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# *Prochlorococcus* viruses—From biodiversity to biogeochemical cycles

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**Abstract** As the dominant primary producer in oligotrophic oceans, the unicellular picocyanobacterium *Prochlorococcus* is the smallest and most abundant photosynthetic phytoplankton in the world and plays an important role in marine carbon cycling. Cyanophages that infect *Prochlorococcus* influence the growth, carbon fixation, diversity, evolution, and environmental adaptation of their hosts. Here, we review studies on the isolation, genomics, and phylogenetic diversity of *Prochlorococcus* viruses and their interactions with *Prochlorococcus*. We also review the potential effects of *Prochlorococcus* viruses on biogeochemical cycling in the ocean.

**Keywords** *Prochlorococcus* viruses, Diversity, Genomics, Biogeochemical significance

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## 1. Introduction

*Prochlorococcus*, a genus of marine cyanobacteria, is the smallest (diameter 0.5–0.7 μm) and most abundant photosynthetic organism on the planet. *Prochlorococcus* is widely distributed between 40°N and 40°S and dominates in oligotrophic oceans, contributing a significant part of marine primary production (Chisholm et al., 1988; Partensky et al., 1999). In some oceanic regions, *Prochlorococcus* is an important contributor (up to 80%) to biomass and primary production (Vaulot et al., 1990; Goericke and Welschmeyer, 1993; Campbell et al., 1997; Liu et al., 1997; Jiao and Yang, 2002; Gérikas-Ribeiro et al., 2016). According to model results, the annual mean global abundance of *Prochlorococcus* is $2.9\pm0.1\times10^{27}$ cells, and the *Prochlorococcus* global net primary production is 4 Gt C $yr^{-1}$, which represents 8.5% of oceanic net primary production. Under future warming of the global ocean surface, the distribution of *Prochlorococcus* will likely extend to the south, with an increase in abundance that is geographically dependent; the estimated mean future increase in cell abundance is 29% (Flombaum et al., 2013). The study of *Prochlorococcus* populations, activity and diversity is important for understanding the responses of marine ecosystems and biogeochemical cycles to global change.

Viruses are the most abundant biological entities in the ocean, with abundances up to $4\times10^{30}$; this value is more than 10 times higher than those of bacteria and archaea (Suttle, 2005, 2007). Viruses are one of the major biological factors affecting *Prochlorococcus* ecological characteristics. Owing to their lethality to their hosts, viruses play a significant role in marine ecology and biogeochemical cycles. For example, viruses can affect the food web structure (Wilhelm and

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Suttle, 1999; Suttle, 2007), host evolution (Rohwer and Thurber, 2009; Avrani and Lindell, 2015), and biogeochemical cycles (Fuhrman, 1999; Wilhelm and Suttle, 1999; Jover et al., 2014; Zhang et al., 2014).

In this short review paper, we summarize the research achievements in the study of *Prochlorococcus* virus isolation, genomics, and phylogenetic diversity and then discuss the potential effects of *Prochlorococcus* viruses on biogeochemical cycling.

## 2. Diversity and genomics of *Prochlorococcus* viruses

### 2.1 Isolation and taxonomy of *Prochlorococcus* viruses

Since *Prochlorococcus* is sensitive to temperature, light and heavy metals, isolating their viruses is challenging. The most widely used isolation technique is a plating method based on ultra-low-melting-point agarose (Sullivan et al., 2003), which involves inoculating Pro99 medium (Moore et al., 2007) with log-phase *Prochlorococcus*, "helper" bacteria EZ55, and filtered seawater. *Prochlorococcus* grows on the solid medium and forms a green mat; however, *Prochlorococcus* cells infected by viruses die, which results in plaques on the solid medium. The bacteria EZ55 can help *Prochlorococcus* growth in liquid or solid media at low cell concentrations and benefit plaque formation (Morris et al., 2008).

To date, 59 dsDNA *Prochlorococcus* viruses have been isolated, including 28 myoviruses, 29 podoviruses, and two siphoviruses (Table 1). Similarly, the major morphotypes of viruses infecting *Synechococcus* (the sister genus of *Prochlorococcus*) and *Roseobacter* (a widely distributed marine heterotrophic bacterial genus) are also myoviruses and podoviruses (Waterbury and Valois, 1993; Yang et al., 2017). Since a global transmission electron microscopy-based analysis showed that the abundances of these three morphotypes are comparable, the lack of isolated siphoviruses from *Prochlorococcus* and other major marine bacterial lineages could be a technique bias.

Based on vertical distribution and light adaptation, *Prochlorococcus* can be divided into high-light-adapted (HL) and low-light-adapted (LL) ecotypes (Biller et al., 2015). The hosts of the isolated cyanophages described thus far are from the HL ecotypes I and II and the LL ecotypes I, IV and II/III. It is important to note that more than 60% of the reported viruses were isolated from three *Prochlorococcus* strains, namely, MED4, NATL1A, and NATL2A (Table 1). This confines the study of *Prochlorococcus* virus diversity, physiology, and ecology and could be the reason that many viral genotypes detected by molecular surveys are not found in isolated cyanophages. Even for *Prochlorococcus* viruses that have been successfully cultured, there is limited knowledge about virus physiology, such as one-step growth curves and adsorption kinetics, because of the difficulty of obtaining enough material for the quantification of active viral particles. Therefore, further studies isolating and investigating the physiology of viruses infecting *Prochlorococcus*, especially strains other than MED4, NATL1A, and NATL2A, are urgently needed.

Interestingly, Sullivan et al. (2003) found that 10 of the 18 viruses isolated from *Synechococcus* can infect *Prochlorococcus*; eight of these viruses infect LL ecotypes, and the remaining two infect both HL and LL ecotypes. However, only three of the 26 viruses isolated from *Prochlorococcus* can infect *Synechococcus*. Because of the limited numbers of isolated *Prochlorococcus* and viruses, it is unknown whether these cross-infection phenomena of *Prochlorococcus* and *Synechococcus* viruses are universal. Nevertheless, answering this question is important for our understanding of cyanobacteria-virus interactions. Enav et al. (2012) proposed that cyanophage tRNAs may play a role in the cross-infectivity of oceanic *Prochlorococcus* and *Synechococcus* hosts. Doron et al. (2016) suggested that phages with broad host ranges that infect multiple hosts are likely dependent on the effectiveness of host defense strategies rather than on the phage infection process.

### 2.2 Genome of *Prochlorococcus* viruses

To date, 16 *Prochlorococcus* myovirus genomes (Sullivan et al., 2005, 2010; Fridman et al., 2017), 13 podovirus genomes (Sullivan et al., 2005; Labrie et al., 2013) and one siphovirus genome (Sullivan et al., 2009) have been published and show a large variation in genomic content. The podoviruses are similar to phage T7, and the myoviruses are similar to phage T4. The podovirus genomes range from 44.9 to 47.7 kb, with 37–40% G+C contents, and are predicted to contain 50–70 open reading frames (ORFs) (Labrie et al., 2013). The myoviruses have a G+C content ranging from 35% to 38% and genome size ranging from 176.4 to 252.4 kb, and the latter is correlated with the ORF number (221–334) (Sullivan et al., 2010). The only known genome of siphovirus P-SS2 has a size of 108 kb with 132 ORFs and a G+C content of 52.3% (Sullivan et al., 2009). P-SS2 gene homologs are rare in the surface ocean, which suggests that P-SS2-like viruses are mainly distributed in deep photic zones (Sullivan et al., 2009).

Many host-like genes, such as pigment biosynthesis genes (*petE*, *petF*, *pebA*), photosystem II (PS II) genes (*psbA, psbD*), photosystem I (PS I) genes (*psaJF*, *psaC*, *A*, *B*, *K*, *E*, *D*), carbon metabolism-related genes (*talC*, *cp12*), phosphate metabolism-related genes (*phoH*, *pstS*), nucleotide metabolism-related genes (*nrd*, *mazG*) and a vitamin B12 biosynthesis-related gene (*cobS*), have been found in *Prochlorococcus* virus genomes (Sullivan et al., 2005;

**Table 1** Summary of publicly available *Prochlorococcus* viruses[a)]

| Phage | Host | Host ecotype | Morphology | Site of isolation | Depth (m) | Genome accession | Reference |
|---|---|---|---|---|---|---|---|
| P-SS1 | MIT 9313 | LLIV | S | Slope | 60 | | Sullivan et al., 2003 |
| P-SS2 | MIT 9313 | LLIV | S | Slope | 83 | NC_013021 | Sullivan et al., 2003 |
| P-ShM1 | MIT 9313 | LLIV | M | Slope | 40 | | Sullivan et al., 2003 |
| P-ShM2 | MIT 9313 | LLIV | M | Slope | 0 | | Sullivan et al., 2003 |
| P-RSM1 | MIT 9303 | LLIV | M | Red Sea | 0 | HQ634175 | Sullivan et al., 2003 |
| P-RSM2 | NATL2A | LLI | M | Red Sea | 50 | | Sullivan et al., 2003 |
| P-RSM3 | NATL2A | LLI | M | Red Sea | 50 | HQ634176 | Sullivan et al., 2003 |
| P-RSM4 | MIT 9303 | LLIV | M | Red Sea | 130 | GU071099 | Sullivan et al., 2008 |
| P-RSM5 | NATL1A | LLI | M | Red Sea | 130 | | Sullivan et al., 2008 |
| P-RSM6 | NATL2A | LLI | M | Red Sea | 50 | HQ634193 | Kelly et al., 2013 |
| P-SSM1 | MIT 9303 | LLIV | M | BATS | 100 | | Sullivan et al., 2003 |
| P-SSM2 | NATL1A | LLI | M | BATS | 100 | AY939844 | Sullivan et al., 2003 |
| P-SSM3 | NATL2A | LLI | M | BATS | 100 | HQ337021 | Sullivan et al., 2003 |
| P-SSM4 | NATL2A | LLI | M | BATS | 10 | AY940168 | Sullivan et al., 2003 |
| P-SSM5 | NATL2A | LLI | M | BATS | 15 | HQ632825 | Sullivan et al., 2003 |
| P-SSM6 | NATL2A | LLI | M | BATS | 40 | | Sullivan et al., 2003 |
| P-SSM7 | NATL1A | LLI | M | BATS | 120 | GU071103 | Sullivan et al., 2008 |
| P-SSM8 | MIT 9211 | LLII/III | M? | SS | 30 | | Sullivan et al., 2008 |
| P-SSM9 | NATL2A | LLI | M? | SS | 0 | | Sullivan et al., 2008 |
| P-SSM10 | NATL2A | LLI | M? | SS | 0 | | Sullivan et al., 2008 |
| P-SSM11 | NATL2A | LLI | M? | SS | 0 | | Sullivan et al., 2008 |
| P-SSM12 | NATL2A | LLI | M? | SS | 95 | | Sullivan et al., 2008 |
| P-TIM3 | MED4 | HLI | M | Red Sea | 20 | | Avrani et al., 2011 |
| P-TIM40 | NATL2A | LLI | M | Pacific Ocean | NA | KP211958 | Enav et al., 2012 |
| P-TIM68 | MIT 9515 | HLI | M | Pacific Ocean | NA | NC_028955 | Fridman et al., 2017 |
| P-HM1 | MED4 | HLI | M | Pacific Ocean | 125 | GU071101 | Sullivan et al., 2010 |
| P-HM2 | MED4 | HLI | M | Pacific Ocean | 125 | GU075905 | Sullivan et al., 2010 |
| MED4-117 | MED4 | HLI | M | HOTS | 125 | NC_020857 | |
| MED4-184 | MED4 | HLI | M | HOTS | 125 | NC_020847 | |
| MED4-213 | MED4 | HLI | M | HOTS | 125 | HQ634174 | Kelly et al., 2013 |
| P-RSP1 | MIT 9215 | HLII | P | Red Sea | 0 | | Sullivan et al., 2003 |
| P-RSP2 | MIT 9302 | HLII | P | Red Sea | 0 | HQ332139 | Sullivan et al., 2003 |
| P-RSP3 | NATL2A | LLI | P | Red Sea | 50 | | Sullivan et al., 2003 |
| P-RSP5 | NATL1A | LLI | P | Red Sea | 130 | GU071102 | Labrie et al., 2013 |
| P-SSP1 | MIT 9215 | HLII | P | BATS | 100 | | Sullivan et al., 2003 |
| P-SSP2 | MIT 9312 | HLII | P | BATS | 120 | GU071107 | Sullivan et al., 2003 |
| P-SSP3 | MIT 9312 | HLII | P | BATS | 100 | HQ332137 | Sullivan et al., 2003 |
| P-SSP4 | MIT 9312 | HLII | P | BATS | 70 | | Sullivan et al., 2003 |
| P-SSP5 | MIT 9515 | HLI | P | BATS | 120 | | Sullivan et al., 2003 |
| P-SSP6 | MIT 9515 | HLI | P | BATS | 100 | HQ634152 | Sullivan et al., 2003 |
| P-SSP7 | MED4 | HLI | P | BATS | 100 | NC_006882 | Sullivan et al., 2003 |
| P-SSP8 | NATL2A | LLI | P | BATS | 100 | | Sullivan et al., 2003 |
| P-SSP9 | SS120 | LLII/III | P | BATS | 100 | HQ316584 | Labrie et al., 2013 |
| P-SSP10 | NATL2A | LLI | P | BATS | 100 | HQ337022 | Labrie et al., 2013 |
| P-SSP11 | MIT 9515 | HLI | P | BATS | 100 | HQ634152 | Sullivan et al., 2003 |
| P-GSP1 | MED4 | HLI | P | Gulf Stream | 40 | HQ332140 | Sullivan et al., 2003 |
| P-SP1 | SS120 | LLII/III | P | Slope | 83 | | Sullivan et al., 2003 |
| P-TIP1 | MED4 | HLI | P | Red Sea | 20 | | Avrani et al., 2011 |
| P-TIP2 | MED4 | HLI | P | Red Sea | 20 | | Avrani et al., 2011 |
| P-TIP34 | MIT 9515 | HLI | P | P-TIP34 | NA* | | Dekel-Bird et al., 2013 |
| P-TIP38 | MED4 | HLI | P | Red Sea | 0 | | Avrani et al., 2011 |
| P-TIP39 | MIT 9215 | HLII | P | Red Sea | NA** | | Dekel-Bird et al., 2013 |
| P-TIP42 | MED4 | HLI | P | Red Sea | NA** | | Dekel-Bird et al., 2013 |
| P-TIP43 | MED4 | HLI | P | Red Sea | NA** | | Dekel-Bird et al., 2013 |
| P-TIP44 | MED4 | HLI | P | Red Sea | NA** | | Dekel-Bird et al., 2013 |
| 10G | MED4 | HLI | P | Red Sea | NA** | | Dekel-Bird et al., 2013 |
| P-HP1 | NATL2A | LLI | P | HOTS | 25 | GU071104 | Thompson et al., 2011 |
| NATL1A-7 | NATL1A | LLI | P | Red Sea | 130 | NC_016658 | |
| NATL2A-133 | NATL2A | LLI | P | HOTS | 25 | NC_016659 | |

a) M, myovirus; P, podovirus; S, siphovirus; SS, Sargasso Sea; BATS, Bermuda Atlantic Time-series station; HOTS, Hawaii Ocean Time-series station. NA, data not available. * coastal region; ** open water

Dammeyer et al., 2008; Fridman et al., 2017). All of these genes, which are known as auxiliary metabolic genes (AMGs), can be expressed in the host cell and are thought to affect host metabolism during infection, providing energy and materials for viral replication (Breitbart et al., 2007; Lindell et al., 2005; Dammeyer et al., 2008; Fridman et al., 2017).

Nearly all the light harvesting-related genes (*ho1*, *pcyA*, *pebS*, *cpeT*), some electron transfer-related genes (*psbD*, *petE*, *petF*, *proX*) and carbon, phosphate and nucleotide metabolism-related genes (*cp12*, *phoH*, *pstS*, *mazG*) appear only in *Prochlorococcus* myovirus genomes (Sullivan et al., 2005, 2010; Labrie et al., 2013). *psbA*, *hli*, *nrd* and *talC* are common in the genomes of podoviruses and myoviruses. In addition, myoviruses contain multiple copies of *hli*, while podoviruses always contain a single copy of *hli*. One of the possible reasons that the AMGs in myoviruses are more diverse than those in podoviruses is that the former usually have a broader host range and more gene exchange opportunities than the latter.

Studies have shown that some host-derived photosythesis-related genes can be expressed and may play an essential role during infection. Lindell et al. (2005) first reported that *Prochlorococcus* podovirus P-SSP7 *psbA* and high-light-inducible (*hli*) genes were expressed when host gene expression decreased. Evidence from *Prochlorococcus* myovirus P-TIM68 showed that cyanophage-derived PS II and PS I genes could be transcribed simultaneously. During infection, the host PS I activity was enhanced, while the host PS II activity was not, suggesting that the phage PS I genes replenished host photosythesis and enhanced host PS I activity, thus maintaining the photosynthetic capacity of the host (Fridman et al., 2017). Pigment biosynthesis gene *pebS* mediated a novel bilin biosynthesis pathway, which is only found in cyanophages and is thought to be more efficient than the host pathway (Dammeyer et al., 2008).

Cyanophages carry the Calvin cycle inhibitor gene *cp12* and the pentose phosphate pathway (PPP) transaldolase gene *talC* (Thompson et al., 2011). It was speculated that, during infection, CP12 proteins inhibit the activity of two key Calvin cycle enzymes, phosphoribulokinase and glyceraldehyde-3-phosphate dehydrogenase, and shift the carbon flux from the Calvin cycle to PPP. The cyanophage *talC* sequence is significantly different from the host transaldolase (TalB) but resembles *Escherichia coli* fructose-6-phosphate (F6P) aldolase. However, the purified product of *talC* shows transaldolase activity *in vitro*, which indicates it might enhance the flux of this key reaction in the host PPP, resulting in the increased production of NADPH and ribose 5-phosphate.

The nucleotide metabolism gene *nrd* (ribonucleotide reductase gene) has been found in most *Prochlorococcus* virus genomes. Phage *nrd* is transcribed with phage DNA replication genes and PPP genes during infection, indicating that *nrd* may help deoxynucleotide production for virus replication (Lindell et al., 2007; Thompson et al., 2011). In addition, a pyrophosphohydrolase/pyrophosphatase gene (*mazG*) has been found in most myovirus genomes and is thought to play a role in virus DNA biosynthesis by degrading host DNA and providing more precursors for viral DNA replication.

*Prochlorococcus* myovirus genomes contain multiple NtcA promoter. NtcA, a 2-oxoglutarate (2OG) dependent nitrogen regulator, which is inactive when 2OG is limited. It was proposed that the cellular nitrogen content of *Prochlorococcus* decreases after viral infection and likely results in 2OG accumulation, thus leading to the expression of viral NtcA promoter involved nitrogen stress genes (Sullivan et al., 2010). It has been determined that *Prochlorococcus* virus genomes have phosphate-inducible genes (*pstS*), which may be part of a possible P-stress response mechanism (Sullivan et al., 2010). Under P-limited conditions, the host P-acquisition gene expression is down-regulated in infected cells, while the viral *pstS* gene expression in infected cells is much higher than that in uninfected cells, which might enhance host P acquisition (Zeng and Chisholm, 2012; Lin et al., 2016).

Cyanophage genome studies have shown that core genes of *Prochlorococcus* myoviruses contain six known functional genes (*psbA*, *mazG*, *phoH*, *hsp20*, *hli03*, *cobS*), phytanoyl-CoA dioxygenase genes, two structural genes, and 16 hypothetical genes (Sullivan et al., 2010). Labrie et al. (2013) showed that *Prochlorococcus* podovirus genomes contain three variable island regions, and most hypervariable genes are located in the C-terminal regions of the genomes. Metagenomic recruitment analysis with 12 cyanopodoviruses showed that podoviruses may represent more than 50% of all recruited reads in samples from the Hawaii Ocean Time-series Station (HOT) and other marine virome databases. Although high-throughput sequencing and metagenome technology provide more *Prochlorococcus* virus sequence information, the functions of many viral genes, including AMGs, are still unknown, which suggests more in-house molecular studies are needed.

Interestingly, the *Prochlorococcus* virus P-SSP7 contains an *int* gene with conserved amino acid motifs and can possibly integrate its genome into the host genome. Additionally, there is a 42 bp fragment located downstream of the *int* gene with the same sequence as part of a noncoding strand of the leucine tRNA gene in the host genome (Sullivan et al., 2005). This suggests P-SSP7 is possibly a prophage. P-SS2 has also been proposed as a possible prophage (Sullivan et al., 2009). However, no *Prochlorococcus* prophage has been found thus far. Given that *Prochlorococcus* lives in oligotrophic environments, it is generally believed that poor nutrient conditions encourage the viruses to choose a lyso-

genic life style. We thus speculate that a *Prochlorococcus* prophage may be discovered when more viruses are isolated, which will open a new window to understanding *Prochlorococcus*-virus interactions.

## 3. Diversity of *Prochlorococcus* viruses

Viruses lack universally conserved genes, leading to difficulties in investigating viral diversity (Paul et al., 2002). However, some genes are conserved in certain viral groups and can be applied to study their diversity (Rohwer and Edwards, 2002). At present, the gene markers used for diversity studies of *Prochlorococcus* viruses include the portal protein gene (*g20*), photosynthetic genes (*psbA*, *psbD*, *psaA*), the major capsid protein gene (MCP gene), and the DNA polymerase gene (DNA *pol*).

A previous study based on *g20* showed cyanomyoviruses can be grouped into four groups (I, II, III, and IV) comprising the isolates and six environmental clades (A–F) with no cultured strains (Sullivan et al., 2008). Group II sequences were found 10 times more often than group I and III sequences in the Global Ocean Survey (GOS) database. We constructed a phylogenetic tree with *g20* sequences from 32 published *Prochlorococcus* myoviruses (Figure 1). When the viral sequence number increased, the four previously established myovirus groups were more clearly differentiated (Figure 1). The six environmental clades still do not have any published isolates, despite being first identified almost 10 years ago. Groups I, II and IV contain myoviruses isolated from *Prochlorococcus*, while group III includes viruses isolated from *Synechococcus* with infectivity to *Prochlorococcus*. In addition, we found *Prochlorococcus* myoviruses in group III can be further divided into four subgroups (III-1, 2, 3, 4). It would be interesting to investigate whether all clade III myoviruses have the ability to infect *Prochlorococcus* and *Synechococcus*.

Based on *psbA* gene analysis, cyanomyoviruses can be classified into three groups, two isolated from *Prochlorococcus* and one isolated from *Synechococcus* (Sullivan et al., 2006). Our analysis of *psbA* genes within newly available *Prochlorococcus* myoviruses demonstrated a similar phylogeny (Figure 2). The cross-infecting myoviruses were located separately in the phylogenetic trees for both structural and functional genes (Figures 1 and 2), suggesting a different evolution pathway than that of the viruses infecting only one cyanobacterial group. The *psbD* gene analysis results were consistant with the *psbA* gene analysis (Sullivan et al., 2006). In addition, the *g20* gene seemingly showed a better resolution for distinguishing *Prochlorococcus* myoviruses than *psbD* and *psbA* and probably reflects a stronger adaptation of viral portal proteins to host ranges.

According to DNA *pol* gene analysis, Labrie et al. (2013) classified nine *Prochlorococcus* podoviruses into two groups (MPP-A, MPP-B) and an outgroup (P-RSP2), which was supported by later studies with more sequences from isolates, PCR amplicons and metagenomics (Dekel-Bird et al., 2013; Huang et al., 2015). The MPP-B group appears to consist of *Prochlorococcus* and *Synechococcus* viruses containing *psbA*, while the MPP-A group does not. Most of the cyanopodophages are clustered into the MPP-B group, which contains more subgroups than the MPP-A group; this indicates MPP-B podoviruses are more common and have a greater diversity than MPP-A podoviruses in the enviroment (Dekel-Bird et al., 2013). In addition, the PCR-amplified viral *psaA* gene revealed cyanophages could be classified into six subgroups by their similarity and G+C% content (Hevroni et al., 2015).

In summary, the study of *Prochlorococcus* virus diversity has mainly focused on isolated viruses, with few PCR amplicon and metagenome data. Our knowledge about the diversity of *Prochlorococcus* viruses in the global ocean is very limited, considering the wide and dynamic distribution of their hosts. The spatial and temporal dynamics of these viruses remain unknown and require large-scale and long-term ecological investigation. Recently, single cell analysis revealed very high cell-to-cell diversity and significant spatial variation in the *Prochlorococcus* genome structure, and these characteristics were tightly related to local environmental conditions, such as nutrients, light, and temperature (Kashtan et al., 2014, 2017; Kent et al., 2016). As obligate parasites, viruses have frequent genetic exchanges with their hosts and therefore may have similar genetic diversification. We expect that newly developed techniques with high genetic resolution, such as microfluidic digital PCR, single virus sorting and genomics, and viral tagging (Allen et al., 2011; Brum and Sullivan, 2015), will help us to elucidate the fine-scale diversity of *Prochlorococcus* viruses.

## 4. The potential biogeochemical role of *Prochlorococcus* viruses

Viruses play an important role in the marine biogeochemical cycle by affecting their host's physiology and ecology. As a key player in the oligotrophic ocean, *Prochlorococcus* contributes an estimated significant part (30–60%) of the total chlorophyll-*a* in the subtropical surface ocean (Partensky and Garczarek, 2010). The fate of this high amount of carbon fixed by *Prochlorococcus* has a significant biogeochemical impact on global carbon cycling. Generally, the *Prochlorococcus* mortalities induced by viral lysis and grazing are similar at low and middle latitudes in the North Atlantic Ocean, with an average rate of 0.14 $d^{-1}$ (range 0.02–0.57 $d^{-1}$) (Mojica et al., 2016). One study from the deep chlorophyll maximum layer in the subtropical Atlantic Ocean showed the


**Figure 1** *Prochlorococcus* myophage neighbor-joining phylogenetic tree based on *g20* amino acid sequences. The *g20* sequence from the phage T4 was used as an outgroup to root this tree. Support values shown at the nodes are neighbor-joining bootstrap values (only values >60 are shown). Group names follow Sullivan et al. (2008). Scale bar, 0.1 substitutions per amino acid position.

virus-induced *Prochlorococcus* mortality rate was $0.02\pm0.03$ $d^{-1}$, accounting for a removal of 3% of the total biomass (Baudoux et al., 2007). In the southern California Current Ecosystem, virus-mediated *Prochlorococcus* mortality rates are up to 0.25 $d^{-1}$ (Pasulka et al., 2015). The viral lysis of *Prochlorococcus* was shown to be related to the growth rate of hosts (Baudoux et al., 2007; Pasulka et al., 2015). In the North Atlantic Ocean, a pattern of reduced viral lysis rates with latitude and a positive relationship between temperature and the viral lysis rate were observed (Mojica et al., 2016), which suggested that the contribution of virus-mediated cell loss to *Prochlorococcus* mortality is likely to increase in the future. According to these limited field data, it was estimated that virus-mediated mortality is responsible for 1% to 60% of *Prochlorococcus* cell loss, with large regional variation. In addition, it should be noted that all of the published virus-mediated *Prochlorococcus* mortality rates have been measured by dilution experiments, which were originally developed to estimate microzooplankton grazing on phytoplankton. The application of the dilution method for studying the viral lysis of *Prochlorococcus* is still questionable owing to the sensitivity of *Prochlorococcus* growth

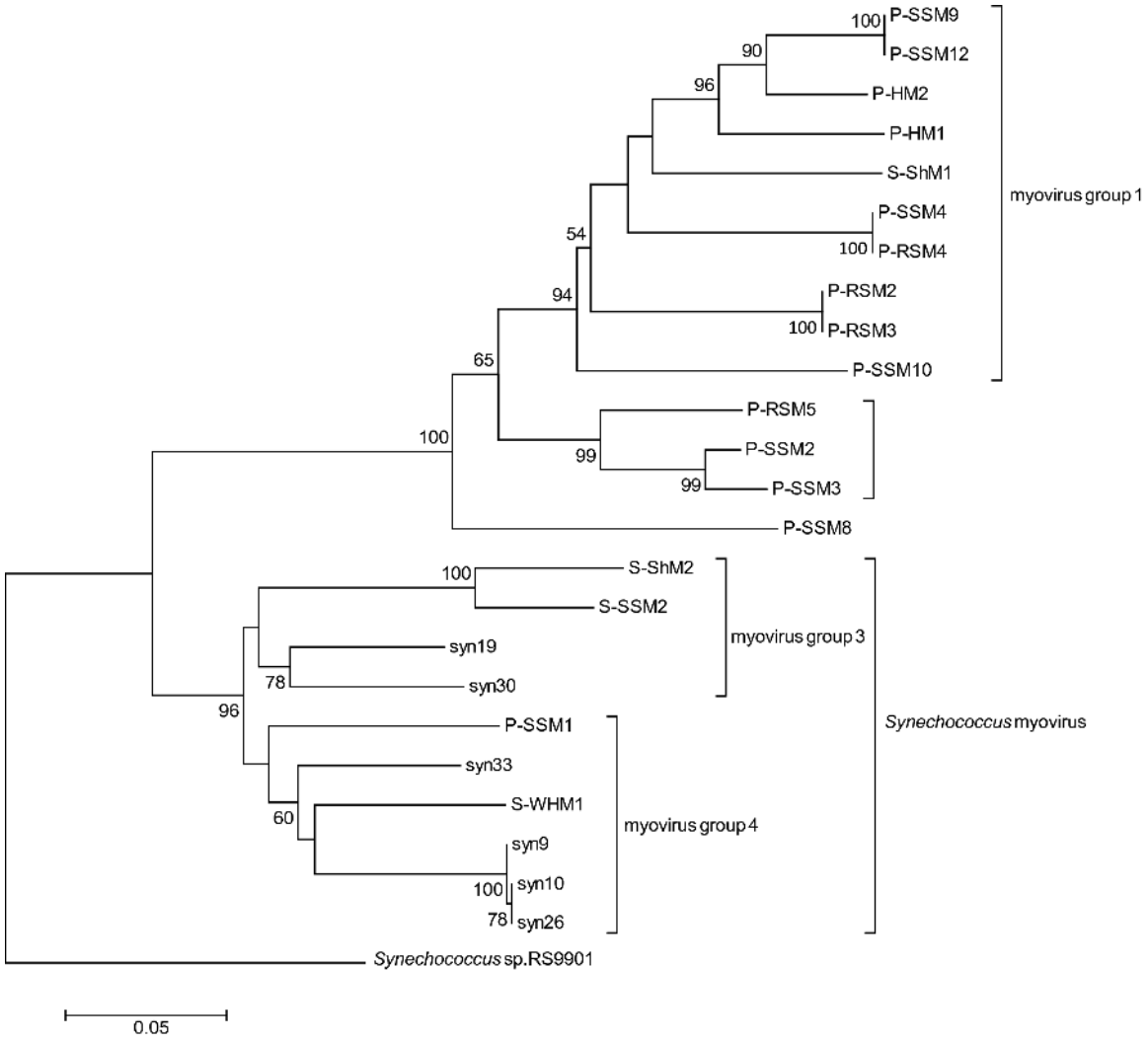


**Figure 2** Maximum likelihood phylogenetic analysis of *psbA* gene sequences from *Prochlorococcus* myophages. The *Synechococcus* RS9901 *psbA* gene sequence was used as an outgroup to root the tree. Bootstrap percentages (values >50) are shown at the nodes. Scale bar, 0.1 substitutions per nucleotide position.

to environmental changes during dilution.

Although the virus-induced mortality rate is not high in *Prochlorococcus* compared with those in other phytoplankton and bacterioplankton, the potential biogeochemical significance of the viral lysis of *Prochlorococcus* should not be ignored because of the wide distribution and large population size of *Prochlorococcus*. Based on an estimated annual abundance of $2.9\pm0.1\times10^{27}$ *Prochlorococcus* cells and celluar carbon quotas ranging from 20 to 60 fg under different nutrient conditions (Bertilsson et al., 2003; Heldal et al., 2003), we estimate 0.6–104.4 Tg C $yr^{-1}$ is released to the environment by the viral lysis of *Prochlorococcus*. Dissolved organic matter (DOM) released by lysis contributes significantly to the marine carbon pool and plays a vital role in the marine biogeochemical cycle (Gobler et al., 1997; Middelboe and Jørgensen, 2006). Recently, Zhao et al. (2017) showed that marine cyanobacteria, including *Prochlorococcus*, release large amounts of fluorescent DOM (FDOM) after viral lysis, which share a similar fluorescence pattern and humic-like composition with deep seawater. This suggests some *Prochlorococcus* lysates may accumulate in the deep ocean, contributing to the marine carbon pool through the microbial carbon pump (Jiao et al., 2010). In addition, during viral infection, the expression of AMGs may regulate host metabolism and impact the associated biogeochemical cycling. For example, Puxty et al. (2016) showed that viral infections could inhibit 20–50% of cyanobacterial $CO_2$ fixation during the latent period and that the reduction in $CO_2$ fixation was estimated to be 0.02–5.39 Pg $yr^{-1}$.

## 5. Perspective

*Prochlorococcus*, the smallest free-living autotrophic organism, is the major primary producer in the oligotrophic ocean. Viruses control the abundance, activity, diversity and community structure of *Prochlorococcus*. However, knowl-

edge about *Prochlorococcus* viruses remains scarce, which limits our understanding of the ecological and biogeochemical roles of *Prochlorococcus* in the ocean. To address this knowledge gap, we propose several areas for future studies:

(1) Isolation of *Prochlorococcus* viruses. At present, the isolation of *Prochlorococcus* viruses is limited to specific hosts (e.g., MED4, NATL1A, and NATL2A) and certain areas (e.g., the Sargasso Sea, the Red Sea), and the isolated viruses and are mainly myoviruses and podoviruses. Metagenome analysis indicates that many types of *Prochlorococcus* viruses do not have cultured strains. To have a more complete view of the biology and ecology of *Prochlorococcus* viruses, it is necessary to isolate viruses infecting more *Prochlorococcus* ecotypes from different ocean regions.

(2) *Prochlorococcus*-virus interactions. Due to the nature of parasitism, the virus activities mainly rely on host-virus interactions. The investigation of interactions between viruses and *Prochlorococcus* is of primary importance for understanding the biological and ecological processes involved. However, information about *Prochlorococcus*-virus interactions is very limited; thus, these interactions should be investigated at genetic, cellular, population and ecosystem levels.

(3) Biogeochemical significance of *Prochlorococcus* viruses. As an important primary producer, *Prochlorococcus* contributes significantly to marine carbon cycling. It is unclear how viruses impact the biogeochemical contribution of *Prochlorococcus* via their interactions at genetic, cellular, population and ecosystem levels. This subject may require the multidisciplinary integration of experimental data, field observations and modeling. In addition, the distribution of *Prochlorococcus* has been projected to extend to higher latitudes in the warming ocean, and the biogeographic patterns of viruses are also tightly coupled with climate and environmental changes. The simulation and prediction of *Prochlorococcus* viruses in the future ocean are of great importance for our understanding of the impacts of climate change on marine ecosystems and biogeochemical cycles.

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