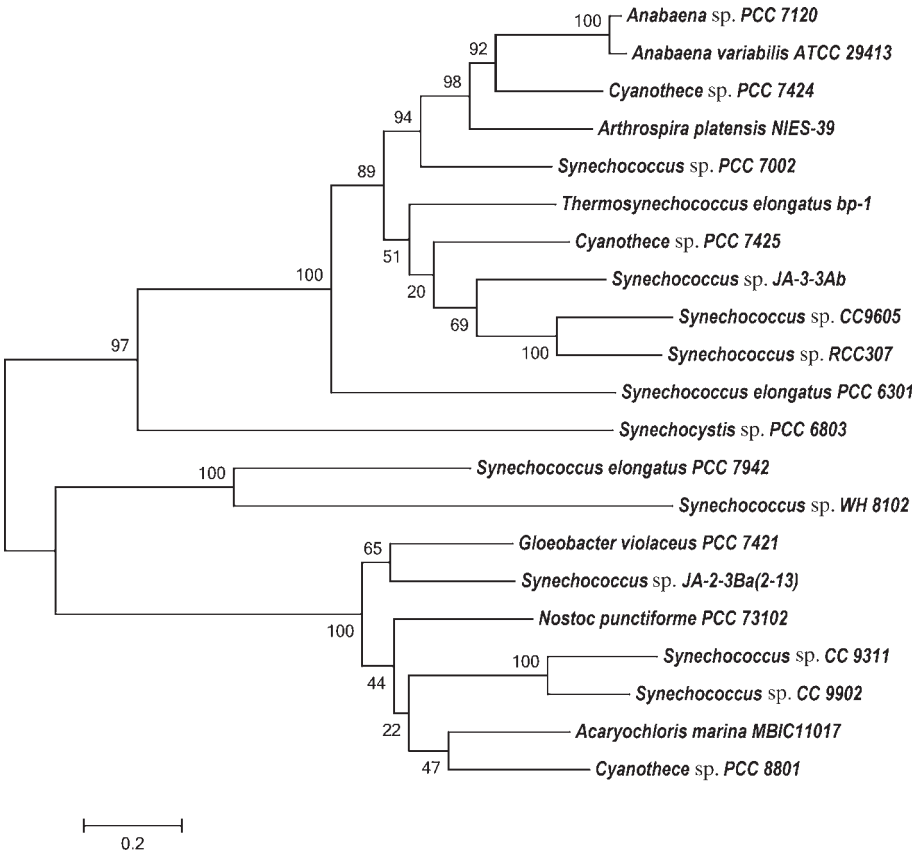


Figure 4. Phylogenetic tree of *Ndh-I4* operon in 21 cyanobacterial species. The trees are constructed using maximum-likelihood method. Bootstrap values (1000 replicates) are shown for confidence levels higher than 50%. The scale bars indicate the number of nucleotide substitutions per site.

in accordance to the changing environment by a functional uptake of certain pre-existing transporters.

Discussion

Diversity in suite of Ci transporters in cyanobacteria is governed by habitation

Cyanobacteria show a diverse collection of transporters depending on Ci availability and the environment in which they dwell. Cyanobacteria which inhabit the marine environments (α-cyanobacteria) possess less varied complement of bicarbonate transporters in comparison to the ones living in fresh water i.e., β-cyanobacteria (Badger *et al.* 2006). BCT1, a high-affinity bicarbonate transporter is absent in marine forms as the marine environment already possesses a substantially high content of bicarbonate which deters the need of a transporter requiring high energy investment (BCT1 requires ATP energization) and favours employment of less energy requiring sodium symporters like BicA and SbtA. Therefore, out of 40 species studied, BCT1 marked its presence only in 16, majority of which are β-cyanobacteria. BicA and SbtA have been reported to be present in almost all cyanobacterial strains but are absent in *G. violaceous* which indicates that these transporters were present before the branching of α-cyanobacterial and β-cyanobacterial clades and were possibly lost from *G. violaceous*. Both CO_2-uptake systems namely, $NdhI_3$ and $NdhI_4$ are present in *Gloeobacter* sp. which suggests that these were acquired during early stages of evolution but the high-affinity $NdhI_3$-uptake system was gradually lost from some marine species. $NdhI_4$ persisted and is still found in many cyanobacterial species inhabiting a wide regime of temperature and Ci fluctuations (Paerl 2000).

Impact of GOE on evolution of Ci transporters

The evolution of the genes related to photosynthesis greatly depends on environmental conditions. Earlier, the environment was CO_2-rich but after GOE, there was a drift from anoxygenic environment to an oxygenic one. The origin of cyanobacteria and the evolution of multicellularity are estimated before or at the beginning of the GOE. Relatively soon after GOE, three major cyanobacterial clades originated which included the unicellular cyanobacteria, terminally differentiated taxa and marine phytoplanktons. The phylogeny

shows that the cyanobacteria which came into existence before the GOE include β-cyanobacteria which comprise of *Gloeobacter*, *Thermosynechococcus* and certain *Synechococcus* species, while the cyanobacteria which evolved after the GOE include species like *Nostoc* and *Anabaena*. Since primitive cyanobacteria-like *Gloeobacter* had evolved before the GOE, it is likely that the CO_2 low-affinity transporters like $NdhI_4$ existed before GOE when the environment was CO_2 rich. But when the environment suffered dramatic change from a reducing to a nonreducing one, the high affinity transporters like $NdhI_3$ must have evolved by acquisition of the function of CO_2 transport probably by the modification of the already existing NAD(P)H dehydrogenase systems which were earlier involved in respiratory activities. Our hypothesis is in consonance with the findings of Howitt *et al.* (1999) who have revealed that the type II NADH dehydrogenases in the cyanobacterium *Synechocystis* PCC 6803 are involved in regulation rather than respiration. One of the cyanobacterial bicarbonate transporter, BicA is also reported to be the member of the SulP superfamily (Saier *et al.* 1999), which includes the plant SulP sulphate transporter family and the mammalian SLC26 family of anion transporters as well as numerous members in a wide range of prokaryotes (Price and Howitt 2011). BCT1, an ABC type transporter shows a strong similarity to the nitrate transporter (NRT1) located on the plasma membrane and its subunits NrtA and NrtC exhibits 46.5 and 30% identity to CmpA and CmpC, respectively (Omata 1991).

According to various reports, it has been found that the two types of CO_2-uptake systems may have been acquired before the branching of α-cyanobacteria, as α-cyanobacteria like *Prochlorochoccus* which dwell in marine habitats do not possess any of the CO_2-uptake systems and only possess HCO_3^--uptake systems (Badger and Price 2003) (HCO_3^- is the predominant form of Ci present in oceans). *Synechococcus* 9902 and *Synechococcus* 8102 possess only the low-affinity CO_2 transporter ($NdhI_4$). After further branching of α-cyanobacteria, the low-affinity CO_2 transporters might have been lost. The high-affinity CO_2-uptake system ($NdhI_3$) is generally absent in marine strains but some marine strains may have acquired these systems by horizontal gene transfer. β-cyanobacteria from freshwater face the greatest immoderations in Ci accessibility, influenced by the conditions of temperature and pH and therefore, these species possess the largest number of different types of transporters.

BCT1 being a high-affinity and high-energy demanding transporter, would not have been in great demand before GOE. The advent of GOE raised the demand of these high-affinity transporters due to scarcity of CO_2 in the vicinity of RuBisCO. Therefore, it can be anticipated that the cyanobacteria which evolved after GOE might have acquired this transporter through modification of the existing transporters like NRT1. $NdhI_4$, being a low-affinity and low-energy investment transporter would have been more prevalent in early environment when CO_2 concentration was higher.

Conclusion and future prospects

The phylogenetic analysis of these transporters in different phyla of cyanobacteria belonging to both α-cyanobacterial and β-cyanobacterial classes suggest that the early environmental conditions have played a major role in shaping the course of evolution of bicarbonate transporters. Depending on the habitat, the cyanobacteria enriched themselves with an array of transporters. As evolution is never a directed one, variations which were found beneficial were incorporated. Since the organization of the genes encoding the transporters exhibited a random order in different cyanobacterial clades, these could also be proposed to have been acquired by horizontal gene transfer comprising of single gene lateral transfer event from an ancestral archaeal, proteobacterial or even a cyanobacterial strain. There is also the possibility that both RuBisCO and carboxysome genes could be inherited by a single lateral gene transfer event as in many cyanobacteria, they are found ordered on contiguous regions of the chromosome (Badger *et al.* 2006).

This research reinforces the knowledge on the evolution of the cyanobacterial transporters namely, BCT1, $NdhI_3$ and $NdhI_4$ which play a major role in CO_2 and bicarbonate acquisition in cyanobacteria. Knowledge of the evolution of these genes can be a way to predict the best candidate cyanobacteria which can be used to contribute genes for the development of efficient carbon-concentrating mechanism even in plant species which lack such mechanisms.

Acknowledgements

Authors are grateful to Birla Institute of Technology and Science, Pilani, Rajasthan, India, for providing infrastructural and logistic support. VT is thankful to DST-inspire fellowship programme of DST, India. GKS is thankful to the UGC-BSR for her fellowship. PN is thankful to CSIR for senior research fellowship. This work was supported by SERB fast track project SERC/LS-0141/2010 sanctioned by the government of India to SM.

References

Badger M. R. and Price G. D. 2003 CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *J. Exp. Bot.* **54**, 609–622.

Badger M. R., Hanson D. T. and Price G. D. 2002 Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. *Funct. Plant. Biol.* **29**, 407–416.

Badger M. R., Price G. D., Long B. M. and Woodger F. J. 2006 The environmental plasticity and ecological genomics of the cyanobacterial CO_2 concentrating mechanism. *J. Exp. Bot.* **57**, 249–265.

Caemmerer S. V. and Evans J. R. 2010 Enhancing C_3 photosynthesis. *Plant Physiol.* **154**, 589–592.

Hall T. A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.

Howitt C. A., Udall P. K. and Vermaas W. F. J. 1999 Type 2 NADH dehydrogenases in the cyanobacterium *Synechocystis* sp. strain PCC 6803 are involved in regulation rather than respiration. *J. Bacteriol.* **181**, 3994–4003.

Mimuro M., Yokono M. and Akimoto S. 2010 Variations in photosystem I properties in the primordial cyanobacterium *Gloeobacter violaceus* PCC 7421. *Photochem. Photobiol.* **86**, 62–69.

Nelissen B., Van de Peer Y., Wilmotte A. and De Wachter R 1995 A nearly origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16S rRNA sequences. *Mol. Biol. Evol.* **12**, 1166–1173.

Omata T. 1991 Cloning and characterization of the nrtA gene that encodes a 45-kDa protein involved in nitrate transport in the cyanobacterium *Synechococcus* PCC 7942. *Plant Cell Physiol.* **32**, 151–157.

Paerl H. W. 2000 Marine plankton. In *The ecology of cyanobacteria* (ed. B. A. Whitton and M. Potts) Kluwer Academic Publishers, Dordrecht, The Netherlands.

Parry M. A. J., Andralojc P. J., Mitchell R. A. C., Madgwick P. J. and Keys A. J. 2003 Manipulation of RuBisCO: the amount, activity, function and regulation. *J. Exp. Bot.* **54**, 1321–1333.

Price G. D. and Howitt S. M. 2011 The cyanobacterial bicarbonate transporter BicA: its physiological role and the implications of structural similarities with human SLC26 transporters. *Biochem. Cell Biol.* **89**, 178–188.

Price G. D., Badger M. R., Woodger F. J. and Long B. M. 2007 Advances in understanding the cyanobacterial CO_2-concentrating mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants. *J. Exp. Bot.* **59**, 1441–1446.

Price G. D., Badger M. R. and Caemmerer S. 2011 The prospect of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis in C_3 crop plants. *Plant Physiol.* **155**, 20–26.

Raven J. A., Giordano M. and Beardall J. 2008 Insights into the evolution of CCMs from comparisons with other resource acquisition and assimilation processes. *Plant Physiol.* **133**, 4–14.

Rudi K., Skulberg O. M., Larsen F. and Jakobsen K. S. 1997 Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequences from the variable regions V6, V7 and V8. *App. Environ. Microbiol.* **63**, 2593–2599.

Saier M. H., Fard B. H., Garg S. J and Hutchinson W. J. 1999 Phylogenetic characterization of novel transport protein families revealed by genome analyses. *Biochim. Biophys. Acta* **1422**, 1–56.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S. 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *J. Mol. Evol.* **28**, 2731–2739.

Received 30 March 2015, in revised form 13 June 2015; accepted 27 July 2015
Unedited version published online: 3 August 2015
Final version published online: 10 February 2016