The ISME Journal (2012) 6, 1617–1620

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SHORT COMMUNICATION

Viral clones from the GOS expedition with an unusual photosystem-I gene cassette organization

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Cyanobacteria have a key role in marine photosynthesis, which contributes to the global carbon cycle and to the world oxygen supply. Genes encoding for photosystem-II (PSII) and photosystem-I (PSI) reaction centers are found in different cyanophage genomes, and it was suggested that the horizontal transfer of these genes might be involved in increasing phage fitness. We have further analyzed a rare viral Global Ocean Sampling (GOS) clone containing PSI genes. This clone contains the unusual PSI gene organization *psaD->C->A*, as opposed to the more frequently observed viral *psaJF->C->A->B->K->E->D* organization, and was detected only once in the GOS metagenome. Our analyses identified more occurrences with similar arrangement and indicate that this PSI viral gene organization (now *psaD->C->A->B*), although rare, is authentic and represents a new PSI gene arrangement.
The ISME Journal (2012) **6,** 1617–1620; doi:10.1038/ismej.2012.23; published online 29 March 2012
Subject Category: integrated genomics and postenomics approaches in microbial ecology
Keywords: photosystem I; phage; cyanobacteria; *Synechococcus*; *Prochlorococcus*

Marine cyanobacteria of the *Synechococcus* and *Prochlorococcus* genera account together for about 25% of global photosynthesis (Li *et al.*, 1993; Liu *et al.*, 1997; Partensky *et al.*, 1999). Several of their cyanophages, from the myo- and podo-viruses families, carry photosynthetic genes, and it was suggested that these genes increase phage fitness (Mann *et al.*, 2003; Lindell *et al.*, 2004, 2005; Millard *et al.*, 2004; Sullivan *et al.*, 2005; Sharon *et al.*, 2007; Dammeyer *et al.*, 2008). Cyanobacterial photosynthetic membranes contain two photosystems, of which PSII mediates the transfer of electrons from water, the initial electron donor, to the plastoquinone pool whereas PSI mediates electron transfer from plastocyanin to ferredoxin, thereby generating reducing power needed for CO_2 fixation in the form of nicotinamide adenine dinucleotide phosphate oxidase. Although PSII is known to be sensitive to photodamage, PSI is considered to be more\able than PSII.

The PSII gene *psbA* coding for the labile D1 protein is readily detected in various cultured and environmental myoviruses and podoviruses infecting *Prochlorococcus* and *Synechococcus* (Mann *et al.*, 2003; Lindell *et al.*, 2004; Zeidner *et al.*, 2005; Sullivan *et al.*, 2006; Sharon *et al.*, 2007). In myoviruses, genes encoding the PSII D2 protein as well as different genes of the electron transport chain are also found (Mann *et al.*, 2003; Lindell *et al.*, 2004; Millard *et al.*, 2004; Sullivan *et al.*, 2005, 2006; Alperovitch *et al.*, 2011; Philosof *et al.*, 2011; Sharon *et al.*, 2011).

Recently, PSI gene cassettes containing whole gene suites (*psaJF, C, A, B, K, E and D*) sufficient to build a monomeric PSI were reported to exist in marine cyanophages from the Pacific and Indian Oceans (Sharon *et al.*, 2009; Alperovitch *et al.*, 2011). This was observed using both the GOS data set (Rusch *et al.*, 2007) and the viral marine biome from the Pacific Line Islands (Dinsdale *et al.*, 2008a, 2008b). These viral PSI gene cassettes were observed in the Pacific and the Indian Oceans. The main gene organization observed was *psaJF->C->A->B->K->E->D* and could be observed in several GOS stations (from the Pacific and Indian Oceans) and in the marine viral biome (from the Pacific Line islands). However, a different GOS arrangement, *psaD->C->A,* was detected only once (clone JCVI_TMPL_1061008099984 (hereafter 9984) in the Pacific open ocean GOS station GS047) and could not be confirmed with the different available viral 454 pyrosequenced biome data sets (Sharon *et al.*, 2009). In addition, this clone's PsaD, PsaC or PsaA possessed long branch topologies in phylogenetic trees as compared with other viral or cyanobacterial

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Received 9 January 2012; revised 16 February 2012; accepted 17 February 2012; published online 29 March 2012

PSI proteins (Sharon *et al.*, 2009). A better understanding of different viral photosynthesis genes and their genomic organizations might help explaining the 'viral photosynthesis' phenomenon (Rohwer and Thurber, 2009).

In order to check whether the rare clone *psaD->C->A* is an authentic event and not a chimeral incident, we checked its %G+C content. As could be seen in the PsaA phylogenetic tree (Figure 1), the clone *psaA* gene's 50% G+C is clearly distinct from its *Synechococcus* tree neighbors 55–60% G+C or from the distant *Prochlorococcus* counterparts (38–42% G+C). Moreover, when %G+C content is checked in the entire *psaD->C->A* cassette,

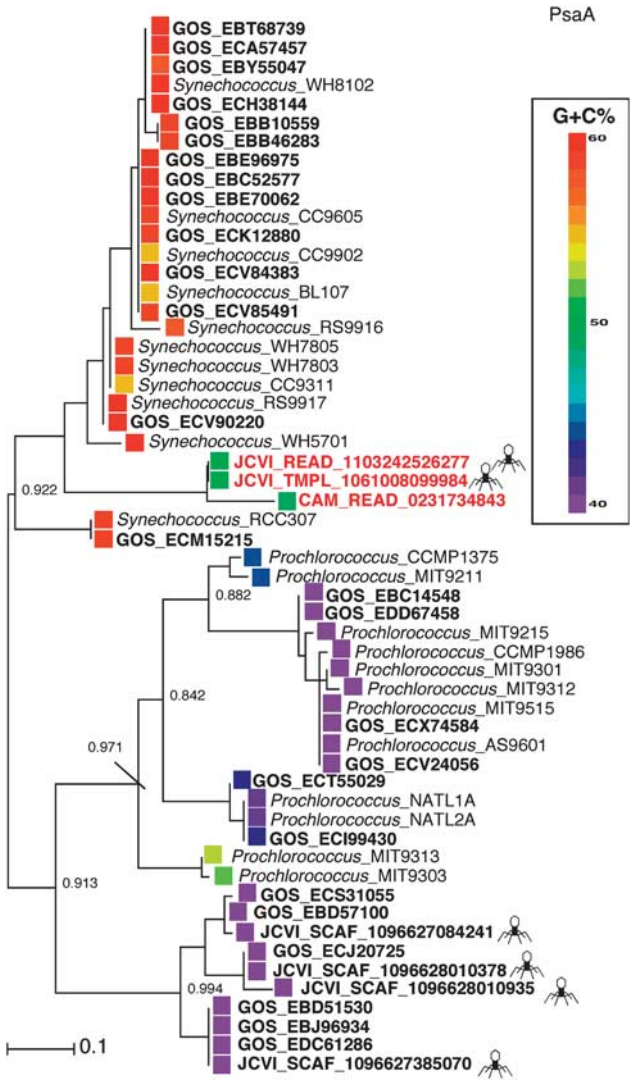

Figure 1 The relationship between *Synechococcus*, *Prochlorococcus* and their GOS phage PsaA proteins and DNA sequences. Following alignment computation (using MUSCLE (Edgar, 2004)), PhyML version 3.0 (Guindon *et al.*, 2009) was used for the calculation of the phylogenetic tree. Sequences from the GOS expedition are shown in bold. For clarity, the tree shows only a subset of the 583 partial PsaA sequences found in the GOS data set. A phage symbol is attached to each GOS sequence identified as also containing structural viral genes. PsaA protein sequences could be found in Supplementary File S1.

similar intermediate %G+C is observed in all three PSI genes (Figure 2). This is clearly distinct from the low ~40–44% G+C observed with the other viral PSI cassettes. Clone 9984 contains the viral neck protein gp13 in the vicinity of the PSI proteins. A phylogenetic protein tree of the gp13 neck protein place clone 9984 gp13 close to myocyanophages (Supplementary Figure S1). In addition, it carries a viral hypothetical gene, which is found in the myocyanophage P-SSM4.

We searched for the *psaD->C->A* gene rearrangements in different publicly available sets in the CAMERA server ((Sun *et al.*, 2011) Database updated: 12 July 2011). Using blastN search and CAMERA default parameters (e-value 1e+1) against in the Sanger sequence-based data sets, a single read (JCVI_READ_1103242526277; raw sequences in Supplementary File S2) from the Indian open ocean GOS station GS112 was detected with a similar *psaD->C->A* arrangement. The PsaA protein from this read was identical to the PsaA protein from clone 9984 and the genes shares similar %G+C content. The other end of the same clone (JCVI_READ_1103242427903) contained a PSI *psaB* gene and a viral major capsid gene gp23 (similar to a gp23 from myocyanophage S-SSM7). Interestingly, this *psaB* gene content was 46% and is clearly distinct from other viral *psaB* genes described so far (Figure 2) and cluster on a long branch within the *Synechococcus* PsaB protein cluster (Figure 3).

When the search was extended to 454 pyrosequence-based sets, a single similar ~500 nt long read (CAM_READ_0231734843) from the Indian Ocean GOS station GS108 (Coccos Keeling, Inside Lagoon) was detected with a partial *psaC->A* arrangement. The new PsaA protein from station GS108 falls next to clone 9984 PsaA protein in the phylogentic tree (Figure 1a) on a long branch and the genes shares similar %G+C content. PCR reactions using primers to target the *psaC->A* arrangement performed on viral concentrates from the Pacific Line Islands (Caroline atoll (Millennium Island)) also confirmed the presence of a *psaC->A* arrangement with similar %G+C content (GenBank accession numbers JQ653152–JQ653153). The PsaA deduced proteins from the PCR products were similar to clone 9984 PsaA protein.

The new viral PSI gene arrangement reported here, *psaD->C->A->B*, is minimal compared with the other known viral arrangement, *psaJF->C->A->B->K->E->D*, however, PSI containing only PsaA, B, C and D proteins are believed to be an important step in the evolution of PSI (Nelson, 2011). It is therefore intriguing that this minimal *psaD->C->A->B* arrangement is the one observed on phages and would suggest that this minimal set is functional.

We have not ruled out the possibility that clone 9984 and related reads originate from yet uncultured cyanobacteria, however, based on the existence of two viral proteins on clone 9984 and one on read

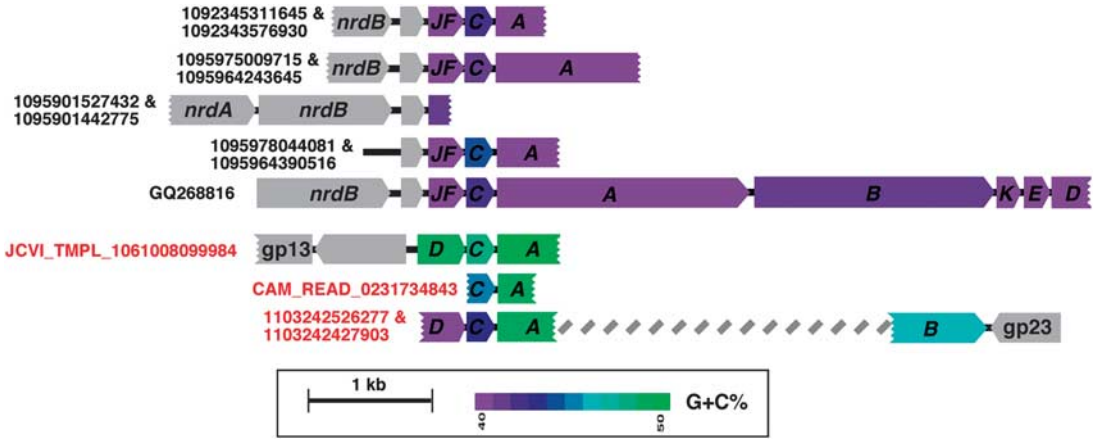

Figure 2 Schematic physical maps of viral GOS clones, reads and long PCRs containing PSI gene cassettes. PSI genes are colored according to their % G+C content. Gray arrows represent viral ORFs. The ORF next to gp13 on clone 9984 is similar to hypothetical protein 133 from *Prochlorococcus* phage P-SSM4. Color code indexes indicate % G+C, the calculations were performed for each gene separately. DNA sequences could be found in Supplementary File S2.

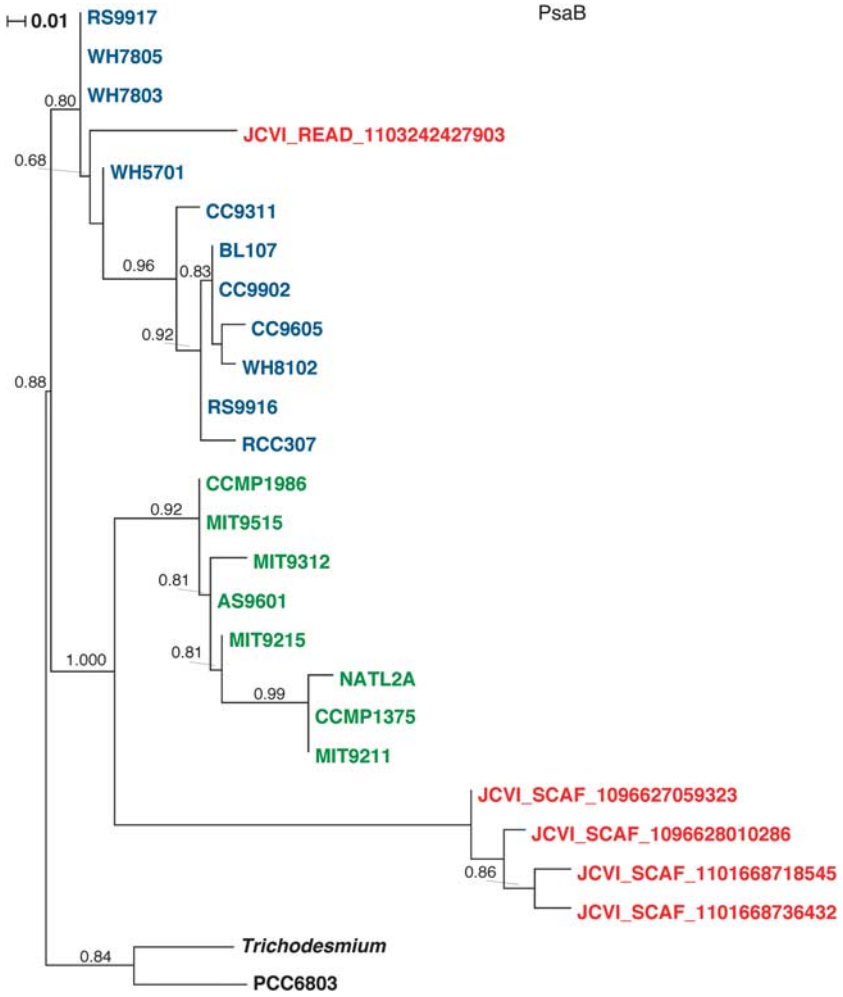

Figure 3 The relationship between *Synechococcus* (cyan), *Prochlorococcus* (green) and viral (red) PsaB proteins. Following alignment computation (using MUSCLE), PhyML was used for the calculation of the phylogenetic tree. PsaB protein sequences could be found in Supplementary File S3.

JCVI_READ_1103242427903, the amplification from viral concentrate, and the unique gene arrangement, we suggest that the observed PSI GOS sequences, although rare, are authentic and represents new viral PSI gene organizations, which needs to be further explored.

Acknowledgements

We thank Forest Rohwer for the phage concentrate sample. This work was supported by grant 580/10 from the Israel Science Foundation.

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Supplementary Informationaccompanies the paper on The ISME Journal website (http://www.nature.com/ismej)