





Genome Sequences of *Synechococcus* sp. Strain MIT S9220 and Cocultured Cyanophage SynMITS9220M01

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ABSTRACT *Synechococcus* bacteria are unicellular cyanobacteria that contribute significantly to global marine primary production. We report the nearly complete genome sequence of *Synechococcus* sp. strain MIT S9220, which lacks the nitrate utilization genes present in most marine *Synechococcus* genomes. Assembly also produced the complete genome sequence of a cyanophage present in the MIT S9220 culture.

Marine *Synechococcus* bacteria are globally distributed picocyanobacteria that contribute to ~17% of annual net marine primary production (1). The genome of *Synechococcus* sp. strain MIT S9220, reported here, adds to the existing six isolate genomes from the CRD1 ecotype, which exhibits physiological and genomic adaptations to its low-Fe niche (2, 3). MIT S9220 was isolated from the equatorial Pacific (4) and cannot grow on nitrate, in contrast to most other marine *Synechococcus* species (4, 5).

DNA was extracted with a phenol-chloroform protocol (6, 7) from a late-exponential-phase, nonaxenic culture of MIT S9220 (obtained from Gabrielle Rocab), propagated in Pro99 seawater-based medium (8) at 23°C with 14:10-h light/dark illumination. Illumina sequencing (Nextera library kit; NextSeq 550) yielded 20,222,448 paired 150-bp reads, and Oxford Nanopore Minlon sequencing (library kit SQK-RAD004; R9.4.1 flow cell) produced 53,387 reads (60 Mb; N_{50} , 1,727 bp). The Illumina reads were quality filtered with Trimmomatic v. 0.38 (9) with the following settings: ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10, LEADING:10, TRAILING:10, SLIDINGWINDOW:4:15, and MINLEN:50. Assembly with Unicycler v. 0.4.7 (10) with default settings using both Illumina and Nanopore reads yielded 13 contigs, 12 of which belong to MIT S9220 (2,406,645 total bp; N_{50} , 1,568,415 bp; GC content, 56.4%; 300× mean coverage) based on high similarity to other CRD1 contigs (blastn; E value, <1e-84; identity, ≥88%). The MIT S9220 genome was annotated with the Prokaryotic Genome Annotation Pipeline (PGAP) v. 4.11 (11), and it contains 2,575 protein-coding genes, 42 tRNAs, and 2 rRNA operons. We estimate that the genome is >99% complete based on it possessing 1,026 homologs (reciprocal best hits; blastp; E value, <1e-10) of 1,033 single-copy core genes shared among *Synechococcus* isolate genomes (3, 12). Like other CRD1 genomes, MIT S9220 possesses a larger repertoire of Fe-related genes than most other *Synechococcus* genomes (3).

MIT S9220 lacks the nitrate utilization genes found in most other marine *Synechococcus* species (Fig. 1) (13). A similar, parallel loss (3) occurs in *Synechococcus* sp. RS9917 (ecotype VIII) (Fig. 1) (14). MIT S9220 provides a valuable resource for further exploring the evolution of nitrogen utilization in these important phytoplankton, especially given similar loss and occasional reacquisition of these genes in closely related *Prochlorococcus* species (15, 16).

The remaining circular, 190,237-bp contig (38.8% GC content, 150× coverage),

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REFERENCES

- Flombaum P, Gallegos JL, Gordillo RA, Rincón J, Zabala LL, Jiao N, Karl DM, Li WKW, Lomas MW, Veneziano D, Vera CS, Vrugt JA, Martiny AC. 2013. Present and future global distributions of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proc Natl Acad Sci U S A* 110:9824–9829. <https://doi.org/10.1073/pnas.1307701110>.
- Sohm JA, Ahlgren NA, Thomson ZJ, Williams C, Moffett JW, Saito MA, Webb EA, Rocap G. 2016. Co-occurring *Synechococcus* ecotypes occupy four major oceanic regimes defined by temperature, macronutrients and iron. *ISME J* 10:333–345. <https://doi.org/10.1038/ismej.2015.115>.
- Ahlgren NA, Belisle BS, Lee MD. 2020. Genomic mosaicism underlies the adaptation of marine *Synechococcus* ecotypes to distinct oceanic iron niches. *Environ Microbiol* 22:1801–1815. <https://doi.org/10.1111/1462-2920.14893>.
- Moore LR, Post AF, Rocap G, Chisholm SW. 2002. Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnol Oceanogr* 47:989–996. <https://doi.org/10.4319/lo.2002.47.4.0989>.
- Fuller NJ, Marie D, Partensky F, Vault D, Post AF, Scanlan DJ. 2003. 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* 69:2430–2443. <https://doi.org/10.1128/aem.69.5.2430-2443.2003>.
- Green MR, Sambrook J. 2017. Isolation of high-molecular-weight DNA using organic solvent. *Cold Spring Harb Protoc* 2017:pdb.prot093450. <https://doi.org/10.1101/pdb.prot093450>.
- Wurch L, Giannone RJ, Belisle BS, Swift C, Utturkar S, Hettich RL, Reyssenbach AL, Podar M. 2016. Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment. *Nat Commun* 7:12115. <https://doi.org/10.1038/ncomms12115>.
- Moore LR, Coe A, Zinser ER, Saito MA, Sullivan MB, Lindell D, Frois-Moniz K, Waterbury J, Chisholm SW. 2007. Culturing the marine cyanobacterium *Prochlorococcus*. *Limnol Oceanogr Methods* 5:353–362. <https://doi.org/10.4319/lom.2007.5.353>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Lee MD, Ahlgren NA, Kling JD, Walworth NG, Rocap G, Saito MA, Hutchins DA, Webb EA. 2019. Marine *Synechococcus* isolates representing globally abundant genomic lineages demonstrate a unique evolutionary path of genome reduction without a decrease in GC content. *Environ Microbiol* 21:1677–1686. <https://doi.org/10.1111/1462-2920.14552>.
- Moreno-Vivian C, Cabello P, Martinez-Luque M, Blasco R, Castillo F. 1999. Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. *J Bacteriol* 181:6573–6584. <https://doi.org/10.1128/JB.181.21.6573-6584.1999>.
- Dufresne A, Ostrowski M, Scanlan D, Garczarek L, Mazard S, Palenik B, Paulsen I, de Marsac N, Wincker P, Dossat C, Ferriera S, Johnson J, Post A, Hess W, Partensky F. 2008. Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. *Genome Biol* 9:R90. <https://doi.org/10.1186/gb-2008-9-5-r90>.
- Berube PM, Biller SJ, Kent AG, Berta-Thompson JW, Roggensack SE, Roache-Johnson KH, Ackerman M, Moore LR, Meisel JD, Sher D, Thompson LR, Campbell L, Martiny AC, Chisholm SW. 2015. Physiology and evolution of nitrate acquisition in *Prochlorococcus*. *ISME J* 9:1195–1207. <https://doi.org/10.1038/ismej.2014.211>.
- Berube PM, Rasmussen A, Braakman R, Stepanauskas R, Chisholm SW. 2019. Emergence of trait variability through the lens of nitrogen assimilation in *Prochlorococcus*. *Elife* 8:e41043. <https://doi.org/10.7554/eLife.41043>.
- Sullivan MB, Huang KH, Ignacio-Espinoza JC, Berlin AM, Kelly L, Weigele PR, DeFrancesco AS, Kern SE, Thompson LR, Young S, Yandava C, Fu R, Krastins B, Chase M, Sarracino D, Osburne MS, Henn MR, Chisholm SW. 2010. Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. *Environ Microbiol* 12:3035–3056. <https://doi.org/10.1111/j.1462-2920.2010.02280.x>.
- Mann NH, Clokie MR, Millard A, Cook A, Wilson WH, Wheatley PJ, Letarov A, Krisch HM. 2005. The genome of S-PM2, a “photosynthetic” T4-type bacteriophage that infects marine *Synechococcus* strains. *J Bacteriol* 187:3188–3200. <https://doi.org/10.1128/JB.187.9.3188-3200.2005>.
- Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW. 2005. Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. *PLoS Biol* 3:e144. <https://doi.org/10.1371/journal.pbio.0030144>.
- Kelly L, Ding H, Huang KH, Osburne MS, Chisholm SW. 2013. Genetic diversity in cultured and wild marine cyanomyoviruses reveals phosphorus stress as a strong selective agent. *ISME J* 7:1827–1841. <https://doi.org/10.1038/ismej.2013.58>.
- Lindell D, Sullivan MB, Johnson ZI, Tolonen AC, Rohwer F, Chisholm SW. 2004. Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. *Proc Natl Acad Sci U S A* 101:11013–11018. <https://doi.org/10.1073/pnas.0401526101>.
- Puxty RJ, Perez-Sepulveda B, Rihtman B, Evans DJ, Millard AD, Scanlan DJ. 2015. Spontaneous deletion of an “ORFanage” region facilitates host adaptation in a “photosynthetic” cyanophage. *PLoS One* 10:e0132642. <https://doi.org/10.1371/journal.pone.0132642>.
- Thompson LR, Zeng Q, Kelly L, Huang KH, Singer AU, Stubbe J, Chisholm SW. 2011. Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. *Proc Natl Acad Sci U S A* 108:E757–E764. <https://doi.org/10.1073/pnas.1102164108>.
- Bryan MJ, Burroughs NJ, Spence EM, Clokie MR, Mann NH, Bryan SJ. 2008. Evidence for the intense exchange of MazG in marine cyanophages by horizontal gene transfer. *PLoS One* 3:e2048. <https://doi.org/10.1371/journal.pone.0002048>.
- Clokie MR, Mann NH. 2006. Marine cyanophages and light. *Environ Microbiol* 8:2074–2082. <https://doi.org/10.1111/j.1462-2920.2006.01171.x>.
- Kneidinger B, Graninger M, Puchberger M, Kosma P, Messner P. 2001. Biosynthesis of nucleotide-activated D-glycero-D-manno-heptose. *J Biol Chem* 276:20935–20944. <https://doi.org/10.1074/jbc.M100378200>.
- Gregory AC, Solonenko SA, Ignacio-Espinoza JC, LaButti K, Copeland A, Sudek S, Maitland A, Chittick L, Dos Santos F, Weitz JS, Worden AZ, Woyke T, Sullivan MB. 2016. Genomic differentiation among wild cyanophages despite widespread horizontal gene transfer. *BMC Genomics* 17:930. <https://doi.org/10.1186/s12864-016-3286-x>.