CyanoHABs: inevitable evolutionary ecological consequence for low nutrient-requiring cyanobacteria in water eutrophication

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Abstract

The mechanism of cyanobacterial harmful algal blooms (CyanoHABs) is complicated and confusing. One major reason is they are studied primarily from an ecological perspective and on bloom-forming species only. This narrow angle loses a broader evolutionary and ecological context in which CyanoHABs occur and fails to provide information on relevant components to achieve a wholistic understanding.

To derive a comprehensive mechanism of CyanoHABs, we examine CyanoHABs through the overlooked evolutionary and ecological lenses: evolutionary radiation, ecological comparison with co-living algae, and recently identified genomic functional repertoire between blooming and non-blooming species. We found key factors: (1) elaborate diverse functional repertoire and low nutrient requirement in cyanobacteria molded by early adaptive evolution, (2) cyanobacteria having lower nutrient requirements than green algae indeed, (3) there is no directed evolution in biological functions toward water eutrophication in cyanobacteria, (4) the CyanoHAB-associated functional repertoire are more abundant and complete in blooming than non-blooming species. These factors lead us to postulate a preliminary mechanism of CyanoHABs as a synergistic quad: superior functional repertoire, established with long adaptive radiation under nutrient-deficient conditions and not evolved toward eutrophic conditions, enables cyanobacteria to efficiently utilize elevated nutrients under current eutrophic regime for excess growth and CyanoHABs thereof, due to their lower nutrient requirements than co-living algae. This preliminary synthesis without doubt needs further empirical testing, which can be undertaken with more comparative studies of multiple species using integrated systems biology approaches.

I. Introduction

CyanoHABs are one of the most profound environmental hazards in modern human history in terms of their global geographical scale (Paerl et al., 2020), longstanding duration (over a century) (Francis, 1878; Lathrop & Carpenter, 1992), and tremendous economic loss (Steffensen, 2008). The mere fact that they are still intensifying and expanding under the global climate change (Chapra et al., 2017; Gobler, 2020; Paerl et al., 2020) attests CyanoHABs are still not fully understood such that their ecology remains 'complicated and confusing' (Wilhelm et al., 2020). Fortunately, a long history of ecological research combined with novel omics techniques has provided plenty of information to piece together and identify a working mechanism.

Provided the scale and complexity of CyanoHABs, one can take a step back and use a holistic approach by considering the fundamental principles of biology. For example, many seemingly complex genomic questions are often reconciled in the light of evolution—"Nothing in biology makes sense except in the light of evolution" (Dobzhansky, 1973)—as shown previously (Avise & Ayala, 2007; Lynch, 2007; Meyer, 2008). Cyanobacteria, the most ancient autotrophs (Boden et al., 2021) with the broadest geographical distribution on modern Earth (Schopf, 2002), have come to this success through various trajectories of adaptive evolution. By examining these trajectories of geological timescales in the context of each niche, one can deduce the key biological functions for their success. More importantly, comparing the early adaptive radiation and present-day ecological dominance of cyanobacteria enables one to identify the differences in factors that contribute to both types of success, respectively. These differences will be our starting points toward proposing a working mechanism of CyanoHABs.

Following this line of reasoning, we first reexamined the biological abilities of early cyanobacteria to survive and thrive in habitats which are physically harsh and nutritiously deficient during evolution on geological timescales. Next, we revisited water eutrophication processes to establish elevated nutrients as the necessary condition for

CyanoHABs and the fact that cyanobacteria have lower nutrient requirements than green algae, which have never bloomed in the current regime of eutrophication. Third, we summarized the biological functions driving bloom formation in eutrophic waters and showed the genes encoding these functions are not under positive (directed) selection but purifying selection. This finding suggests their biological capabilities are established before the era of water eutrophication. All these steps above led us to postulate the preliminary working mechanism of CyanoHABs.

II. Cyanobacteria: evolutionary champions

Originating in an anoxic biosphere of early Earth, cyanobacteria are likely to evolve in scarcity of macronutrients such as phosphorus and nitrogen (Bjerrum & Canfield, 2002; Falkowski, 1997; Kipp & Stüeken, 2017; Reinhard et al., 2017) and trace metals (Anbar & Knoll, 2002; Saito et al., 2003). This humble beginning of life leads to primitive metabolic networks (Goldford & Segrè, 2018), which suggests that cyanobacteria could only obtain minimal energy and matter in both variety and amount to support cell survival and division (Schopf, 2002). Arguably, their nutrient requirement for life is likely to be very low. Besides nutrient deficiency, early cyanobacteria also faced various types of stresses which are 'unforeseeable' for them. One type is the rising oxygen level they caused themselves through oxygenic photosynthesis, for which they developed various counterattack strategies in modern cyanobacteria (Moore et al., 2017; Raymond & Segrè, 2006). Ultraviolet light is another early stress which has persisted until now, but some cyanobacteria evolved against it by producing sunscreens (Garcia-Pichel et al., 2019). These global stresses were also intertwined with landscape changes through geophysical processes (e.g., volcanism and climatic shifts). Here, we demonstrate the remarkable adaptive ability of cyanobacteria by showcasing their evolution of biological capability and morphology under the rising oxygen levels.

Since origin, particularly after the Great Oxidation Event (GOE) (Figure 1), ancestral cyanobacteria quickly acquired versatile antioxidant capabilities against reactive oxygen species (ROSs), derived from O_2 produced as a result of oxygenic photosynthesis. Different classes of superoxide dismutases (SODs) and other types of antioxidant

enzymes are evolved to deal with ROSs throughout their entire history (Boden et al., 2021). Based on the availability of metal cofactors (another case of adaptation), three types of SODs—NiSOD, CuZnSOD, and MnSOD/FeSOD—have been found in four types of aquatic environments (Figure 1A). Besides direct enzymatic removal of ROSs, cyanobacteria have also evolved other strategies, e.g., in photosynthetic reaction center (RC) which split into two types as a response to the rising oxygen (Orf et al., 2018). Another adaptation lies in the redundancy (heavy investment) of key functions. For example, in photosystem I, cyanobacteria have two electron transporters that evolved separately: copper-dependent plastocyanin (PC) and iron-dependent cytochrome c_6 ($Cytc_6$). Distinct in the primary sequence and tertiary structure of protein/amino acid, their expression is controlled by a BlaI/CopY-family transcription factor (PetR) and a BlaR-membrane protease (PetP), depending on the availability of the metal cofactors (García-Cañas et al., 2021).

With a continuous rise of oxygen level, morphological diversification was called upon to provide an extra dimension for functional expansion. It has been confirmed that the evolution of multicellular morphotypes and the rate of morphological diversification coincide with the GOE onset (Schirrmeister et al., 2013). Heterocyst became a specialized cell form for nitrogen fixation, in which the oxygen level is reduced to pre-GOE levels (Figure 1B). Nitrogen fixation is viewed as a leap forward in promoting marine primary production and contributed to increased O_2 levels, coinciding with the rise of animals (Lyons et al., 2014). Similarly, cyanobacteria also obtained a specific photosynthetic structure for carbon fixation, carboxysome, in which CO_2 is increased from 10-15μM to 40mM outside and greatly improved the efficiency of enzymatic fixation of carbon dioxide (Mangan & Brenner, 2014). After the evolution of geological timescales (Figure 1C and 1D), modern cyanobacteria are among the most diverse prokaryotic phyla, with morphotypes ranging from unicellular to multicellular filamentous forms, including those able to terminally (i.e., irreversibly) differentiate in form and function (Hammerschmidt et al., 2020; Schirrmeister et al., 2013).

Billions of years of adaptive evolution have allowed structure elaboration and functional

diversification in coping with nutrient deficiency, environmental extremes and constraints (Paerl, 2014). This evolutionary divergence has enabled cyanobacteria to occupy all geographic habitats in modern Earth, including terrestrial and aquatic ecosystems, ranging from deserts to tropical rain forests, soils and limestones, and from open oceans and brackish waters to freshwaters and hydrothermal vents (Figure 2). This broad array of biological functions underlying the adaptive radiation in cyanobacteria is recorded in their genomes (Chen et al., 2021). A large proportion of the genes in their genomes are associated with adaption to the specific habitat they occupy, but not to any other habitats. Therefore, the genomes produce a pangenome with most genes being unique and only a small set of 323 genes being common among cyanobacteria (Shi & Falkowski, 2008). These core genes are mainly involved in housekeeping functions, as opposed to interacting with environment, e.g., ribosomal proteins, photosynthetic apparatus, ATP synthesis, chlorophyll biosynthesis, and the Calvin cycle. This small stable core and large variable shell of genomes suggest most of the non-overlapping functions are specialized in adaptation to distinct habitats.

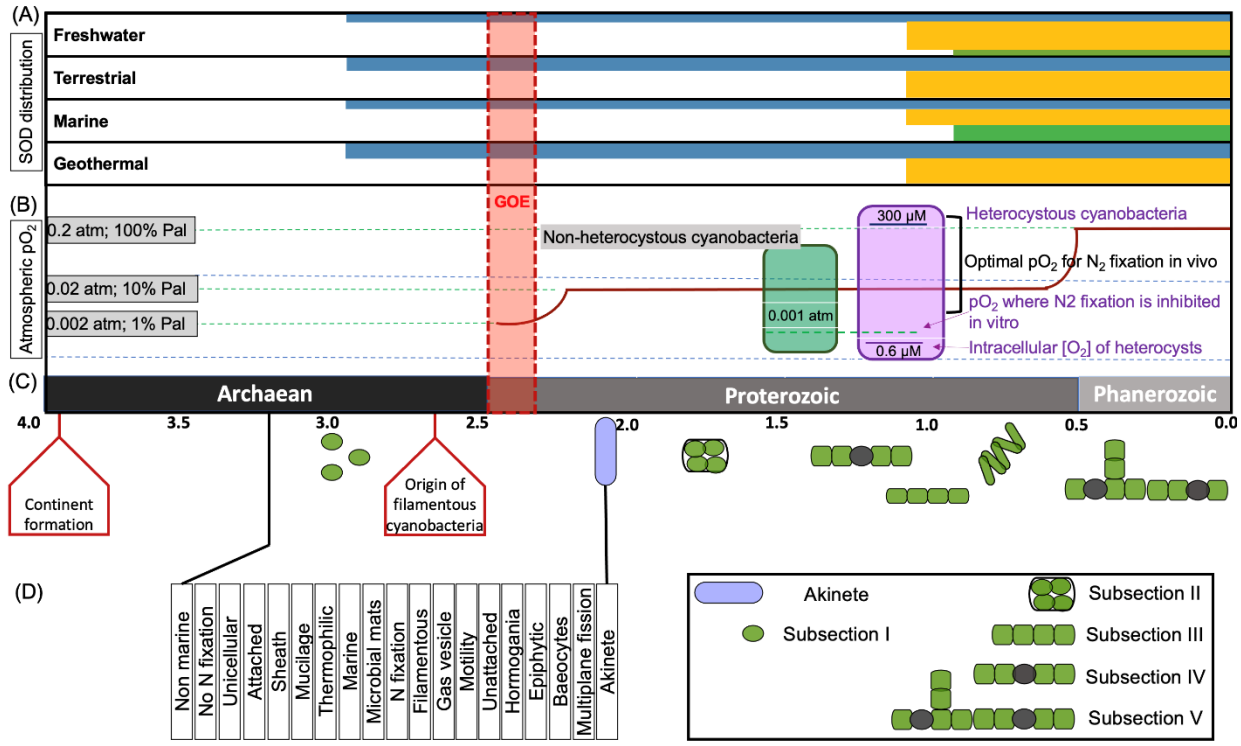

Figure 1. The evolutionary trajectory of cyanobacteria in the presence of oxygen. (A) The origin and distribution of SODs in different habitats. The timing of origin and habitat distribution of SOD genes

among taxa is created based on Boden et al (Boden et al., 2021). Starting points of horizontal bars represent time of origin and color SOD types: CuZnSOD (blue), NiSOD (green), and Fe- and Mn-utilizing SODs (yellow). (B) The oxygen level in atmosphere and nitrogen fixation in heterocysts in cyanobacteria in the presence of elevated oxygen levels. The level of oxygen is based on (Tomitani et al., 2006). (C) and (D) The temporal morphological diversification cyanobacteria is based on Schirrmeistera et al (Schirrmeister et al., 2013).

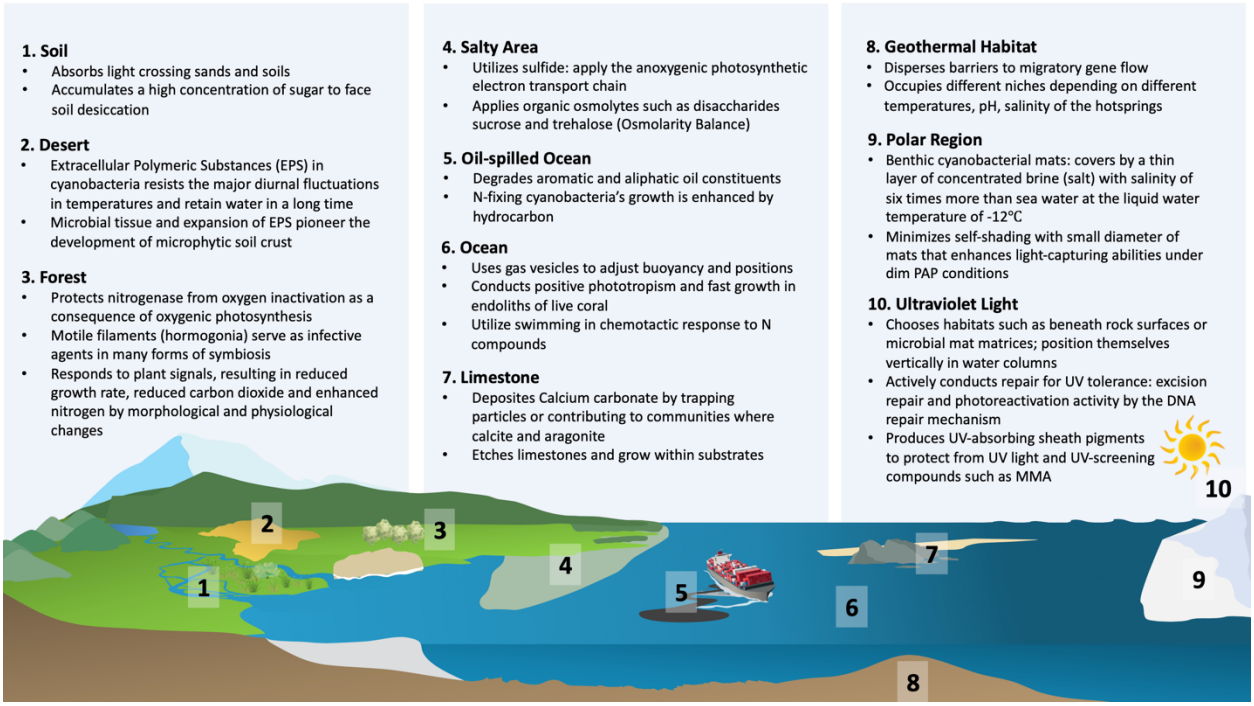

Figure 2. Cyanobacterial adaptation strategies in nine environments, namely soil, desert, forest, salty area, oil-spilled ocean, open ocean, limestone, geothermal water, and polar region. Cyanobacteria also cope with the damage caused by ultraviolet light.

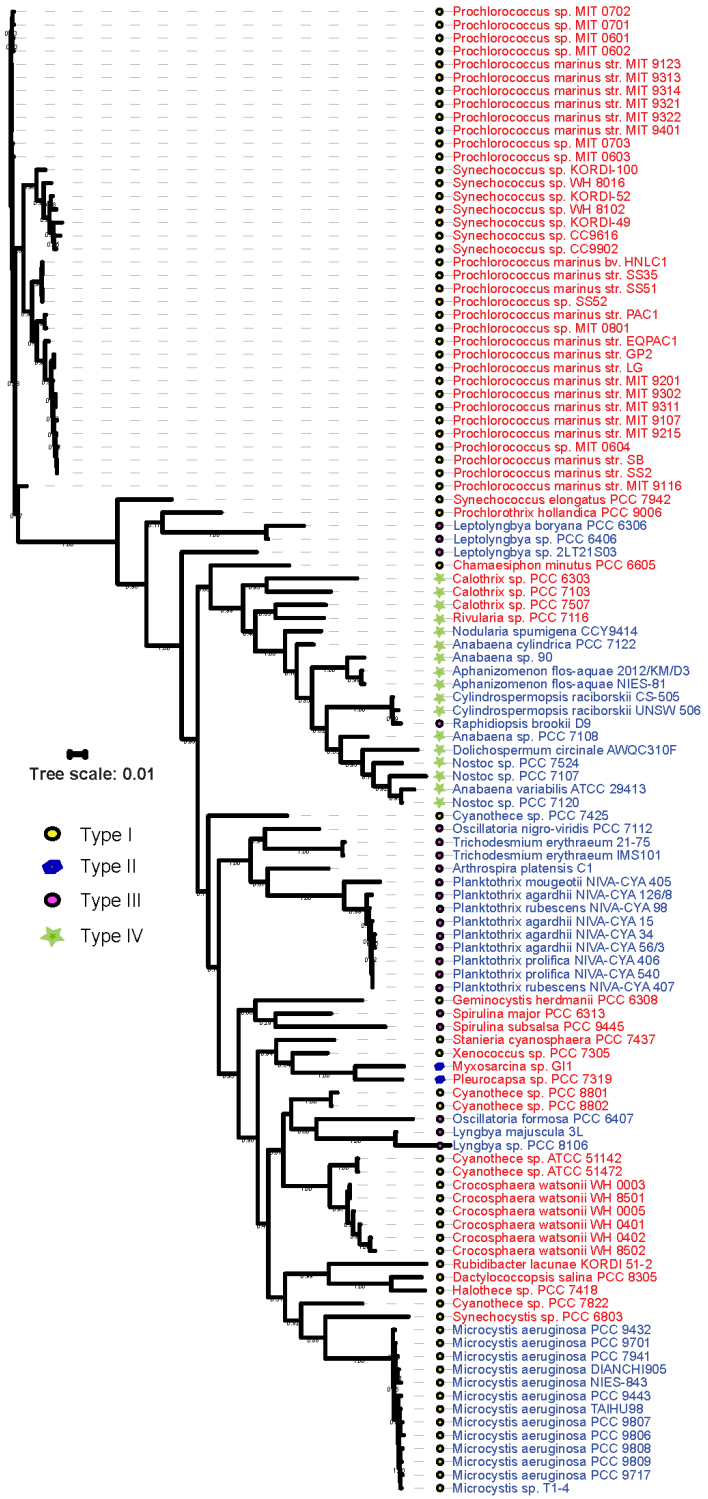

Figure 3. The phylogeny of cyanobacteria based on 16S ribosomal genes. Blue labels: bloom-forming cyanobacteria; and red labels: non-bloom-forming cyanobacteria. The shapes of different colors represent they types of morphology.

III. CyanoHABs: an inevitable consequence when low-nutrient requirement meets water eutrophication

Anthropogenic eutrophication, elevated loading of nutrients into waters from human activities on a global scale, is an 'unforeseeable' surprise to cyanobacteria which have been evolving, but not forming blooms, in physically harsh and nutrient-deficient waters. Apparently, eutrophic waters present themselves as a new but favorable condition, in which cyanobacteria dominate over co-living algae in phytoplankton communities and form CyanoHABs. To achieve a wholistic mechanism of CyanoHABs, three types of questions should be addressed: First, are there direct causality between eutrophication and CyanoHABs to prove eutrophication as the cause of CyanoHABs? Second, why is it cyanobacteria, not other co-living algae (e.g., green algae), that form blooms? Do other algal species have higher nutrient requirements than cyanobacteria, which cannot be met by current eutrophic levels? Last, what are the superior biological functions of blooming cyanobacteria that take advantage of eutrophic conditions compared to the non-blooming cyanobacteria?

3.1 Water eutrophication—a consequence of complex landscape alteration—as the cause for CyanoHABs

Despite natural eutrophication, few CyanoHABs in natural waters were reported before the era of eutrophication. Water eutrophication is not only a result of nutrient loading but also a process during which favorable factors such as hydrology are brought about by anthropogenic landscape changes. The driving role of eutrophication in CyanoHABs is well established (Heisler et al., 2008). Its causality is manifested in two ways: fast bloom formation in response to nutrient elevation and decline in blooming under de-eutrophication. Immediate bloom formation has been repeatedly observed in laboratory simulations (Lürling et al., 2018) and field studies (Molot et al., 2021; Wilkinson et al., 2018). Here we provide two examples to show that the transition from oligotrophic to eutrophic state is swift and CyanoHABs follow in a short period of time.

One example is the 'most studied lake in the world', Lake Mendota (Brock, 1985; Kitchell, 1992). Started as a clear oligotrophic lake prior to Euro-Americans colonization

in the 1830s in Madison, Wisconsin (Riera et al., 2001), Lake Mendota had CyanoHABs in 1882 and later 1890s (Lathrop & Carpenter, 1992) due to eutrophication from combined landscape changes of agricultural expansion in area and crops (corn in place of wheat and oats), wetlands drainage, lake-level rise and slow water flow, and soil erosion due to the damming lake outlet. Arguably the most convincing evidence for the cause of eutrophication for CyanoHABs is from Lake Erie, where CyanoHABs not only form following eutrophication but also decline with de-eutrophication, as summarized by Steffen et al (2014). Briefly, an increase in loading of phosphorus and other macronutrients to Lake Erie led to CyanoHABs (so-called autumnal maximum) dominated by filamentous cyanobacteria (*Anabaena* spp., *Aphanizomenon* spp., *Lyngbya* spp.), which quickly succeeded to *Microcystis* sp. With the phosphorus identified as the key nutrient and subsequent implementation of the Great Lakes Water Quality Agreement in 1972, the total phosphorus loading goal was reached in 1981 and continued to mid-1990s. Accordingly, a consistent decline in phytoplankton was observed. A return of *Microcystis aeruginosa* blooms was observed in 1995 (Conroy et al., 2005) due to external loading of soluble reactive phosphorus (Joosse & Baker, 2011) and CyanoHABs have persisted until now.

Besides freshwater ecosystems, eutrophication-led CyanoHABs were also found in brackish waters, e.g., Baltic Sea (Bianchi et al., 2000; Gustafsson et al., 2012) and coastal waters and seas (Anderson et al., 2008; Malone & Newton, 2020; Rahav & Bar-Zeev, 2017; Sabeur et al., 2016). The fact that CyanoHABs form on the entire spectrum of salinity indicates that salinity is not an effective deterring factor, which is a confirmation that cyanobacteria have long adapted to each of these habitats under oligotrophic conditions (Figure 2).

3.2 CyanoHABs: continued success of low nutrient-requiring cyanobacteria in unforeseeable eutrophic conditions

As observed in the studies of causality between eutrophication and CyanoHABs, it is cyanobacteria, not green algae, that form blooms in response to nutrient addition. Considering the adaptive radiation of cyanobacteria, one can reason that cyanobacteria

have lower requirements for nutrients than green algae. As nutrients increase in originally oligotrophic test waters, they meet the optimal needs of cyanobacteria first and further increases will meet the larger needs of green algae to drive them to form blooms.

To test this hypothesis, two analyses were performed. First, we compared the recipes of synthetic media for cyanobacteria and green algae and found those for green algae have much higher ionic concentrations than those for cyanobacteria (Figure 4). Second, we summarized the results which directly compared the growth of cyanobacteria and green algae in the field, laboratory, and cosmos by supplying external nutrients (Table 1). Five groups of studies support our hypothesis: low nutrients (TP, TN, DIN, urea-N, Ca, etc.) support dominance of cyanobacteria and high nutrients support dominance of green algae and even brown algae. Another support is that cyanobacteria tend to have a higher stress resistance in N limitation than other green algae. For example, *M. aeruginosa* can increase their photosynthetic performance which helps maintain their population size during seasonal N limitation (Jin, 2020), when the photochemical efficiency of PSII and the activity of PSII reaction centers (RCs) in *Porphyridium Cruentum* decreases under nitrogen starvation and thermal dissipation and ability to tolerate and resist high photon flux densities were also weakens (Zhao, 2017). In *Nannochloropsis Gaditana*, its RNA sequence proved that 20 putative regulators of lipid production were downregulated under nitrogen deprivation (Vecchi, 2020). Third, cyanobacteria can tolerate lower levels of nitrogen stress than green algae. *M. aeruginosa* has better tolerance against N limitation, with increased photosynthetic performance, which is decreased in green alga *Porphyridium Cruentum* under the same N limitation (add the Jin 2020 citation here). All these evidence clearly shows that cyanobacteria indeed have low requirements than green algae and thus bloom first when nutrient levels increase.

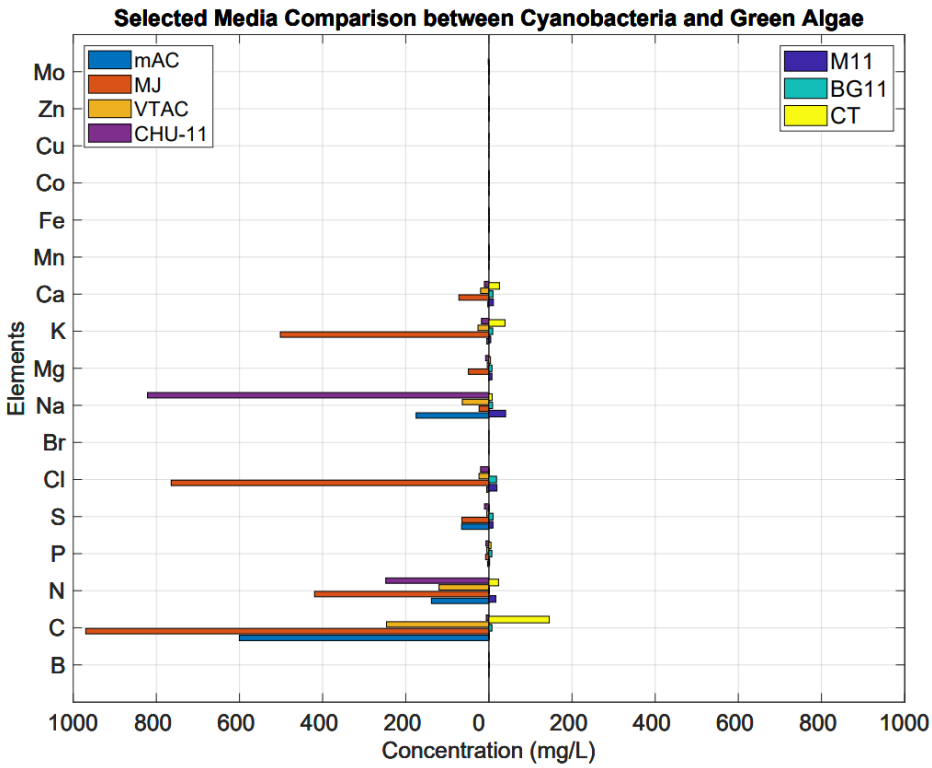

Figure 4. Lower nutrient requirements by cyanobacteria than green algae in aquatic environment. Among seven selected media, the recipes of mAC, MJ, VTAC, and CHU-11 (left panel) are used for culturing green algae and the recipes of M11, BG11 and CT (right panel) are used for culturing cyanobacteria.

Table 1. A list of case studies which show cyanobacteria have lower nutrient requirements than green algae. B: cyanobacteria; G: green algae. DIN: dissolved inorganic N; SRP: soluble reactive phosphorus; TN: total nitrogen; and TP: total phosphorus.

	Study Type	Study Site(s)	Indicator(s)	Concentration(s)	Dominance	Dominant Species	References
Group 1	Mesocosm	Wascana Lake, Canada	Urea-N	1-8 mg N L^{-1}	B	Colonial cyanobacteria	(Bogard *et al.*, 2020)
				>8 mg N L^{-1}	G	Chlorophytes	
	Laboratory	N/A	NH_4^+ and NO_3^-	32µM (NO_3) after 48h; 32µM NH_4^+ after 48h	B	*Synechococcus 6301*	(Hyenstrand *et al.*, 2000)
				32µM NO_3 after 25h; 32µM NH_4 after 18h	G	*Scenedesmus sp.*	
Group 2	*In situ*	210 Danish lakes	TP	0.25-0.8 mg P L^{-1}	B	Non-heterocystous cyanobacteria	(Jensen *et al.*, 1994)
				>1.0 mg P L^{-1}	G	Chlorophytes	
Group 3	*In situ*	Vela Lake, Central Portugal	DIN and SRP	0.66-2.42 mg L (DIN); 0-0.24 mg L (SRP)	B	*Aphanizomenon flos-aquae, Ch roococcus limneticus, M. aeruginosa* and *Pseudanabaena sp.*	(Figueiredo *et al.*, 2006)
				0.7-3.3 mg L (DIN); 0.19-0.93 mg L (SRP)	G	*Coelastrum reticulatum* var. *reticulatum, Kirchneriella lunaris, Monoraphidium contortum, Scenedesmus acuminatus* var. *acuminatus* and *Pediastrum boryanum* var. *boryanum*	
	In situ	Murchison Bay, Lake Victoria	TN and TP	1127 ug L (TN); 91 ug L(TP)	B	*Anabaena sp., M. wesenbergii, Gomphosphaeria aponina and Microcystis aeruginosa, Planktolyngbya circumcreta*	(Haande *et al.*, 2011)
	Laboratory and *in situ*	Lake Tai, China	N and P	3 mg/L (TN) and 0.2 mg/L (TP)	B	*M. aeruginosa*	(Zhu, Wan, & Zhao, 2010)
				10, 15, or 20 mg/L (TN), and 1 mg/L (TP)	G	*Scenedesmus quadricauda*	
Group 4	Mesocosm	Archipelago Sea	N:P Ratio	1N:1P 1.7 µg L^{-1}: 1.7 µg L^{-1}	B	*Aphanizomenon* sp., *Nodularia spumigena*, *Anabaena* spp., *Synechococcus* spp.	(Vuorio *et al.*, 2005)
				7N:1P 12 µg L^{-1}:1.7 µg L^{-1}	G	*Dictyosphaerium subsolitarium*, *Monoraphidium contortum*, and *Monoraphidium contortum, Oocystis* spp..	
	Mesocosm	Wascana Lake, Canada	N:P ratio	15:1 to >24:1	G	*Micractinium, Oocystis*	(Finlay *et al.*, 2010)
Group 5	*In situ* and laboratory	Taipei Feitsui Reservoir	Ca	7-9 mg L^{-1}	B	*Microcystis* spp. and *Aphanocapsa delicatissima*.	(Wu & Kow, 2010)
				9,11,13,15,18 mg L^{-1}	G	*Eutetramorus*, *Coelastrum*, *Coenocystis*, and *Dictyosphaerium*	

3.3 CyanoHABs: global dominance enabled by superior functional repertoire without prior local adaptation

Given their global presence and persistence for over a century (Francis, 1878; Lathrop & Carpenter, 1992), one may wonder whether there is prior adaptive evolution in cyanobacteria to form bloom in eutrophic waters, or CyanoHABs are simply ecological consequences of synergistic interaction between superior pre-equipped biological functions and elevated nutrients. We think the latter is more likely than the former. First, short-time whole-lake experiments in fertilization or nutrient reduction (Molot et al., 2021; Schindler et al., 2016; Wilkinson et al., 2018) have indisputably shown the causality of eutrophication for CyanoHABs on an ecological time scale, allowing no time for evolution. Second, anthropogenic eutrophication is unforeseeable to cyanobacteria, and therefore they cannot proactively prepare themselves through directed evolution.

Third, we confirmed there is no directed evolution toward CynaoHABs, by calculating the metric, dN/dS (ratio of non-synonymous to synonymous mutations), for all homologous genes in 20 blooming species of *M. aeruginosa* genomes from different countries. Indeed, almost all homologous genes display dN/dS < 0.8, with a median of 0.32 (Figure 5A). When grouped into the functional pathways curated previously (Cao et al., 2020), the homologous genes associated with CyanoHABs displayed the levels of purifying selection pressure as those in core metabolism and housekeeping processes (e.g., translation and transcription regulation) (Figure 5B). This strong purifying selection suggests these gene functions not only are important functions such that cells cannot afford them to be comprised by mutations. The levels of function are sufficient in the metabolic networks for blooming cyanobacteria to thrive in oligotrophic conditions and dominate in eutrophic conditions.

Last, the timescales observed between eutrophication and first blooms permit no evolution. We can calculate the positive mutation generated by mutations in one annual cycle. The spontaneous mutation rate (μ) in cyanobacteria is estimated at 10^{-7} per genome per generation (García-Villada et al., 2004; López-Rodas et al., 2007; Osburne et al., 2011; Segawa et al., 2018). For a lake of a million m^3, with cyanobacterial 20

cells/L growing to 10^8 cells/L after 25 doublings (t) at a growth rate 0.05-1.1 d^{-1} in an annual cycle bottlenecked in fall and winter, this gives an effective population size (N_e) of 10^8 cells, based on the equation 15 in (Wilson Alan et al., 2006). So, the number of beneficial mutations to be fixed can be approximated by, assuming a beneficial rate (s) at 0.01 and a rate of fixation ($2s$) at 0.05, $N_e \mu t 2s$ ($10^8 \times 10^{-7} \times 25 \times 2 \times 0.05 = 0.12$). Therefore, each year there is less than one beneficial mutant will occur, which makes prior adaptive evolution highly unlikely.

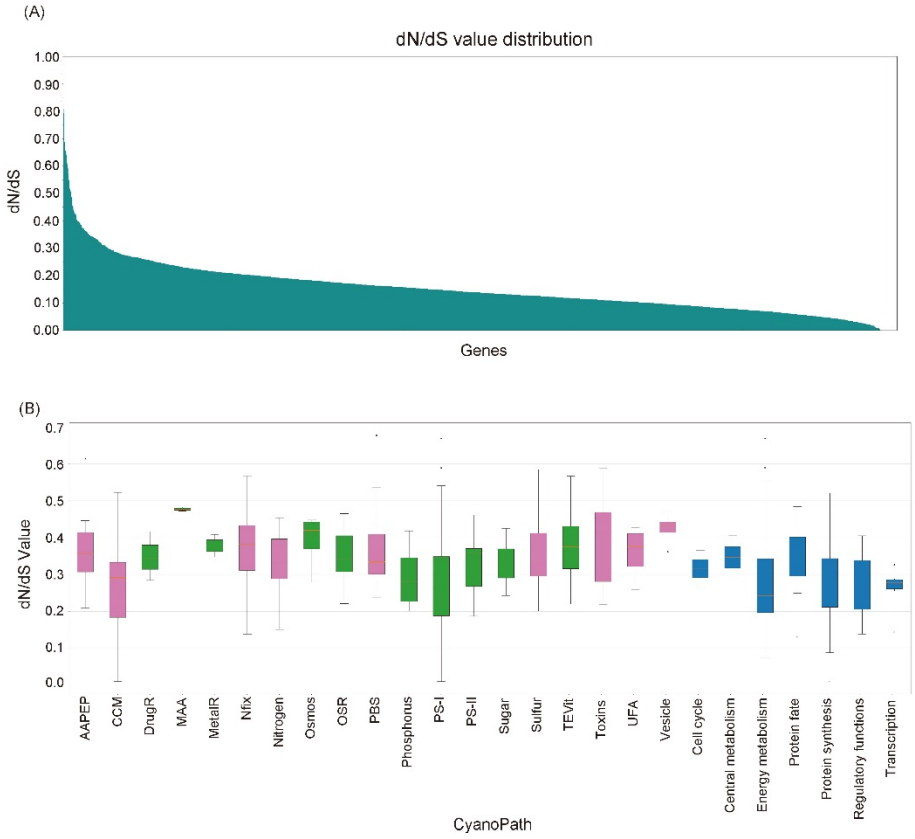

Figure 5. dN/dS for all homologous genes in 20 genomes of *M. aeruginosa*. (A) An overall distribution of dN/dS for all homologous genes; and (B) the dN/dS for genes in different groups of functions. dN/dS values were calculated by CODEML from the PAML 4.9j package with default settings (Yang, 2007). AAPEP: uptake of amino acids and peptides; CCM: CO_2-concentrating mechanism; DrugR: antibiotics resistance; MAA: UV radiation; MetalR: heavy metal resistance; Nfix: nitrogen fixation; Nitrogen: nitrogen utilization; Osmos: osmos

homeostasis; OSR: oxidative stress resistance; PBS: phycobilisome; Phosphorus: inorganic/organic utilization; PS-I/PS-II: photosystem I and photosystem II; Sugar: sugar assimilation; TEVit: assimilation of trace metals and vitamins; Toxins: cyanotoxins; UFA: unsaturated fatty acids; Vesicle: gas vesicles. The last seven columns colored dark blue represent essential gene sets (Rubin *et al.*, 2015).

IV. Biological functions associated with CyanoHABs

Elevated nutrients in eutrophic waters—macro or trace, persistent or pulse—are the drivers of CyanoHABs (Heisler et al., 2008). The relevant biological functions assimilating and converting them into biomass, as synthetic media converted into cells in lab cultures, have been recently identified by comparative genomics between blooming and non-blooming species (Cao et al., 2020). They are also made available as a database, CyanoPATH (Du et al., 2020) (Figure 6). These pathways have one-on-one correspondence with nutrients or stressors, which also show varying degrees of hierarchical structure. Below we detail the major pathways associated with CyanoHABs.

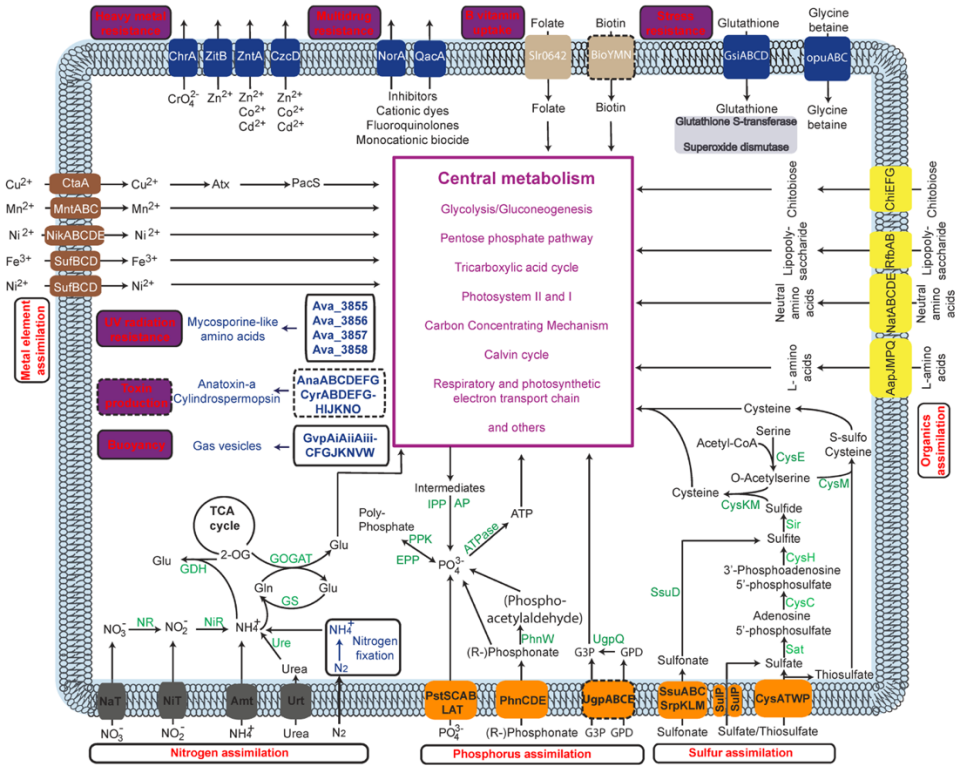

Figure 6. The core and query pathways in *Aphanizomenon flos-aquae* NIES-81. The pathways labeled with dashed borders are not complete (due to the absence of required components) in this strain but may be complete in others.

4.1 Gas vesicles

Gas vesicles are hollow protein structures made of gene products of *gvpA* and *gvpC* of the *gvp* gene cluster, consisting of 14 genes in several operons (Walsby, 1972, 1994). Gas vesicles are well-known for their roles in buoyancy regulation, allowing cyanobacteria to position themselves in the water column for optimal resource utilization (Carey et al., 2012; Walsby et al., 1997). This pathway is more enriched in blooming than non-blooming species and correlated with the blooming incidence of cyanobacteria recorded in the literature (Cao et al., 2020): nearly all blooming species have at least one copy of the *gvpA* and *gvpC* genes, and the genes *gvpFGJKNVWX*, while all but one non-blooming species do not have any of these genes (Figure 7A). This pathway operates independently of other pathways, without a direct association with central metabolism.

4.2 Functions for macronutrient utilization

Phosphorus and nitrogen are recognized as the top two macronutrients for CyanoHABs by empirical studies (Heisler et al., 2008; Molot et al., 2021; Schindler et al., 2016) and statistical analyses (Beaulieu et al., 2013). Indeed, the reconstructed pathways show that bloom-forming cyanobacteria invest heavily in these two functions, as they are limiting in the history of Earth (Kipp & Stüeken, 2017; Lyons et al., 2014; Reinhard et al., 2017). For phosphorus, both organic and inorganic phosphorus can be utilized. Inorganic phosphate can be internalized via ABC transporter *pstSCAB* or low facilitator affinity transporter *pitA*/*pitB* (Harris et al., 2001). Strikingly, nearly all cyanobacteria have one or two *pstSCAB* operons, and the globally dominant species *M. aeruginosa* has three. Organic phosphonate is imported via an ABC transporter PhnCDE and converted into phosphate by aminotransferase PhnW. The phosphate obtained has two destinations: incorporated into ATP as an energy molecule or converted to polyphosphate by polyphosphate kinase (PPK). Additionally, phosphate can be internally recycled, released from phosphate-containing compounds by inorganic pyrophosphatase and alkaline phosphatase. Interestingly, despite its verified driving role in CyanoHABs, the phosphate utilization pathway is not significantly more enriched (abundant or complex) in blooming than non-blooming species (Cao et al., 2020). This underscores two points. First, phosphorus has been a global limiting factor historically (Elser et al., 2007; Maberly et al., 2020; Schindler, 1977) such that all cyanobacteria have at least one and often two ABC transporters; phosphate appears to be only a necessary but not sufficient driver for CyanoHABs, this is probably why total nitrogen rather than total phosphorus is a better CyanoHAB predictor (Beaulieu et al., 2013). Second, this suggests the biological machinery for CyanHABs is complicated and more likely to operate in a systems manner (Kirschner, 2005).

Total nitrogen and water temperature are recognized as the best predictors of cyanobacterial biomass (Beaulieu et al., 2013). Indeed, as the nitrogen utilization pathway reconstructed with transporters and enzymes shows, cyanobacteria can use four forms of nitrogen: nitrogen gas, inorganic nitrate/nitrite, ammonium, and organic urea (Figure 7C). Key transporters for nitrate/nitrite (*nrtABCD*), ammonium (amt), urea

(*urtABCDE*) and key nitrate/nitrite reductive enzymes *narB*, *nirB*, and urease (*ureABCDE*) are more enriched in blooming than non-blooming cyanobacteria (Figure 7D). Interestingly, the genes involved in incorporating nitrogen into amino acids (e.g., gdhA or gltB/gltD) are not significantly more enriched in blooming species; this suggests that superior nitrogen utilizing ability mainly lies in the uptake from the extracellular environment, other than intracellular incorporation. Other metabolic pathways for macronutrients include those for sulfur (Figure 6), which is also enriched in blooming than non-blooming species (Cao et al., 2020).

4.3 Utilization of organic compounds

Organic compounds were not initially considered, even though cyanobacteria were known to utilize organic nutrients under light (Baalen et al., 1971; Rippka, 1972; Stanier, 1973). As CyanoHAB research progresses, organics gained some attention but were limited to organic nitrogen and phosphorus in a few species (Berman & Chava, 1999; Chaffin & Bridgeman, 2014). The transporters internalizing organic compounds, such as amino acids and sugars, have been identified systematically (Figure 6) and ABC amino acid transporters are found more enriched in blooming than non-blooming species (Cao et al., 2020). Two transporters, AaPJMPQ for L-amino acids and NatABCDE for neutral amino acids, are found to be encoded in nearly all blooming species. Strikingly, the top blooming species, *M. aeruginosa* have additional transporters, HisJMPQ (for histidine), ArtIJMQP (arginine), DppABCDF (d,d-dipeptide), GlnHPQ (Glutamine), GltIKJL (Glutamate/aspartate), LivFGHKM (leucine, isoleucine, and valine), GluABCD (glutamate), and AotJMPQ (arginine/ornithine). Experimentally, this has been verified with lab studies: glutamic acid, aspartic acid, leucine, alanine and arginine can be assimilated by *M. aeruginosa* at varying rates for the biosynthesis of microcystin (Dai et al., 2009). Besides amino acids, some types of sugars can also be internalized by blooming cyanobacteria, such as the ABC transporter RfbAB for lipopolysaccharides and ChiEFG for chitobiose.

4.4 CO_2-Concentrating Mechanism (CCM)

CCM is the step concentrating CO_2 in carboxysomes to high levels (~ 40μM) for

effective incorporation into ribulose bisphosphate in the Calvin cycle. CCM is more enriched in blooming than non-blooming species (Cao et al., 2020) *et al.*, 2020). The CCM genes encode four types of functions: *ccmKLMNO* (structural proteins forming the carboxysome), *icfA* (carbonic anhydrase), *bicA* and *sbtA* (bicarbonate facilitator), *cdnF*$_3$*D*$_3$*chY*, *cdnF*$_4$*D*$_4$*chX*, and *cmpABCD* (ABC transporter for HCO_3^-), and *rbcLS* (ribulose bisphosphate carboxylase/oxygenase). All but one (*bicA*) have more gene copies in blooming than non-blooming species (Figure 7E). This enrichment is understandable because heavy drawdown of CO_2 can easily lead to carbon limitation during a bloom, particularly when a diffusion limitation effect is associated with high population density of cyanobacteria in the water. Meanwhile, elevated CO_2 level has been found to intensify CyanoHABs (Verspagen et al., 2014) and CCM transporters and regulators respond quickly (Ma et al., 2019; Sandrini et al., 2016).

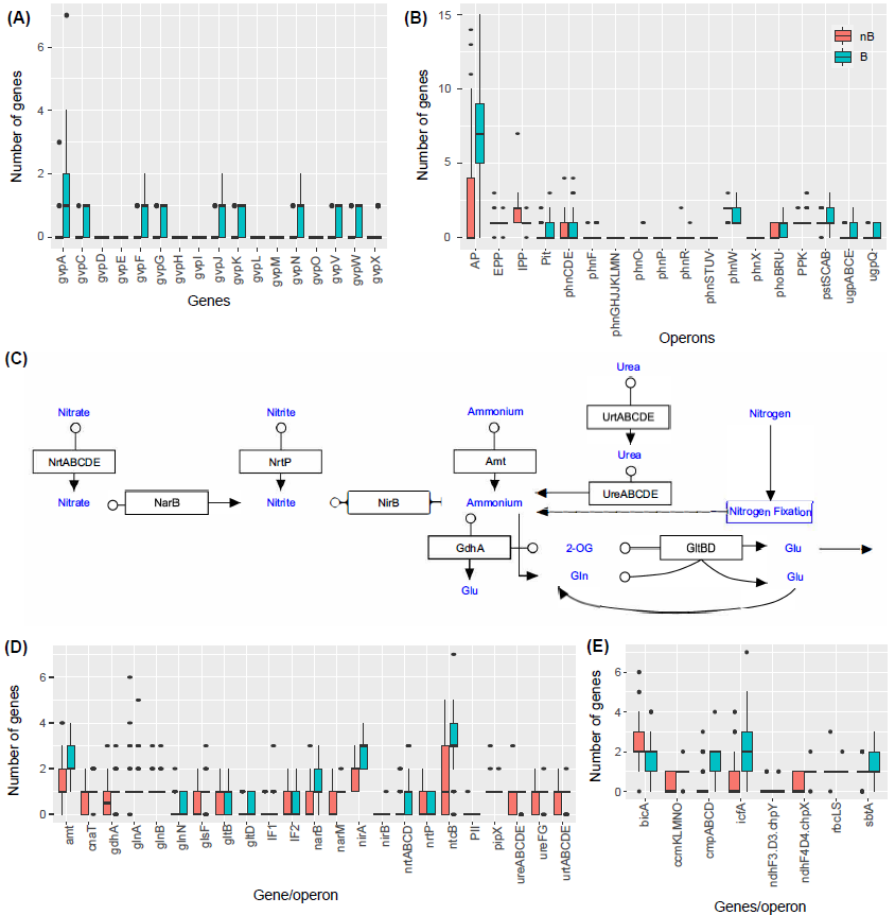

Figure 7. Some important CyanoPATHs associated with CyanoHABs in blooming vs. non-blooming cyanobacteria. (A): gas vesicle genes; (B): genes for phosphorus

utilization; (C): nitrogen utilization pathway; (D): nitrogen utilization genes; and (E): CCM genes.

4.5 Disputed roles of cyanotoxins

Cyanotoxins are conventionally viewed as deterrents for anti-predation/anti-grazing of cyanobacteria (Zanchett & Oliveira-Filho, 2013). Recent research suggests their functions are not limited to deterrents: their toxicity may as well be a coincidence, and its real roles are rather different. First, a new study suggests a direct role in antioxidant response leading to reduced ROSs outside and inside of *M. aeruginosa* cells (Malanga et al., 2019). Second, a protein-binding role has been identified for microcystin-LR (MC-LR), which binds several enzymes of the Calvin cycle, phycobiliproteins and two NADPH-dependent reductases. The binding, strongly enhanced under high irradiance and oxidative stress, is a protection and increases cell growth (Zilliges et al., 2011). Third, they act as a signaling molecule in maintaining algal colonies and the dominance of *Microcystis* spp. (Gan et al., 2012). Last, some cyanotoxins, e.g., microcystins, has a deep evolutionary origin, which predates that of predators/grazers and eliminates the possibility of directed evolution for cyanotoxins toward intoxicating animals (Rantala et al., 2004).

4.6 Other functions

There are two additional types of functions associated with CyanoHABs: assimilation of trace elements and stress resistance. Despite their importance, few specific functions of this pathway are enriched in blooming than non-blooming species.

Trace elements and vitamins. This pathway mainly consists of various classes of transporters for their uptake. The trace elements for which utilization pathways are reconstructed include magnesium, ferric/ferrous ions, copper, nickel, manganese, and cobalt (Ahern et al., 2008; Kosakowska et al., 2007). Among these elements, iron has the most types of transporters for siderophore-based high-affinity iron-uptake *fecBCDE*, *feoABC* (for ferrous iron), *fhuBCD*, *futABC*, *sfuABC*, and *sufBCD*. For example, iron has the most elaborate set of transporters for both ferric and ferrous iron. Despite these

driving roles reported for some of these trace elements (Berman-Frank et al., 2001; Facey et al., 2019; Gu et al., 2020; Molot et al., 2014), there are no significant changes in gene copy number between blooming and non-blooming species (Cao et al., 2020). For vitamins, the transporters are for riboflavin, nicotinamide riboside, biotin (vitamin B-7), and cobalamin (vitamin B12). Most bloom-forming species are vitamin B1 and B12 auxotrophs and their transporters have been identified BioYMN (biotin), YbaA/PnuX/ImpX (for riboflavin), PnuC (for nicotinamide riboside), and BtuFCD (cobalamin).

Stress resistance. Some stressors in eutrophic waters include heavy metals, antibiotics, and dyes (Devarajan et al., 2015; Guo et al., 2020; Li et al., 2020). Despite the expected inhibitive effects of heavy metals on cyanobacterial growth (Richardson et al., 2018), some antibiotics are found to be promoting in two recent studies (Li et al., 2021; Xu et al., 2021). Here, the biological functions of cyanobacteria in countering these three types of stress are summarized in CyanoPATH: heavy metal and antibiotics, and mycosporine-like amino acids (MAAs) to shield ultraviolet light. For heavy metals and antibiotics resistance, the main metabolic functions identified so far are either ATP-powered efflux ABC transporters or simple facilitator outward pumps relying on cross-membrane gradients as drive. The identified efflux transporters include ChrA (chromate efflux), CnrABC (cobalt/nickel), CopABC (copper efflux ATPase), CueO (Multicopper oxidase), CusABCF (Cu^+/Ag^+ efflux), CzcABC and ZitB (Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} efflux), CzcD (Co^{2+} and Zn^{2+} efflux), NccABC and NreB (Ni^{2+}), and ZntA (Zn^{2+} and Cd^{2+} P-type ATPase exporter) (Cao et al., 2020). The antibiotic resistance genes are mostly responsible for exporting toxic compounds and antibiotics out of cells. These transporters are EmrBA-TolC (uncouplers and antibiotics (nalidixic acid)), LmrP (cationic dyes, tetracycline, and daunomycin/macrolides), MdfA (cationic dyes, chloramphenicol/fluoroquinolone), NorA (fluoroquinolones/cationic dye/inhibitors), QacA/B (monocationic biocide and dyes), EmrE (lipophilic cations), AcrB/D-A-TolC, MexBA-OprM, MexDC-OprJ, and MexFE-OprN (multidrugs, e.g, tetracycline, chloramphenicol, fluoroquinolones, novobiocin, erythromycin), MexYX-OprM (aminoglycosides).

Another type of stress is universal, UV light, since early Earth (Garcia-Pichel et al., 2019). Cyanobacteria have been equipped with the capabilities to produce sunscreen compounds MAAs (Jain et al., 2017; Sinha et al., 2001). The operons encoding the biosynthetic genes have been identified in *Anabaena variabilis* and other species (Balskus & Walsh, 2010; Miyamoto et al., 2014). A gene cluster of four synthetases catalyzes the formation of shinorine from sedoheptulose 7-phosphate in the pentose phosphate pathway. The synthetases are nonribosomal peptide synthetase (ava-3855), ATP-grasp family enzyme (ava-3856), O-methyltransferase (ava_3857), and a dehydroquinate synthase (ava_3858). Interestingly, only some of the blooming species have one cluster whereas non-blooming species do not contain any (Cao et al., 2020).

V. Synthesis on CyanoHAB mechanism and outlook

Through the lens of adaptive radiation, four factors of the ecology of CyanoHABs stand out: (1) elaborate diverse functional repertoire and low nutrient requirement in cyanobacteria molded by early adaptive evolution, (2) cyanobacteria having lower nutrient requirements than green algae indeed, (3) there is no directed evolution in biological functions toward water eutrophication in cyanobacteria, (4) the CyanoHAB-associated functional repertoire are more abundant and complete in blooming than non-blooming species. Based on all the discussion above and with the four factors, we propose a preliminary comprehensive mechanism of CyanoHABs as a synergistic quad: These factors lead us to postulate a preliminary mechanism of CyanoHABs as a synergistic quad: superior functional repertoire, established with long adaptive radiation under nutrient-deficient conditions and not evolved toward eutrophic conditions, enables cyanobacteria to efficiently utilize elevated nutrients under current eutrophic regime for excess growth and CyanoHABs thereof, due to their lower nutrient requirements than co-living algae. One prominent feature of the quad mechanism is the relative nutrient requirement of different algae. This boils down to the chemical composition of the cellular machinery and how the composition can be achieved under various growth conditions. In this sense, this quad mechanism has profound implications for essentially all fields of life sciences.

Considering the multiple factors in the mechanism, further empirical tests and theoretic explorations are needed to make a better synthesis of the mechanism. Two types of research should be given priority: (1) comparative studies between cyanobacteria and co-living algae in nutrient requirement and growth in lab and field settings; and (2) the integrated systems biology assessment of the relative roles in promoting growth in cyanobacteria. The latter can be best achieved by genome-scale metabolic networks.

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Figure Legend

Figure 1

Figure 1. The evolutionary trajectory of cyanobacteria in the presence of oxygen. (A) The origin and distribution of SODs in different habitats. The timing of origin and habitat distribution of SOD genes among taxa is created based on Boden et al (Boden et al., 2021). Starting points of horizontal bars represent time of origin and color SOD types: CuZnSOD (blue), NiSOD (green), and Fe- and Mn-utilizing SODs (yellow). (B) The oxygen level in atmosphere and nitrogen fixation in heterocysts in cyanobacteria in the presence of elevated oxygen levels. The level of oxygen is based on (Tomitani et al., 2006). (C) and (D) The temporal morphological diversification cyanobacteria is based on Schirrmeistera et al (Schirrmeister et al., 2013).

Figure 2

Figure 2. Cyanobacterial adaptation strategies in nine environments, namely soil, desert, forest, salty area, oil-spilled ocean, open ocean, limestone, geothermal water, and polar region. Cyanobacteria also cope with the damage caused by ultraviolet light.

Figure 3

Figure 3. The phylogeny of cyanobacteria based on 16S ribosomal genes. Blue labels: bloom-forming cyanobacteria; and red labels: non-bloom-forming cyanobacteria. The shapes of different colors represent they types of morphology.

Figure 4

Figure 4. Figure 4. Lower nutrient requirements by cyanobacteria than green algae in aquatic environment. Among seven selected media, the recipes of mAC, MJ, VTAC, and CHU-11 (left panel) are used for culturing green algae and the recipes of M11, BG11 and CT (right panel) are used for culturing cyanobacteria

Figure 5

Figure 5. dN/dS for all homologous genes in 20 genomes of M. aeruginosa. (A) An overall distribution of dN/dS for all homologous genes; and (B) the dN/dS for genes in different groups of functions. dN/dS values were calculated by CODEML from the PAML 4.9j package with default settings (Yang, 2007). AAPEP: uptake of amino acids and peptides; CCM: CO_2-concentrating mechanism; DrugR: antibiotics resistance; MAA: UV radiation; MetalR: heavy metal resistance; Nfix: nitrogen fixation; Nitrogen: nitrogen utilization; Osmos: osmos homeostasis; OSR: oxidative stress resistance; PBS: phycobilisome; Phosphorus: inorganic/organic utilization; PS-I/PS-II: photosystem I and photosystem II; Sugar: sugar assimilation; TEVit: assimilation of trace metals and vitamins; Toxins: cyanotoxins; UFA: unsaturated fatty acids; Vesicle: gas vesicles. The last seven columns colored dark blue represent essential gene sets (Rubin et al., 2015).

Figure 6

Figure 6. The core and query pathways in *Aphanizomenon flos-aquae* NIES-81. The pathways labeled with dashed borders are not complete (due to the absence of required components) in this strain but may be complete in others.

Figure 7

Figure 7. Some important CyanoPATHs associated with CyanoHABs in blooming vs. non-blooming cyanobacteria. (A): gas vesicle genes; (B): genes for phosphorus utilization; (C): nitrogen utilization pathway; (D): nitrogen utilization genes; and (E): CCM genes.