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SHORT COMMUNICATION

Genomic potential for nitrogen assimilation in uncultivated members of *Prochlorococcus* from an anoxic marine zone

This article has been corrected since Advance Online Publication and an erratum is also printed in this issue

Marcia Astorga-Eló^{1,2}, Salvador Ramírez-Flandes^{1,3}, Edward F DeLong^{4,5,6} and Osvaldo Ulloa¹

¹Departamento de Oceanografía and Instituto Milenio de Oceanografía, Universidad de Concepción, Concepción, Chile; ²Programa de Magíster en Bioquímica y Bioinformática, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile; ³Programa de Doctorado en Ingeniería de Sistemas Complejos, Universidad Adolfo Ibáñez, Santiago, Chile; ⁴Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA; ⁵Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA and ⁶Daniel K. Inouye Center for Microbial Oceanography: Research and Education, University of Hawaii, Honolulu, HI, USA

Cyanobacteria of the genus *Prochlorococcus* are the most abundant photosynthetic marine organisms and key factors in the global carbon cycle. The understanding of their distribution and ecological importance in oligotrophic tropical and subtropical waters, and their differentiation into distinct ecotypes, is based on genetic and physiological information from several isolates. Currently, all available *Prochlorococcus* genomes show their incapacity for nitrate utilization. However, environmental sequence data suggest that some uncultivated lineages may have acquired this capacity. Here we report that uncultivated low-light-adapted *Prochlorococcus* from the nutrient-rich, low-light, anoxic marine zone (AMZ) of the eastern tropical South Pacific have the genetic potential for nitrate uptake and assimilation. All genes involved in this trait were found syntenic with those present in marine *Synechococcus*. Genomic and phylogenetic analyses also suggest that these genes have not been acquired recently, but perhaps were retained from a common ancestor, highlighting the basal characteristics of the AMZ lineages within *Prochlorococcus*.

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Cyanobacteria of the genus *Prochlorococcus* are the most abundant photosynthetic microorganisms inhabiting the oceans, key factors in the carbon cycle and a model organism in environmental microbiology (Partensky and Garczarek, 2010). They can be broadly classified into high-light and low-light (LL)-adapted ecotypes (Rocap *et al.*, 2002). These ecotypes exhibit distinct distributions both vertically in the water column and geographically across oligotrophic tropical and subtropical waters (Bouman *et al.*, 2006; Johnson *et al.*, 2006; Zwirgmaier *et al.*, 2008).

In past years, the genomes of over a dozen isolates of *Prochlorococcus* have been fully sequenced (for example, Kettler *et al.*, 2007) and over a hundred

single-cell-amplified partial genomes have been described (Malmstrom *et al.*, 2013; Kashtan *et al.*, 2014). All of them have revealed that they cannot use nitrate as a nitrogen source. However, new uncultivated lineages of *Prochlorococcus* have been identified in the environment using culture-independent techniques based on the sequencing of the 16S rRNA gene and related genomic regions (Lavin *et al.*, 2010; West *et al.*, 2011; Mühling, 2012; Malmstrom *et al.*, 2013). On the other hand, nitrate assimilation rates were reported for uncultivated deep populations of *Prochlorococcus* in the Western Atlantic Ocean (Casey *et al.*, 2007). In addition, genes necessary for nitrate assimilation associated to *Prochlorococcus* were identified in the global ocean sampling metagenomic database (Martiny *et al.*, 2009) and in metagenomes of flow-cytometry-sorted *Prochlorococcus* populations (Batmalle *et al.*, 2014).

Important uncultivated *Prochlorococcus* lineages include those thriving in anoxic marine zones (AMZs), where oxygen concentrations fall below the detection limit of modern sensors, light is scarce,

Correspondence: O Ulloa, Departamento de Oceanografía and Instituto Milenio de Oceanografía, Universidad de Concepción, Casilla 160-C, Concepción 4070386, Chile.
E-mail: oulloa@udec.cl

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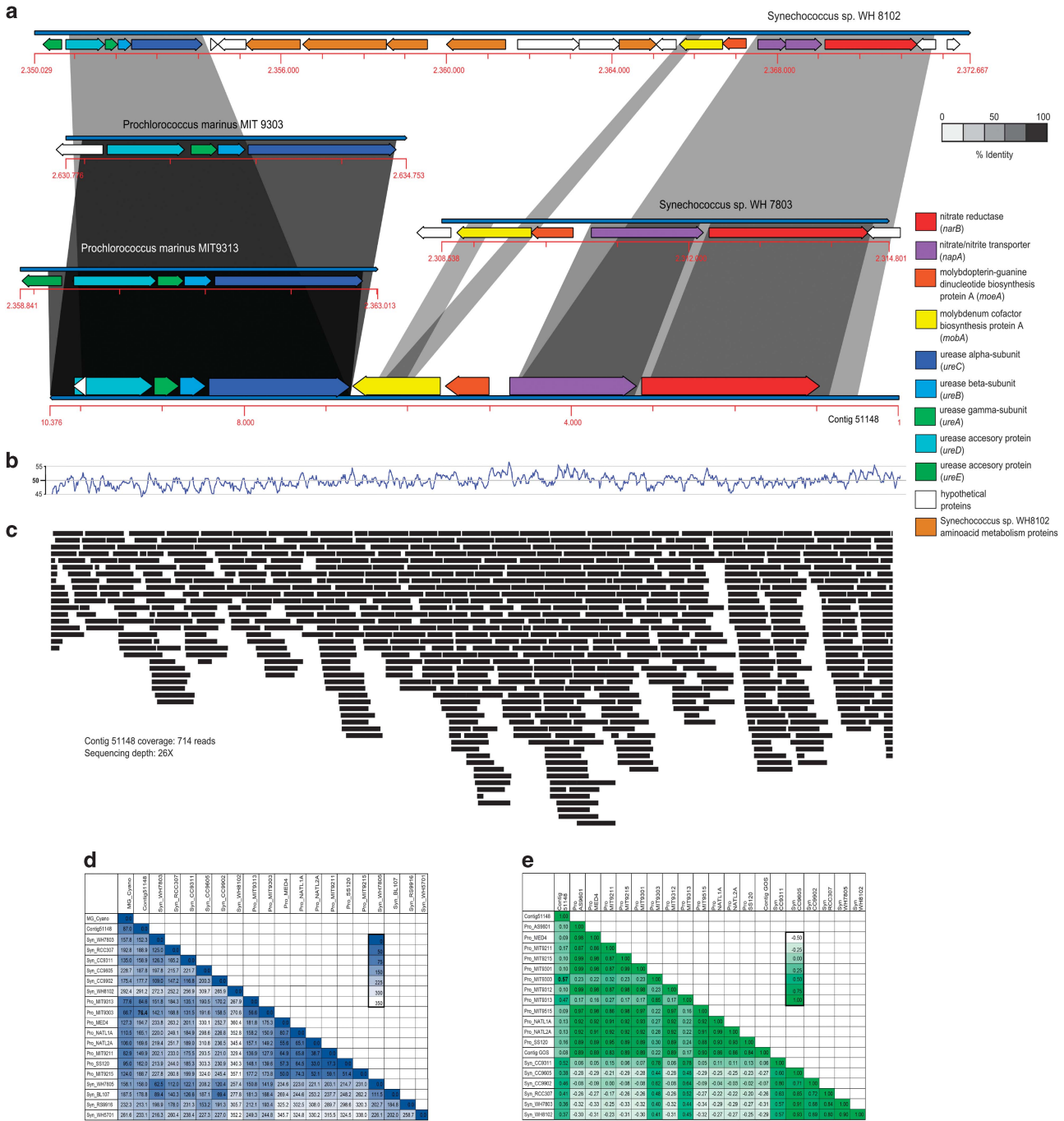


Figure 1 Genomic characteristics of the nitrogen assimilation operon found in contig 51148. (a) Schematic representation of synteny among contig 51148, *Prochlorococcus* MIT9303 and MIT9313 genomes, and *Synechococcus* WH7803 and WH8102 genomes centered on nitrate and urea assimilation genes. Identities (%) among sequences are shown in gray. (b) GC content. (c) Contig coverage. (d) Proximity matrix (Euclidean distance) of the difference in codon usage pattern for the genomes of *Prochlorococcus* (Pro) and *Synechococcus* (Syn), and of contig 51148. The shortest distance (dark blue) indicates the highest proximity. (e) Spearman rank-order correlation between tetranucleotide frequency of contig 51148 and those of genomes of marine *Prochlorococcus* (Pro) and marine *Synechococcus* (Syn). The highest correlation is shown in dark green.

but inorganic nutrients are plentiful (Goericke *et al.*, 2000; Ulloa *et al.*, 2012). Phylogenetic analysis using the 16S–23S rRNA internal transcribed spacer region revealed that the AMZ-associated *Prochlorococcus* assemblages are mainly composed of two novel LL ecotypes (termed LL-V and LL-VI), which correspond to basal groups linking *Prochlorococcus*

with marine *Synechococcus* (Lavin *et al.*, 2010), the other dominant marine picocyanobacterium. However, no genomic or physiological information exists for these AMZ lineages.

Here we report results from a metagenomic analysis carried out on environmental genomic sequences retrieved from a sample collected at

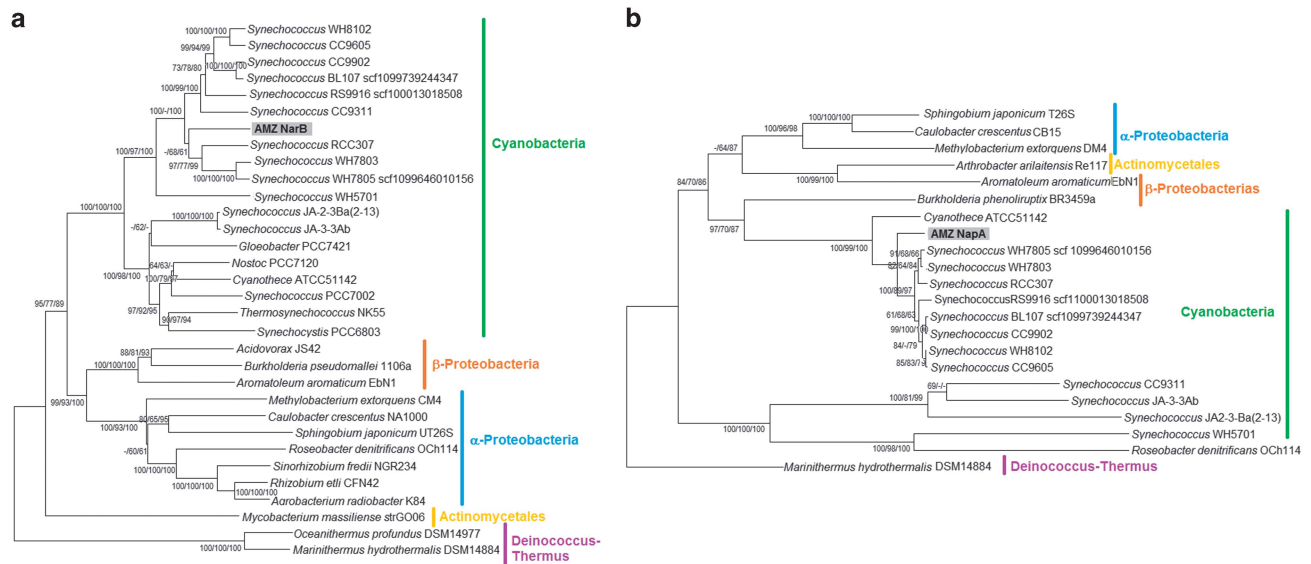


Figure 2 Phylogenetic trees for nitrate assimilation and uptake genes. Maximum-likelihood phylogenetic trees of (a) *narB*- and (b) *napA*-predicted amino acid sequences found in contig 51148. Evolutionary history was inferred using neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML). Bootstrap support values for 100 replications are shown at the nodes (NJ/MP/ML).

60 m depth within the AMZ of the eastern tropical South Pacific (Supplementary Figure S1), where dissolved oxygen was undetectable and inorganic nutrients were abundant (Supplementary Figure S2a; Thamdrup *et al.*, 2012). The microbial community was enriched in *Prochlorococcus*, shown to comprise ~10% of cell abundance, versus ~0.7% of *Synechococcus*, assessed by flow cytometry (Supplementary Figure S2b). Blast analysis of the taxonomic affiliation of sequences matching the *rpoC* region 1, a taxonomic marker for cyanobacteria based on a single-copy gene (Palenik, 1994), showed an *rpoC* gene relative abundance of 86% for *Prochlorococcus* and 14% for *Synechococcus* (Supplementary Table S1), supporting the flow cytometry results. Moreover, of the 15% protein-coding sequences assigned to cyanobacteria, 10% binned with *Prochlorococcus* and 5% with *Synechococcus* (Supplementary Figure S3). Of those assigned to *Prochlorococcus*, 90% were related to the LL ecotypes MIT9313 and MIT9303, the closest reported relatives to the AMZ lineages with genomes fully sequenced (Lavin *et al.*, 2010). General statistics of this AMZ metagenome are shown in Supplementary Tables S2 and S3.

Analysis of *de novo*-assembled contigs revealed the presence of several large contigs that binned with *Prochlorococcus*. In particular, a single contig was found to encode genes related to urea and nitrate uptake and assimilation (contig 51148, GenBank accession number KM282015; 10 300 bp; Figure 1), in synteny with those in *Synechococcus* WH8102. The genes in the urease gene cluster (*ureABCD*) presented high identity to those described for *Prochlorococcus* MIT9313 and MIT9303 (Rocap *et al.*, 2003; Supplementary Figure S4). Notably, the nitrate/nitrite transporter *napA* and assimilatory nitrate reductase *narB* were also found within the same contig

(Figure 1a), as well as the genes *moeA* and *mobA* (Supplementary Figure S5) involved in the biosynthesis of the Mo-cofactor and necessary for the *narB* function (Flores *et al.*, 2005). None of these genes have been found in any of the genomes of *Prochlorococcus* sequenced and described so far. However, homologues that presumably come from uncultivated relatives of *Prochlorococcus* have been found in the global ocean sampling database (Martiny *et al.*, 2009) and in metagenomes of uncultured, sorted *Prochlorococcus* populations (Batmalle *et al.*, 2014).

The GC content of contig 51148 was ~51.1% (Figure 1b) and similar to that of LL *Prochlorococcus* and some marine *Synechococcus* (Kettler *et al.*, 2007). Likewise, the *narB* gene had a GC content of 52%, which is less than the ~60% of those in the marine *Synechococcus* strains WH8102 and WH7803 (to which it presented the highest nucleotide identity), but significantly higher than the ~40% GC of the global ocean sampling high-light *Prochlorococcus narB* (Supplementary Figure S6). Analysis of codon usage patterns (Yu *et al.*, 2012) and tetranucleotide frequencies (see Supplementary Material and Methods) showed that the cyanobacterial portion of the metagenome and contig 51148 exhibit the highest similarity with LL *Prochlorococcus* MIT9303 (Figures 1d and e). Additionally, nucleotide identities and phylogenetic analysis confirmed that the urease genes of contig 51148 were associated more closely with *Prochlorococcus* than *Synechococcus* (Supplementary Table S4 and Supplementary Figure S4).

The homogeneous GC content of contig 51148, the differences in codon usage bias with *Synechococcus* and phylogenetic analyses of AMZ *narB* and *napA* (Figures 2a and b) all suggest that the genetic potential for nitrate uptake and assimilation was not obtained recently by horizontal gene transfer, but instead potentially were retained from a common ancestor

with *Synechococcus*. Mapping the presence/absence of the different nitrate utilization genes onto the cyanobacteria 16S rRNA phylogenetic tree is consistent with this hypothesis (Supplementary Figure S7).

In summary, our results indicate that AMZ *Prochlorococcus* lineages have the genetic potential for urea and nitrate assimilation, likely an adaptation to the unique nutrient-rich environment where they thrive. Additional genomic characteristics that could explain their high abundance in the oxygen-deficient and very-LL waters of AMZs remain to be assessed.

Conflict of Interest

The authors declare no conflict of interest.

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