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Precipitation of calcium carbonate mineral induced by viral lysis of cyanobacteria: evidence from laboratory experiments

Hengchao Xu et al.

Correspondence to: Xiaotong Peng (xtpeng@idsse.ac.cn)

Methods

Synechococcus sp. PCC 7177

Cyanobacteria was isolated from the surface seawater from Sanya Bay following the methods of Waterbury (2006). Briefly, 1.0 mL of the seawater sample was inoculated to enrich cyanobacteria in 250 ml conical flasks, containing 100 mL of F/2 medium. The flasks were incubated at 25℃ under a photon irradiance of 6000 lux, with a 12 h light/dark cycle. Adequate growth was observed after 20 d; then, the culture was examined under a microscope (Leica DM6B) to determine the presence of cyanobacteria by autofluorescence. A 1.0 ml aliquot of the enrichment culture was added to 9.0 ml of sterile water and further diluted by 10^4-fold. From each dilution, 100 μL was spread onto F/2 plates. Individual colonies were isolated after 15-20 d and checked for morphology. One of the coccoid to rod-shaped cyanobacteria was recognized as the *Synechococcus* group and was further purified three times on plates. The *Synechococcus* group was selected for calcification because it is very widespread in the marine environment. Isolated cells are coccoid-shaped and ~1.3 μm in diameter. 16S rRNA genes of the strain were amplified by PCR using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR products were sequenced by the Beijing Liuhehuada Biological Service in China. 16s rDNA sequence analysis shows that the strain is *Synechococcus* sp. PCC 7177.

Viruses that infect *Synechococcus* sp. PCC 7177

Viruses that infect cyanobacteria were isolated using the double-layer agar technique with methods modified from Millard (2009). Water samples were collected from Sanya Bay and filtered immediately through a 0.2 μm filter to remove any bacteria and were kept in the dark at 4 ℃.

25-30 ml of fresh exponentially growing host cells were harvested by centrifugation at 6000 × g for 15 min at 25 ℃. Cell pellets were resuspended with 500 μL growth medium by pipetting. Aliquots of filtered seawater samples were added to the cell awaiting adsorption at 25 ℃ in constant light for 1 h. Cells were gently mixed with cooled molten 0.4% (w/v) agar. This mixture were poured onto the top of a solid 1% agar plate. The agar was allowed to set for 1 h before inverting the plate and placing it in an incubator. A single plaque was picked and resuspended in 1 ml of fresh SM buffer (Sinopharm chemical reagent Co., Ltd) for at least 1 h at 4 ℃ in the dark to allow the viruses

to diffuse into the fresh medium to form a lysate. A dilution series of the lysate was made in a second round of double-layer agar processes. This process has to be repeated 3 times to ensure that a clonal virus has been isolated.

Viruses were recovered from liquid lysates and concentrated by iron chloride precipitation following the methods from John et al., (2011). Briefly, Approximately 2 L of liquid culture medium was filtered followed by a 0.22 µm pore size polycarbonate membrane, 142 mm diameter filter, then 2 ml of 10 g/L Fe stock solution were added for viruses concentration, the $FeCl_3$ treated filtrate was filtered by 1.0 µm, 142 mm polycarbonate membrane. The virus concentrates were resuspended with the fresh 0.1 M EDTA-0.2 M $MgCl_2$ Buffer, 10 % solid PEG 8000, and NaCl was added to a final concentration of 1M (Thurber et al., 2009). Resuspension took overnight under the 4 degree fridge with no light. After resuspension, the virus concentrates were centrifuged for 30 min at 13,000 g and 4 degree to pellet the viruses, then discard the supernatant and resuspended the pellet in TE buffer. Virus DNA was extracted followed by the method of Wizard Columns. Briefly, Mix 1 ml Wizard DNA Purification Resin (Promega #A7181) with 0.5 ml virus sample, the resin with virus sample was loaded to Wizard Mini Columns (Promega #A7211) with 3 ml sterile syringe. 2 ml of 80 % isopropanol were used to wash the resin, and 100 µl 80 degree ultrapure water was used for elute viral DNA. Viral metagenomic libraries were constructed for Illumina Hiseq 4000 platform sequencing (paired-end 150 bp reads) at Novogene (Tianjing, China). Metagenomic libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's protocols. A total of 10 Gb raw sequence data was generated. Raw data were first processed by Trimmomatic 0.36 for adapter removal and moderate quality trimming to obtain "clean" data for subsequent data analysis (Bolger et al., 2014). Reads were assembled to contigs using metaSPAdes from SPAdes v3.10 (Bankevich et al., 2017). The contigs more than 5 kb were applied to ran the Virsorter (Roux et al., 2015), the viral contigs (categorise 1 and 2) were retained for next analysis. The sequence coverage of each contig (%) from viral lysates was calculated by Bowtie 2 (Langmead and Salzberg, 2012), SAMtools (Li et al., 2009). Note that contig_1 has the highest coverage with a majority (~85%) of the reads being assigned to it. Then we used vConTACT (Bolduc et al., 2017) to identified and classified this contig.

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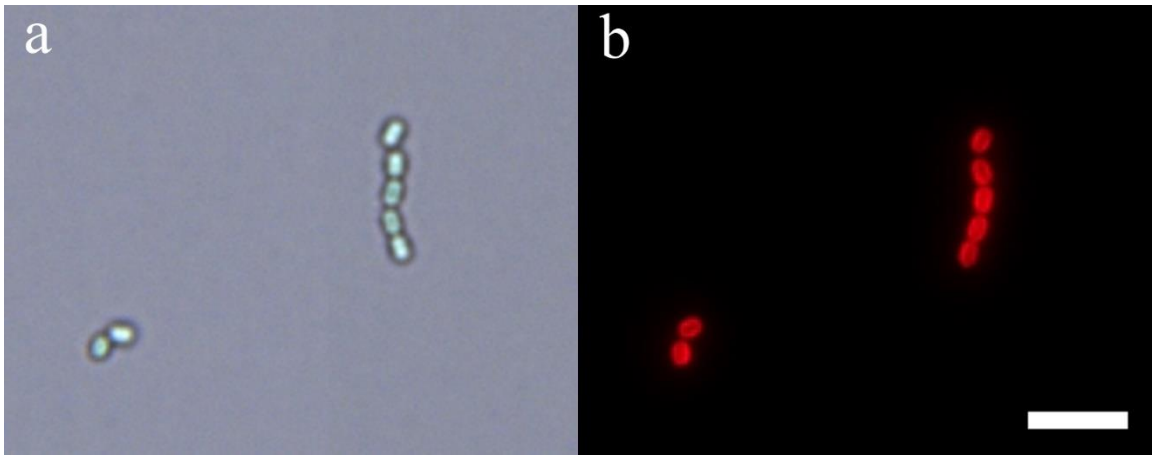

Figure S1: Strain of *Synechococcus* spp. isolated from Sanya Bay. Scale bar = 10 μm.

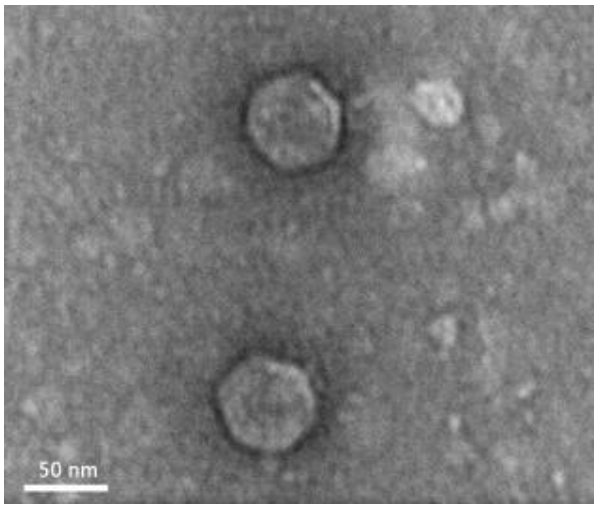

Figure S2: Virus isolated from Sanya Bay.

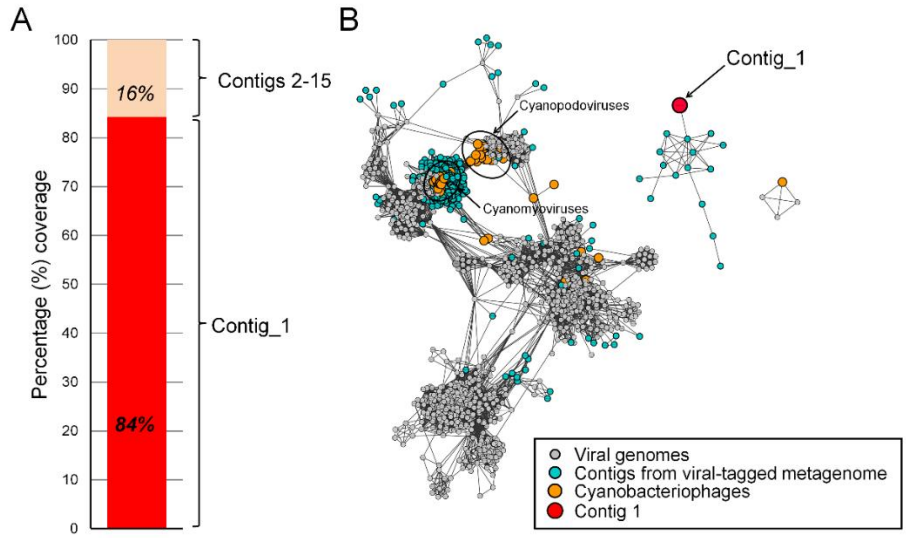

Figure S3. Identification and characterization of lysate-associated viral genomic sequences. (A) The sequence coverage of each contig (%) from viral lysates was calculated by Bowtie 2, SAMtools and BamM. Note that contig_1 has the highest coverage with a majority (~85%) of the reads being assigned to it. (B) Left, a weighted gene-sharing network was built to characterize the relationships of contig_1 to other viruses, in which nodes represent viral genomes/contigs and edges represent the similarity between genomes/contigs based on the number of shared gene (i.e., homologous protein clusters, PCs). As the reference, a total of 2,010 bacterial and archaeal virus genomes (VirRefSeq v75) as well as Synechococcus-specific viral contigs retrieved from viral-tagging (VT) data (Deng et al., 2014) were used. For clarity, only 44,948 edges from 1,442 viral genomes/contigs are shown. Right, matrix view shows a list of clustered proteins (PCs) of contig_1, which are limited to those of cyanophages. A red-colored cell indicates the presence of homologs across the contig_1, cyanophages, and/or *Synechococcus*-specific contigs from VT data.

Time	salinity		Mg				Ca				Mg/Ca		Bacteria				Virus	TA				DIC	
d	%		mg/L	error	mg/L	error	mg/L	error	mg/L	error	atomic ratio		number/ml	error	number/ml	error	number/ml	uM/KG	error	uM/KG	error	uM/KG	uM/KG
	C	C+P	C	C	C+P	C+P	C	C	C+P	C+P	C	C+P	C	C	C+P	C+P	C+P	C	C	C+P	C+P	C	C+P
1	27.68	27.45	ND	ND	1132.09	2.59	ND		319.90	1.23		5.90	3.06E+05	1.28E+07	5.07E+05	1.31E+06	/	3828.66	8.91	3866.33	17.21	2101.38	2062.20
2	28.14	27.45	1143.79	3.31	1143.91	3.76	321.59	1.40	323.78	2.09	5.93	5.89	3.85E+05	6.77E+05	5.52E+05	3.60E+06	/	3767.55	26.10	3830.81	8.11	ND	ND
3	27.60	27.52	1134.11	2.64	ND	2.42	322.78	2.00	ND		5.86		6.08E+05	2.59E+06	1.25E+06	2.41E+06	/	3820.79	30.32	3724.66	13.05	2014.60	2053.91
4	27.83	27.75	1137.09	0.00	1126.30	1.46	320.27	1.01	318.93	1.35	5.92	5.89	9.80E+05	2.07E+06	4.47E+06	7.10E+06	/	3845.98	22.90	3828.40	24.80	ND	ND
5	27.37	27.22	1143.84	0.85	1141.46	1.85	321.95	0.39	324.07	0.72	5.92	5.87	1.99E+06	1.27E+07	1.10E+07	8.54E+06	/	3896.67	37.31	3841.96	24.56	ND	ND
6	27.29	27.22	1143.84	0.85	1125.75	3.01	324.23	0.79	319.70	2.07	5.88	5.87	8.04E+06	1.51E+07	2.36E+07	1.28E+07	9.94E+06	3950.84	32.59	4059.58	49.63	1922.16	1891.77
7	27.29	27.22	1134.74	3.73	1128.37	3.20	322.92	1.96	319.90	1.65	5.86	5.88	2.39E+07	7.90E+06	2.81E+07	1.05E+07	ND	4031.05	52.23	4010.64	44.07	ND	ND
8	27.45	27.29	1144.22	2.17	1139.71	1.47	325.90	1.06	323.82	0.92	5.85	5.87	3.55E+07	5.01E+07	3.34E+07	2.75E+07	1.45E+07	4122.63	40.32	3664.81	34.40	1602.91	1467.02
9	27.52	27.60	1127.19	1.09	1087.77	2.66	303.63	1.22	306.45	0.60	6.19	5.92	4.30E+07	2.49E+07	2.52E+07	2.85E+07	3.92E+07	3747.68	38.70	2811.36	88.07	ND	ND
10	27.60	27.52	1075.69	3.66	1060.40	1.49	310.62	0.41	303.65	0.63	5.77	5.82	7.50E+07	9.42E+06	3.61E+07	3.52E+07	3.42E+07	1851.05	50.40	2286.03	86.14	1003.85	945.82
12	27.52	27.45	1090.97	1.04	1061.64	2.23	318.51	0.84	302.28	0.97	5.71	5.85	1.03E+08	4.21E+06	3.97E+07	2.61E+06	1.28E+07	1451.85	47.20	2139.96	69.90	819.35	626.45
14	27.45	27.29	1095.33	0.64	1074.11	3.34	320.61	0.83	291.88	1.17	5.69	6.13	1.30E+08	1.20E+07	2.82E+07	2.50E+06	5.82E+06	1251.92	59.44	2936.08	133.63	473.23	1006.99
16	27.52	27.45	1092.76	1.85	1125.69	1.81	318.31	0.99	291.17	0.73	5.72	6.44	1.40E+08	6.23E+06	2.28E+07	3.12E+06	5.23E+06	1401.50	97.72	2807.01	85.41	582.99	1402.72
19	27.52	27.68	1102.16	3.79	1132.81	3.42	321.16	1.75	291.60	1.52	5.72	6.47	1.35E+08	3.12E+06	1.76E+07	9.12E+05	7.26E+06	1780.46	67.10	3012.41	142.34	719.01	1904.46

Table S1: Experimental conditions of experiments performed in this study. C represent the treatment without virus. C+P represents the treatment inoculated with virus.