




Presence of toxin-antitoxin systems in picocyanobacteria and their ecological implications

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Abstract

Picocyanobacteria (mainly *Synechococcus* and *Prochlorococcus*) contribute significantly to ocean's primary production. Toxin-Antitoxin (TA) systems present in bacteria and archaea are known to regulate cell growth in response to environmental stresses. However, little is known about the presence of TA systems in picocyanobacteria. This study investigated complete genomes of *Synechococcus* and *Prochlorococcus* to understand the prevalence of TA systems in picocyanobacteria. Using the TAfinder software, Type II TA systems were predicted in 27 of 33 (81%) *Synechococcus* strains, but none of 38 *Prochlorococcus* strains contain TA genes. *Synechococcus* strains with larger genomes tend to contain more putative type II TA systems. The number of TA pairs varies from 0 to 42 in *Synechococcus* strains isolated from various environments. A linear correlation between the genome size and the number of putative TA systems in both coastal and freshwater *Synechococcus* was established. In general, open ocean *Synechococcus* contain no or few TA systems, while coastal and freshwater *Synechococcus* contain more TA systems. The type II TA systems inhibit microbial translation *via* ribonucleases and allow cells to enter the “dormant” stage in adverse environments. Inheritance of TA genes in freshwater and coastal *Synechococcus* could confer a recoverable persister mechanism important to survive in variable environments.

Introduction

Picocyanobacteria are small unicellular cyanobacteria, and they contribute greatly to carbon fixation in the aquatic ecosystem. Marine picocyanobacteria contain two major genera, *Prochlorococcus* and *Synechococcus*, which together can contribute about 25% of net primary production in the ocean [1, 2]. While *Prochlorococcus* is more restricted to warm oligotrophic water, *Synechococcus* is widely distributed in various aquatic environments ranging from open oceans to freshwater [3]. The average cell size of *Synechococcus* (0.9 μm) is larger than that of *Prochlorococcus*

(0.6 μm) [4]. In addition, the average genome size of *Prochlorococcus* (1.8 Mb) is also smaller than that of *Synechococcus* (2.9 Mb) [5]. Genome streamlining provides less ecological flexibility to marine *Prochlorococcus*, on the other hand, the relatively large genome size of *Synechococcus* provides more genomic plasticity which enables them to adapt to more variable habitats [6–9].

Diverse *Synechococcus* strains have been isolated from freshwater, estuarine, coastal, and oceanic water [2, 10–12], suggesting that *Synechococcus* can adapt to distinct aquatic environments. In the estuarine environment, picocyanobacteria (mostly *Synechococcus*) can make up 20–40% of phytoplankton chlorophyll a and up to 60% of primary production in summer [13]. Freshwater *Synechococcus* can also play an important role in carbon fixation and nutrient cycling in ponds, lakes, and rivers [14–16]. Phylogenetic analyses of freshwater and marine *Synechococcus* show that *Synechococcus* is polyphyletic [3, 17–19]. Molecular systematics has challenged the traditional taxonomy of *Synechococcus* in the past 20 years [17, 20, 21]. Genetic diversity of *Synechococcus* has been studied in various aquatic environments [22–25]. In marine waters, three subclusters of *Synechococcus* have been defined [10, 26]

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and subdivided into 28 clades based on the ITS sequences [27]. In the freshwater system, 6–8 clusters of *Synechococcus* have been identified based on the 16S rRNA gene or other genetic markers [27–31]. Freshwater *Synechococcus* are deeply branched and are less congruent compared to marine *Synechococcus* [12]. Because of their ubiquity in aquatic systems, *Synechococcus* contain highly diverse phylotypes and ecotypes.

Comparative genomics of cyanobacteria has greatly advanced our understanding of molecular evolution, metabolic potential and ecological adaptation of different cyanobacterial types [10, 26]. Unicellular cyanobacteria with smaller genomes (<3.3 Mb) appear to have relatively more genes involved in amino acid metabolism, but fewer genes for environmental sensing (signal transduction) and cell motility compared to cyanobacteria with larger genomes (>3.3 Mb) [8]. In the marine environment, ecological adaptation of *Synechococcus* to different niches is evident at the genomic level. The first complete marine *Synechococcus* genome (strain WH8102) was sequenced in 2003 [32]. By comparing the genomes of a coastal *Synechococcus* strain (CC9311) and the oceanic *Synechococcus* strain (WH8102), Palenik et al. showed that the coastal strain has a greater capacity to sense and respond to changes in their environment compared to its oceanic counterpart [33]. Open ocean *Synechococcus* ('specialists') tend to have smaller genomes and less genome islands than coastal *Synechococcus* ('opportunists' or 'generalists') [10, 26]. Coastal *Synechococcus* strains have an increased tolerance for copper and oxidative stress through distinct transcriptional responses and genomic features [34, 35]. Coastal *Synechococcus* genomes contain a large portion of accessory and unique genes which provide them considerable flexibility to adapt to diverse habitats [26]. Novel genes on picocyanobacterial genome islands can provide selective advantage for niche adaptation [10]. Recently, genome sequencing of a Chesapeake Bay *Synechococcus* strain CB0101 unveiled its increased capacity in environmental sensing, transportation, regulation, and stress response [36]. The presence of toxin-antitoxin (TA) genes and their functional assignment in *Synechococcus* CB0101 suggests that TA systems can be important to the high environmental endurance of estuarine *Synechococcus* [37].

TA systems are known to be involved in stress responses in microbes, but little is known about TA systems in picocyanobacteria. TA systems are genetic modules comprised of a toxin, which often arrests translation and subsequent growth, and a cognate antitoxin which negates the interruption caused by the toxin [38, 39]. TA system activation often results in persister cell formation which can be advantageous for bacterial survival in highly variable environments. While TA systems have been broadly described as ubiquitous in nearly all bacterial species, TA

systems in cyanobacteria have only recently been described. TA systems have been predicted in freshwater cyanobacteria including *Microcystis aeruginosa* [40], *Synechocystis* PCC6803 [41], and *Synechococcus* PCC7942 [42]. Only a few TA genes, *i.e.* VapB, VapC, and PemK were reported in the freshwater cyanobacterial strains, no systematic survey on TA genes was performed at the genomic level on those strains. The first chromosomal TA system in marine *Synechococcus* was described in the estuarine *Synechococcus* strain CB0101 [37]. CB0101, isolated from the Chesapeake Bay, belongs to *Synechococcus* subcluster 5.2 [43]. Transcriptomic analysis of CB0101 reveals a tight coupling between the upregulation of particular toxins, such as *relE*, with environmental stressors like zinc heavy metal toxicity and high light intensity [37]. Marsan et al. showed that TA systems can be important to the environmental stress response in *Synechococcus* [37]. However, little is known about the occurrence, diversity, evolution, and ecological functions of type II TA systems in *Synechococcus* and other picocyanobacteria.

The goal of this study is to investigate the presence of TA genes in picocyanobacteria using the TAfinder software [44]. Our search comprised of 71 complete picocyanobacterial genomes, including 33 *Synechococcus* and 38 *Prochlorococcus* genomes. An interesting linear relationship between the number of TA pairs and genome size was found in *Synechococcus*.

Methods

Complete *Synechococcus* ($n = 33$) and *Prochlorococcus* ($n = 38$) genomes were downloaded in September, 2019 from both the National Center for Biotechnology Information (NCBI) RefSeq database [45, 46] and the Joint Genome Institute genome portal [47]. To ensure quality, we omitted incomplete genomes from this study. The *Synechococcus* and *Prochlorococcus* genomes included in this study cover the majority of major known phylogenetic clades and sub-clusters (Table S1).

Toxin-antitoxin systems were predicted using the TAfinder software which utilizes the Toxin-Antitoxin Database (TADB) [44, 48]. Genomes that were not included in the TAfinder's available genome list were downloaded locally and manually uploaded to TAfinder. TAfinder was used to predict type II TA pairs in *Synechococcus* (freshwater, estuarine, coastal, and open ocean strains) and marine *Prochlorococcus* genomes using default settings (BLAST *e*-value = 0.01, HMMer = 1, Maximum length = 300 aa, Distance = -20_150). *Synechococcus* strains were classified into habitats based on literature searches for original isolation information. Because estuarine strains, *Synechococcus* CB0101 and PCC7002,

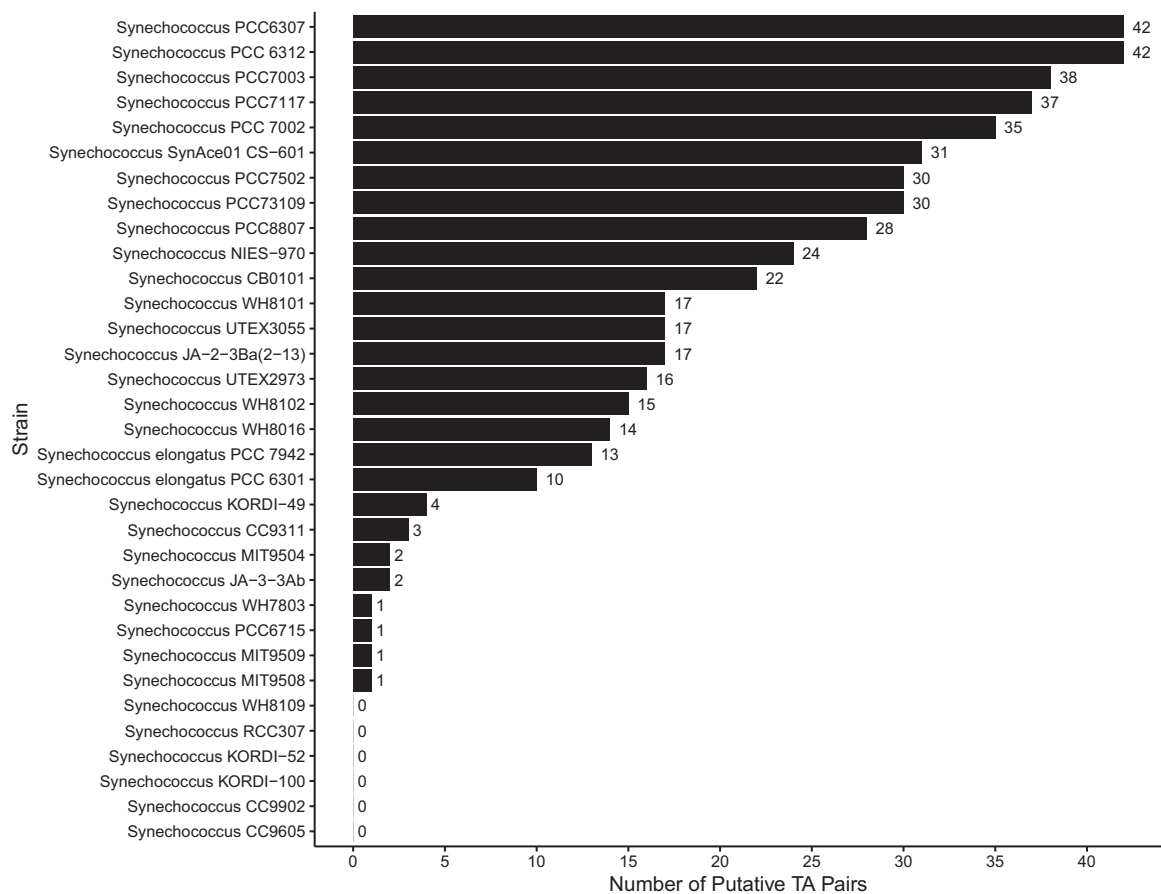


Fig. 1 Occurrence frequency of putative TA systems in 33 strains of *Synechococcus* isolated from various aquatic environments. Numbers shown at the end of bars are the number of putative TA systems.

are underrepresented, they were categorized into the coastal habitat category (Table S1) for the purpose of linear regression data analysis.

To estimate relative diversity of the putative TA families, predicted amino acid sequences were searched against the NCBI conserved domain database (version CDD v3.18 - 55570 PSSMs) [49]. Short names for conserved domains were manually reviewed and determined to be of a consensus of a major TA family. If the gene did not fall into one of the traditional TA families, it was categorized as “Other” for the consensus. If the predicted amino acid sequence did not have a significant match to the conserved domain database, it was categorized as “Unknown”.

Linear regression and linear models were completed using Rstudio software [50] and figures were made using ggplot2 [51]. Genome Island regions were predicted using IslandViewer 4 software [52].

Results

TAfinder predicted at least one TA pair in 27 of 33 *Synechococcus* genomes (81%). The number of TA systems in

Synechococcus varies from 0 to 42 (A toxin-antitoxin system is normally comprised of one toxin gene and one cognate antitoxin gene). Only five strains of *Synechococcus* did not contain putative TA systems. A total of 986 putative toxin and antitoxin genes were predicted, constituting 493 TA systems, in 27 complete *Synechococcus* genomes. The occurrence frequency of TA systems in *Synechococcus* is shown in Fig. 1. The 27 TA-containing *Synechococcus* strains were isolated from various aquatic environments including freshwater, Antarctic (cold adapted), hot spring (thermophile), estuarine, coastal, and oceanic waters. Representative *Synechococcus* strains belong to diverse phylogenetic lineages (Table S1).

TAfinder did not predict any TA systems in any of the *Prochlorococcus* genomes ($n = 38$). These *Prochlorococcus* genomes were representative of many clades from both high light and low light adapted strains. These queried *Prochlorococcus* genomes ranged in size from 1.6 to 2.7 Mb.

Freshwater and coastal *Synechococcus* contained many putative TA systems. For example, freshwater *Synechococcus* strains PCC6312 and PCC6307 both contained 42 putative TA systems. These 84 genes accounted for ~1.2%

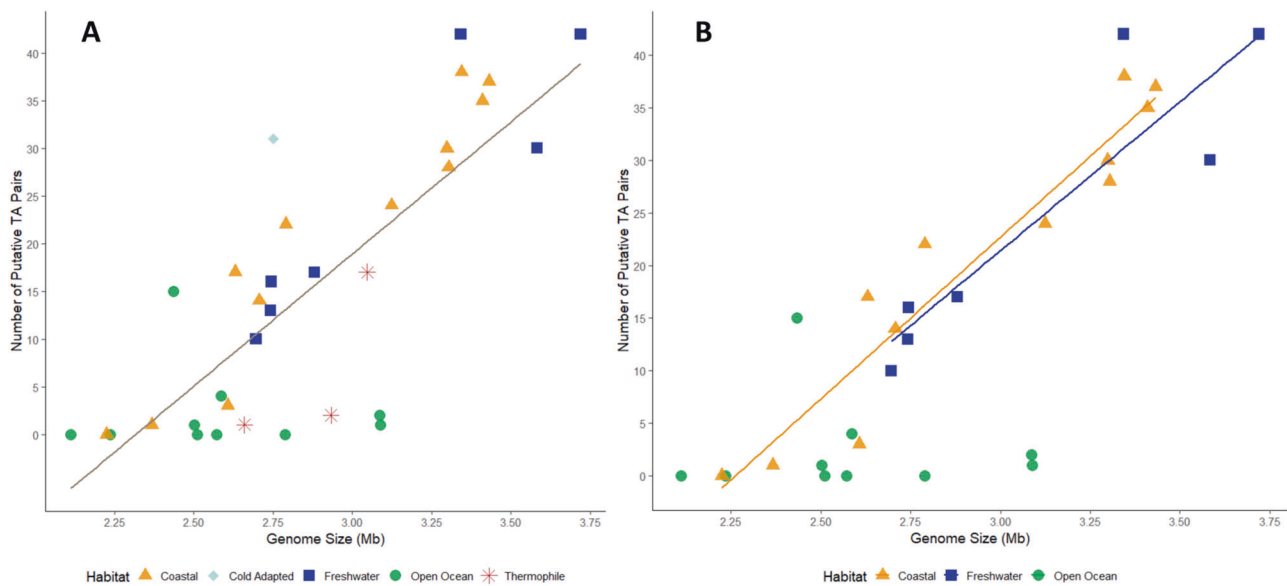


Fig. 2 Relationship between genome size and the number of putative TA pairs in *Synechococcus*. **a** Linear correlation between genome size and putative TA systems for all complete genomes of *Synechococcus* strains isolated from all habitats ($r^2 = 0.6235$, $p < 0.0001$); **b** Linear correlation between genome size (Mb) and the

number of putative TA pairs in coastal (gold) and freshwater (royal blue) *Synechococcus*, ($r^2 = 0.9152$, $p < 0.00001$ and $r^2 = 0.8296$, $p < 0.005$ respectively). No such correlation was found in open ocean *Synechococcus* (green).

of their total coding sequences (Table S1). Coastal strains PCC7003, and PCC7117 contained 38, and 37 TA pairs, respectively, accounting for 1.24% and 1.17% of their coding sequences.

In general, *Synechococcus* living in coastal, estuarine, and freshwater environments tend to have larger genomes compared to their counterparts living in the open ocean. It appears that *Synechococcus* with larger genomes contain more TA genes than *Synechococcus* with smaller genomes. Interestingly, a good linear correlation between the genome size and the number of putative TA pairs ($r^2 = 0.6235$, $p < 0.0001$) (Fig. 2a) was found in *Synechococcus*, further confirming the above observation that larger *Synechococcus* genomes contain more TA genes. This apparent relationship between genome size and putative TA pairs becomes more clear in cases when endemic ecological conditions are considered; specifically, for coastal and freshwater *Synechococcus*. When analyzed separately, better linear regressions ($r^2 = 0.9152$, $p < 0.00001$ and $r^2 = 0.8296$, $p < 0.005$) between genome size and putative TA pairs were found in coastal and freshwater *Synechococcus* (Fig. 2b). Conversely, the general correlative trend between genome size and the number of TA pairs in all *Synechococcus* strains was not found when only the open ocean strains were analyzed. *Synechococcus* toxin genes contained more known conserved domains than antitoxins (Fig. 3). About 77% of toxin genes had a known conserved domain with an annotation of a traditionally named TA system. The most common toxin gene included a conserved PIN domain which is characteristic of the VapC toxin which cleaves

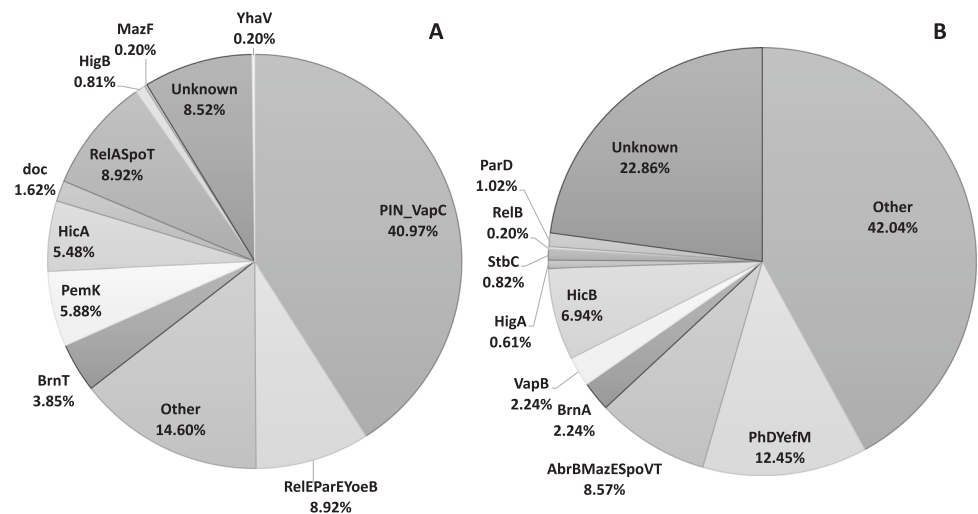
tRNAs or rRNAs [53]. Nearly 41% of putative toxin genes contained the conserved domain for VapC.

Putative antitoxin sequences contained fewer NCBI conserved domains than toxins. Only 35% of antitoxin genes had a conserved domain with a traditionally annotated TA system. Many conserved domains in putative antitoxin genes had generic names such as “domains of unknown function” (DUF) or clusters of orthologous groups (COG).

Discussion

A survey on picocyanobacterial TA systems leads to an interesting finding that *Synechococcus* strains with larger genome size contain more TA systems. Although many genetic features of picocyanobacterial genomes have been explored [10, 11], little is known about the prevalence of TA genes in picocyanobacteria. *Synechococcus* has a remarkable adaptation capability, which is reflected by their occupancy in diverse environments ranging from lakes, rivers, estuaries, coastal and oceanic water. The presence of a specific group or genus over such a wide range of habitats makes *Synechococcus* an ideal model to explore the relationship between their ecological adaptation and genomic features. TA systems have been well studied in bacteria and archaea. One well-known function of TA systems is dormancy, or the ability to initiate a persister state under stressed conditions and recover when the adverse stresses are released [39, 40, 54]. While the actual functions of

Fig. 3 Conserved domain regions of putative toxins and antitoxins. a Conserved domains in toxins. **b** Conserved domains in antitoxins. Putative toxin and antitoxin sequences that contained a conserved domain that was not a traditional TA system were categorized as ‘Other’. Sequences that did not contain a conserved domain were categorized as ‘Unknown’.



Synechococcus TA genes have not been tested, it is believed that inheritance of more TA genes may allow some *Synechococcus* strains to endure more variable environments, which could confer a competitive advantage against other less resilient picocyanobacteria. Coastal, estuarine, and freshwater environments are characterized by rapid changes in environmental conditions, the higher occurrence frequency of TA genes may provide adaptive advantages for *Synechococcus* living in these types of aquatic habitats.

TA systems have been shown to provide recoverable persister states when *Synechococcus* cells were exposed to conditions to induce oxidative stress [37]. These conditions are expected in rapidly changing environments such as estuaries, coastal, and some freshwater environments. In another cyanobacterial species, *Synechocystis* PCC 6803, TA systems have been found and were predicted to have RNase activity which could have a drastic effect on transcriptomic remodeling [55]. Such a remodeling is possible through RNA degradation as a result of toxin over-expression, which can have a significant impact on slowing translation. Type II TA systems can have other methods of actions in other bacteria including post segregational killing and abortive infection [54]. Unfortunately, these remain poorly studied and understood in picocyanobacterial systems.

Interestingly, a linear correlation was found between the genome size and the number of TA genes in *Synechococcus*. Such a linear correlation has not been found in bacteria [56]. When testing 2,181 genomes of prokaryotes (archaea and bacteria from both obligate intracellular species to free living species) [56] and 65 genomes of *Acetobacter* (with sources ranging from fermented food, to fruits, to symbiotes in the fruit fly *Drosophila melanogaster*) [57], the number of TA gene pairs does not increase linearly with increased genome size. The clear linear trend seen in *Synechococcus* is likely related to larger genome

sizes having a wide array of CDS. For strains with expanded genetic capacity, it may be advantageous to retain a multitude of TA systems in aquatic habitats with highly variable chemical and physical features. While *Synechococcus* is ubiquitous in nearly all aquatic ecosystems, the presence of *Synechococcal* TA systems is not; this suggests that TA systems are advantageous in some, but not all, aquatic environments. The genome size of *Synechococcus* available for this in this study ranges from 2.1 to 3.7 Mb. *Synechococcus* genomes have previously been shown to correlate strongly with the length of hypervariable genome island regions [26]. In *Synechococcus*, TA genes can be located on these genome islands, but the majority of the TA pairs are not located on hypervariable genome islands (Table S1).

The lack of TA systems in *Prochlorococcus* is likely related to their relatively stable habitats. The endemic habitat of *Prochlorococcus* is the pelagic ocean which is characterized by its stable, nutrient limiting environment coupled with a predictably high cellular density [5]. The genus *Prochlorococcus* is a highly diverse group comprised of 12 specialized clades with genomic features uniquely adapted to specific conditions in oceanic ecosystems [6]. High light adapted group II has some of the smallest genomes (~1.7 Mb) and lowest GC content (~33%), which is indicative of genomic reduction [9]. Some *Prochlorococcus* strains (such as low light adapted group IV) have relatively large genomes (2.4–2.6 Mb) and many unique genes [58]. Regardless of their large genetic capacity, no TA genes were detected in the genomes of group IV *Prochlorococcus* strains. Despite the diversity of *Prochlorococcus* ecotypes, TA systems may not be needed due to *Prochlorococcus* specific adaptation to the oligotrophic ocean.

Among the 33 *Synechococcus* strains examined in this study, 11 are open ocean strains. Oceanic *Synechococcus* strains in general contain no TA genes or only a few TA

genes. The five open ocean *Synechococcus* strains that are void of TA genes are WH8109, KORDI-52, KORDI-100, CC9605, and CC9902, while four oceanic *Synechococcus* strains (MIT9504, MIT9508, MIT9509, and KORDI-49) contain few (1 to 5) putative TA pairs. The exception to this is open ocean *Synechococcus* strain WH8102, which contains 15 putative systems. WH8102 was originally isolated from the Sargasso Sea, and its genome is more indicative of a 'generalist' with features acquired *via* horizontal gene transfer [59]. Like marine *Prochlorococcus*, oceanic *Synechococcus* may not need TA genes due to their acclimation to the stable oligotrophic environment.

Along with genome size, endemic ecological conditions and habitats are an important indicator of the prevalence of TA systems in *Synechococcus*, and more broadly picocyanobacteria. *Synechococcus* strains from more variable environments like coastal and freshwater locations tend to have more TA pairs than open ocean strains that are streamlined to a stable pelagic lifestyle. This phenomenon may also explain the broader pattern of TA system distribution in picocyanobacteria; the prevalence of TA pairs in picocyanobacteria living in the nutrient rich and dynamic habitats and the rareness and complete absence of TA in picocyanobacteria living in the oligotrophic open ocean. The presence of TA systems may be one of the many genetic features that allow *Synechococcus* to inhabit a wide array of aquatic ecosystems and achieve a cosmopolitan distribution. The lack of TA systems in *Prochlorococcus* is consistent with their reduced genomes and oligotrophic lifestyle [60].

Originally, 7 TA pairs were predicted in CB0101 using BLASTCLUST [61] and confirmed using the RASTA-bacteria [62] and TADB [48]. More recently, the TAFinder search tool was used to search whole genomes [44], rather than specific gene pairs to predict type II TA systems. Due to the ever-expanding TADB and improved prediction methods like TAFinder, 22 TA systems, including the original 7 pairs, were found in CB0101. These new pairs were confirmed manually, and conserved domains were predicted using NCBI's conserved domain database and Interpro for protein functional analysis.

The scope of this study is constrained by the use of TAFinder. TAFinder is capable of predicting type II TA systems, which are the most well studied and characterized TA systems. Type II TA systems comprise 99% of the TA genes in the TADB [48]. To ensure that other, less known TA families (I, II-VI) were not overlooked, a blast search for those few systems against all the genomes of *Synechococcus* and *Prochlorococcus* was completed. No significant matches were reported using default settings. Antitoxin sequences contained fewer conserved domains than toxins. Antitoxin sequences appear to be highly diverse and variable among *Synechococcus* strains. Multiple antitoxin

structures may function to bind their cognate toxin. When the paired gene can sufficiently neutralize the toxin, it acts as an antitoxin and selection for highly conserved sequences may be relaxed. Although more toxins contain conserved domains than antitoxins, it is important to note that TA systems are not present in all *Synechococcus* and they are highly variable in terms of the number and type of TA systems. Even within the closely related *Synechococcus* strains, it is difficult to identify suitable genetic markers for phylogenetic analysis due to the overall poor TA gene conservation. VapC, and its cognate VapB antidote, are the largest family of bacterial toxin-antitoxin modules [63]. A wide variety of toxin functionality is represented in *Synechococcus* as both ribosomal-dependent mRNA endonucleases like RelE and ribosomal-independent mRNA endonucleases like HicA and MazF were predicted [54].

Conclusion

The tight correlation of genome size and the number TA genes in coastal and freshwater *Synechococcus* suggest that the retention of TA systems could be advantageous for *Synechococcus* living in highly variable environments. All the tested *Prochlorococcus* genomes ($n = 38$) do not contain any TA genes, regardless of their genome sizes (1.6 to 2.7 Mb). This result suggests that *Prochlorococcus* do not have a TA system mediated dormancy in response to changing environments. This also applies to some *Synechococcus* living in open oceans where chemical and hydrological conditions are relatively stable compared to coastal, estuarine, and freshwater environments. It is interesting that the number of TA pairs is linearly correlated with the increasing genome sizes of *Synechococcus*. It appears that the acquisition and retention of TA genes in *Synechococcus* is not only influenced by genome size, but also environmental stability. *Synechococcus* strains with large genomes, especially those that inhabit dynamic ecosystems (coastal, estuarine, and freshwater) have more TA systems than strains with smaller genomes that are present in stable environments like the open ocean. Compared to *Prochlorococcus*, *Synechococcus* has a relatively large genome, with space for more coding sequences, ample TA systems, and a wide variety of environmental response genes that allow for their ubiquitous distribution in diverse aquatic environments. TA systems in *Synechococcus* could confer an ability to enter persister states in the presence of stressful stimuli, which is advantageous to *Synechococcus* living in freshwater and coastal estuary where environmental conditions are more variable compared to open oceans.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

- Flombaum P, Gallegos JL, Gordillo RA, Rincón J, Zabala LL, Jiao N, et al. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proc Natl Acad Sci*. 2013;110:9824–9.
- Li WKW, Uhl S. Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting. *Limnol Oceanogr*. 1994;39:169–75.
- Dvořák P, Casamatta DA, Pouličková A, Hašler P, Ondřej V, Sanges R. *Synechococcus*: 3 billion years of global dominance. *Mol Ecol*. 2014;23:5538–51.
- Morel A, Ahn YH, Partensky F, Vaulot D, Claustre H. *Prochlorococcus* and *Synechococcus*: a comparative study of their optical properties in relation to their size and pigmentation. *J Mar Res*. 1993;51:617–49.
- Partensky F, Blanchot J, Vaulot D. Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review. *Bull l'Institut océanographique*. 1999;19:457–75.
- Biller SJ, Berube PM, Lindell D, Chisholm SW. *Prochlorococcus*: the structure and function of collective diversity. *Nat Rev Microbiol*. 2015;13:13–27.
- Sun Z, Blanchard JL. Strong Genome-Wide Selection Early in the Evolution of *Prochlorococcus* Resulted in a Reduced Genome through the Loss of a Large Number of Small Effect Genes. *PLoS ONE*. 2014;9:e88837.
- Larsson J, Nylander JAA, Bergman B. Genome fluctuations in cyanobacteria reflect evolutionary, developmental and adaptive traits. *BMC Evol Biol*. 2011;11:187.
- Dufresne A, Garczarek L, Partensky F. Accelerated evolution associated with genome reduction in a free-living prokaryote. *Genome Biol*. 2005;6:R14.1–R14.10.
- Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, et al. Ecological genomics of marine picocyanobacteria. *Microb Mol Biol Rev*. 2009;73:249–99.
- Scanlan DJ. Marine Picocyanobacteria. In: Whitton B. (eds). *Ecology of Cyanobacteria II: Their Diversity in Space and Time*. Springer Netherlands: Dordrecht, Netherlands 2012, pp. 503–33.
- Sánchez-Baracaldo P, Hayes PK, Blank CE. Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. *Geobiology*. 2005;3:145–65.
- Wang K, Wommack KE, Chen F. Abundance and distribution of *Synechococcus* spp. and cyanophages in the Chesapeake Bay. *Appl Environ Microbiol*. 2011;77:7459–68.
- Callieri C. Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. *Freshw Rev*. 2008;1:1–28.
- Stockner JG. Phototrophic picoplankton: an overview from marine and freshwater ecosystems. *Limnol Oceanogr*. 1988;33:765–75.
- Callieri C, Stockner JG. Freshwater autotrophic picoplankton: a review. *J Limnol*. 2002;61:1–14.
- Honda D, Yokota A, Sugiyama J. Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. *J Mol Evol*. 1999;48:723–39.
- Rippka R, Deruelles J, Waterbury JB. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol*. 1979;111:1–61.
- Wilmotte AMR, Stam WT. Genetic relationships among cyanobacterial strains originally designated as '*Anacystis nidulans*' and some other *Synechococcus* strains. *J Gen Microbiol*. 1984;130:2737–40.
- Coutinho F, Tschoeke DA, Thompson F. Comparative genomics of *Synechococcus* and proposal of the new genus *Parasynechococcus*. *PeerJ*. 2016;4:e1522 1–18.
- Robertson BR, Tezuka N, Watanabe MM. Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. *Int J Syst Evol Microbiol*. 2001;51:861–71.
- Zwirgmaier K, Jardillier L, Ostrowski M, Mazard S, Garczarek L, Vaulot D, et al. Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. *Environ Microbiol*. 2008;10:147–61.
- Rocap G, Distel DL, Waterbury JB, Chisholm SW. Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. *Appl Environ Microbiol*. 2002;68:1180–91.
- Toledo G, Palenik B. *Synechococcus* diversity in the California Current as seen by RNA polymerase (rpoC1) gene sequences of isolated strains. *Appl Environ Microbiol*. 1997;63:4298–303.
- Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF, Scanlan DJ. Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. *Appl Environ Microbiol*. 2003;69:2430–43.
- Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, et al. Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. *Genome Biol*. 2008;9:R90.
- Huang S, Wilhelm SW, Harvey HR, Taylor K, Jiao N, Chen F. Novel lineages of *Prochlorococcus* and *Synechococcus* in the global oceans. *ISME J*. 2012;6:285–97.
- Callieri C, Coci M, Corno G, Macek M, Modenutti B, Balseiro E, et al. Phylogenetic diversity of nonmarine picocyanobacteria. *FEMS Microbiol Ecol*. 2013;85:293–301.
- Crosbie ND, Pockl M, Weisse T. Dispersal and phylogenetic diversity of nonmarine picocyanobacteria inferred from 16S rRNA gene and cpcBA-intergenic spacer sequence analyses. *Appl Environ Microbiol*. 2003;69:5716–21.
- Jasser I, Królicka A, Karnkowska-Ishikawa A. A novel phylogenetic clade of picocyanobacteria from the Mazurian lakes (Poland) reflects the early ontogeny of glacial lakes. *FEMS Microbiol Ecol*. 2011;75:89–98.
- Ernst A, Becker S, Wollenzien UIA, Postius C. Ecosystem-dependent adaptive radiations of picocyanobacteria inferred from 16S rRNA and ITS-1 sequence analysis. *Microbiology*. 2003;149:217–28.
- Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, et al. The genome of a motile marine *Synechococcus*. *Nature*. 2003;424:1037–42.
- Palenik B, Ren Q, Dupont CL, Myers GS, Heidelberg JF, Badger JH, et al. Genome sequence of *Synechococcus* CC9311: Insights into adaptation to a coastal environment. *Proc Natl Acad Sci*. 2006;103:13555–9.
- Stuart RK, Dupont CL, Johnson DA, Paulsen IT, Palenik B. Coastal strains of marine *Synechococcus* species exhibit increased tolerance to copper shock and a distinctive transcriptional response relative to those of open-ocean strains. *Appl Environ Microbiol*. 2009;75:5047–57.

35. Stuart RK, Brahamsha B, Busby K, Palenik B. Genomic island genes in a coastal marine *Synechococcus* strain confer enhanced tolerance to copper and oxidative stress. *ISME J.* 2013;7:1139–49.
36. Fucich D, Marsan D, Sosa A, Chen F. Complete genome sequence of Subcluster 5.2 *Synechococcus* sp. strain CB0101, isolated from the Chesapeake Bay. *Microbiol Resour Announc.* 2019;8:6–8.
37. Marsan D, Place A, Fucich D, Chen F. Toxin-antitoxin systems in estuarine *Synechococcus* strain CB0101 and their transcriptomic responses to environmental stressors. *Front Microbiol.* 2017;8:1–11.
38. Page R, Peti W. Toxin-antitoxin systems in bacterial growth arrest and persistence. *Nat Chem Biol.* 2016;12:208–14.
39. Unterholzner SJ, Poppenberger B, Rozhon W. Toxin-antitoxin systems. *Mob Genet Elem.* 2013;3:e26219 1–13.
40. Makarova KS, Wolf YI, Koonin EV. Comprehensive comparative-genomic analysis of type 2 toxin-antitoxin systems and related mobile stress response systems in prokaryotes. *Biol Direct.* 2009;4:19.
41. Kaneko T, Nakamura Y, Sasamoto S, Watanabe A, Kohara M, Matsumoto M, et al. Structural analysis of four large plasmids harboring in a unicellular cyanobacterium, *Synechocystis* sp. PCC 6803. *DNA Res.* 2003;10:221–8.
42. Chen Y, Holtman CK, Magnuseon RD, Youderian PA, Golden SS. The complete sequence and functional analysis of pANL, the large plasmid of the unicellular freshwater cyanobacterium *Synechococcus elongatus* PCC 7942. *Plasmid.* 2011;23:1–7.
43. Chen F, Wang K, Kan J, Suzuki MT, Wommack KE. Diverse and unique picocyanobacteria in Chesapeake Bay, revealed by 16S-23S rRNA internal transcribed spacer sequences. *Appl Environ Microbiol.* 2006;72:2239–43.
44. Xie Y, Wei Y, Shen Y, Li X, Zhou H, Tai C, et al. TADB 2.0: An updated database of bacterial type II toxin-antitoxin loci. *Nucleic Acids Res.* 2018;46:D749–D753.
45. O’Leary NA, Wright MW, Brister JR, Ciufu S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.* 2016;44:D733–D745.
46. Agarwala R, Barrett T, Beck J, Benson DA, Bollin C, Bolton E, et al. Database resources of the national center for biotechnology information. *Nucleic Acids Res.* 2017;45:D12–D17.
47. Nordberg H, Cantor M, Dusheyko S, Hua S, Poliakov A, Shabalov I, et al. The genome portal of the Department of Energy Joint Genome Institute: 2014 updates. *Nucleic Acids Res.* 2014;42:26–31.
48. Shao Y, Harrison EM, Bi D, Tai C, He X, Ou HY, et al. TADB: a web-based resource for Type 2 toxin-antitoxin loci in bacteria and archaea. *Nucleic Acids Res.* 2011;39:606–11.
49. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, et al. CDD/SPARCLE: Functional classification of proteins via sub-family domain architectures. *Nucleic Acids Res.* 2017;45:D200–D203.
50. R Core Team. R: A Language and Environment for Statistical Computing. 2018. Vienna, Austria.
51. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag; 2009.
52. Bertelli C, Laird MR, Williams KP, Lau BY, Hoad G, Winsor GL, et al. IslandViewer 4: Expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Res.* 2017;45:W30–W35.
53. Winther KS, Gerdes K. Enteric virulence associated protein VapC inhibits translation by cleavage of initiator tRNA. *Proc Natl Acad Sci.* 2011;108:7403–7.
54. Harms A, Brodersen DE, Matarai N, Gerdes K. Toxins, targets, and triggers: an overview of toxin-antitoxin biology. *Mol Cell.* 2018;70:768–84.
55. Kopfmann S, Roesch S, Hess W. Type II toxin-antitoxin systems in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803. *Toxins.* 2016;8:228.
56. Leplae R, Geeraerts D, Hallez R, Guglielmini J, Drze P, Van Melderen L. Diversity of bacterial type II toxin-antitoxin systems: a comprehensive search and functional analysis of novel families. *Nucleic Acids Res.* 2011;39:5513–25.
57. Xia K, Bao H, Zhang F, Linhardt RJ, Liang X. Characterization and comparative analysis of toxin – antitoxin systems in *Acetobacter pasteurianus*. *J Ind Microbiol Biotechnol.* 2019;46:869–82.
58. Biller SJ, Berube PM, Berta-Thompson JW, Kelly L, Roggensack SE, Awad L, et al. Genomes of diverse isolates of the marine cyanobacterium *Prochlorococcus*. *Sci Data.* 2014;1:1–11.
59. Palenik B, Barahamsha B, Larimer FW, Land M, Hauser L, Chain P, et al. The genome of a motile marine *Synechococcus*. *Nature.* 2003;424:1037–42.
60. Scanlan DJ, West NJ. Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microbiol Ecol.* 2002;40:1–12.
61. McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, et al. Analysis tool web services from the EMBL-EBI. *Nucleic Acids Res.* 2013;41:W597–W600.
62. Sevin EW, Barloy-Hubler F. RASTA-bacteria: a web-based tool for identifying toxin-antitoxin loci in prokaryotes. *Genome Biol.* 2007;8:R155.1–R155.14.
63. Robson J, McKenzie JL, Cursons R, Cook GM, Arcus VL. The vapBC operon from mycobacterium smegmatis Is an auto-regulated toxin-antitoxin module that controls growth via inhibition of translation. *J Mol Biol.* 2009;390:353–67.