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Authors

Wittmers, Fabian
Comstock, Jacqueline
Poirier, Camille
et al.

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Non-photosynthetic lineages sibling to Cyanobacteria associate with eukaryotes in the open ocean

Fabian Wittmers[1,2], **Jacqueline Comstock**[3], **Camille Poirier**[2], **David M. Needham**[2], **Frederik Schulz**[4], **Rex Malmstrom**[4], **Craig A. Carlson**[3], and **Alexandra Z. Worden**[1,2,*]

Margulisbacteria are elusive uncultivated bacteria that have illuminated evolutionary transitions in the progenitor of Cyanobacteria, the latter being a critically important phylum that underpins oxygenic photosynthesis[1,2]. The non-photosynthetic Margulisbacteria were discovered in a sulfidic spring[3] and later in other habitats[4–6]. Currently, this candidate phylum partitions into the Riflemargulisbacteria, primarily from sediments and groundwater, the Termititenax from insect gut microbiomes, and the Marinamargulisbacteria, from marine samples[4–6]. We found that Marinamargulisbacteria amplicons were unusually distributed in size-fractionated samples from the sunlit photic and dark twilight zones of the ocean. Sequencing of wild marine protists rendered genomic information for distinct marinamargulisbacterial clades co-associated with uncultivated, non-photosynthetic Stramenopila and Opisthokonta protists. Phylogenomic analyses combining these data and available metagenome-assembled genomes (MAGs) and single-amplified genomes (SAGs) from sorted bacteria revealed new Marinamargulisbacteria lineages. The lineages delineate by their environment, forming clades comprising freshwater, marine pelagic, or sediment/hypoxic taxa. The remarkable diversity of Margulisbacteria indicates success in colonizing various habitats, potentially in a conserved strategy involving eukaryotic cells.

Margulisbacteria were identified as a unique group (ZB3) in environmental 16S rRNA gene surveys[3]. However, it was through phylogenomic analyses that their relationship with Cyanobacteria was realized, along with that of several other non-photosynthetic groups[2,4,5]. The Melainabacteria and Sericytochromatia are sister to the Cyanobacteria, whereas the Saganbacteria (formerly WOR-1) and Margulisbacteria (formerly ZB3 or RBX1) comprise candidate phyla adjacent to the former trio. Collectively, these groups help trace metabolic features that influenced the evolution of aerobic respiration, a vital step connected to development of oxygenic photosynthesis[1,5]. Still, relatively little is known about margulisbacterial niches, since most data come from bulk water collection, where combined biomass from many liters is sequenced, limiting assembly of genomes from microdiverse microbes and obfuscating detection of cell–cell interactions.

We examined Marinamargulisbacteria distributions in V4 16S rRNA gene amplicons using size-fractionated samples from the Bermuda Atlantic Time-series Study (BATS) site. Marinamargulisbacteria were present from the photic zone through 500 m in nearly all samples from all size fractions, including 0.2–1.2 μm, where free-living bacteria like SAR11 and *Prochlorococcus* dominate, to 1.2–5, 5–20 and >20 μm, where particles and living protists are captured (Figure 1A). The even distribution of Marinamargulisbacteria across size fractions suggests they have a free-living life stage, consistent with recovery of a Marinamargulisbacteria SAG (AG-410-N11) from a sorted Atlantic bacterial cell[5] ,and a particle/protist-associated stage, or that they facultatively occupy both niches.

Host-associated lifestyles have been reported for some Margulisbacteria. The tiny Termititenax live attached to ectosymbiotic (spirochete) bacteria that reside on phagotrophic Metamonada ('Excavata') protists that live inside termite guts[4]. Additionally, intracellular symbionts living in the placozoan *Trichoplax*, a simple marine animal, include an uncultivated Marinamargulisbacterium (*Ruthmannia eludens*) that localizes to epithelial cells[6]. Margulisbacteria

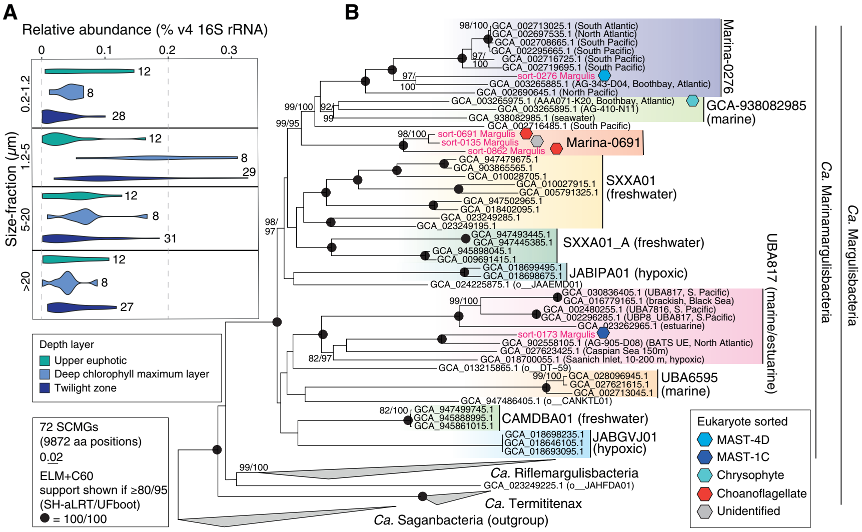

Figure 1. Diversity and evolutionary relationships of new Margulisbacteria lineages.
Relative abundances of marine Margulisbacteria at BATS. Photic zone (100–0.1% of incident light level) samples were partitioned to upper euphotic zone (UE), the deep chlorophyll maximum (DCM), and twilight zone (here <0.1% incident light level to 500 m). Seawater was sequentially filtered *in situ* to generate size fractions during summer and autumn, resulting in 195 individual samples (indicated by size fraction beside plotted data). Margulisbacteria V4 16S rRNA gene amplicons were detected in 92 ± 14%, 97 ± 5%, 92 ± 8%, 85 ± 17% of samples from the smallest to largest size fractions. (B) Maximum-likelihood phylogenomic reconstruction of Margulisbacteria using our data and publicly available MAGs and SAGs (Supplemental methods). Marinamargulisbacteria clades are colored based on GTDB order-level taxonomy. Metagenomic bins of Marinamargulisbacteria (pink text) from our phagotrophic protist sorts and corresponding protist taxonomy (hexagons) are indicated. Note, in our reconstruction, GCA_002713045 replaces the lower quality prior UBA6595 representative (GCA_002433595; see Figure S1B). Topology was similar in results from a non-mixture model (Figure S1B).

amplicons have also been reported in oyster gill tissues[7]. Our amplicon results indicating a possible co-association of Marinamargulisbacteria with eukaryotes in the larger size fractions of the sunlit and dark ocean (Figure 1A) are compatible with these studies on associations with phagotrophic eukaryotes. Thus, we hypothesized that relationships occur between Marinamargulisbacteria and phagotrophic protists in the pelagic ocean.

To examine our hypothesis, we targeted cells of non-photosynthetic protists in the photic zone using fluorescence-activated cell sorting (FACS) and whole-genome amplification. Assemblies from several individual protists included margulisbacterial assemblies, and the proteins predicted were used to examine evolutionary relationships (Figure 1B and Data S1A). Phylogenomic reconstruction resolved distinct Marinamargulisbacteria clades affiliated with divergent protists (Figure 1B). Sort-0276 was a stramenopile from the widespread MAST-4D[8]. The co-associated Margulisbacterium was placed in a supported clade that included MAGs or SAGs from several oceans, with a coastal Atlantic assembly (AG-343-D04) forming its sister branch (Figure 1B). Another deep-branching clade was in an adjacent position and included AAA071-K20 and AG-410-N11. These two purportedly branch inside and basal to *R. eludens*[6] (we could not find *R. eludens* genome data to include). Additionally, they form one of four marinamargulisbacterial groups recognized previously, and AAA071-K20 is from a sorted protist[5].

The other Marinamargulisbacteria captured with FACS-sorted protists herein belonged to separate clades. One branched deep within UBA817 (sort-0173), which contains MAGs and SAGs from marine and estuarine samples (Figure 1B). Sort-0173 was co-associated with a multi-cell predator sort with a complete 18S rRNA gene from MAST-1C, another phagotrophic stramenopile[8], and is most closely related to a MAG from BATS, where both MAST-1C and MAST-4D are common (see also Figure S1A and Data S1B). The third group was co-associated with choanoflagellates, the closest living unicellular relatives of animals[8], and formed its own clade. In addition to revealing protist-associated clades we expose a broader diversity of Marinamargulisbacteria, including multiple marine clades, freshwater lineages (CAMDBA01, SXXA01, SXXA01_A), and two from hypoxic seawater samples (JABIPA01, JABGVJ01) (Figure 1B).

Overall, the topology of our phylogenomic reconstruction supported Saganbacteria and Margulisbacteria being separate phyla[2,5], contrasting with earlier work[6]. The Termititenax were in a supported position basal to the sediment-associated fermentative Riflemargulisbacteria and the putatively aerobic[5] Marinamargulisbacteria. A limitation is that only four high-quality Marinamargulisbacteria MAGs exist, two from SXXA01 and two deep-branching UBA817 members (average 91.5% complete, Data S1A). However, some ‘universal’ proteins used to generate completeness estimates are absent from characterized symbionts[9]. Hence, available marinamargulisbacterial assemblies potentially reflect some gene losses that require future identification. Finally, using 16S rRNA gene sequences from our MAGs and environmental clones, we demonstrate that the protist-associated clades identified herein are present in the Atlantic Ocean, Pacific Ocean, and China Sea (Figure S1A).

We show that most truly marine clades of Marinamargulisbacteria have associations with protists that appear to be facultative, given even distributions across size-fractions (Figure 1). Although little is known about lifestyles within the non-photosynthetic sibling lineages of Cyanobacteria, our findings, combined with those from Margulisbacteria in animals (placozoans, oysters, and termites), underscore a predilection for eukaryotic associations. Together with the cyanobacterial lineage that putatively gave rise to eukaryotic plastids[10], the evolution of a eukaryote-associated lifestyle has thus occurred multiple times in this part of the bacterial tree. Co-association of three Marinamargulisbacteria lineages with pelagic phagotrophic protists, and their continuous presence through the water column, provide first insights into their interconnected roles in ocean ecosystems.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental information including one figure, experimental procedures, data availability, accessions, metadata, acknowledgements, author contributions, references and one data file can be found with this article online at https://doi.org/10.1016/j.cub.2024.09.009.

REFERENCES

1. Oliver, T., Sánchez-Baracaldo, P., Larkum, A.W., Rutherford, A.W., and Cardona, T. (2021). Time-resolved comparative molecular evolution of oxygenic photosynthesis. Biochim. Biophys. Acta Bioenerg. *1862*, 148400.
2. Hug, L.A., Baker, B.J., Anantharaman, K., Brown, C.T., Probst, A.J., Castelle, C.J., Butterfield, C.N., Hernsdorf, A.W., Amano, Y., Ise, K., *et al.* (2016). A new view of the tree of life. Nat. Microbiol. *1*, 16048.
3. Elshahed, M.S., Senko, J.M., Najar, F.Z., Kenton, S.M., Roe, B.A., Dewers, T.A., Spear, J.R., and Krumholz, L.R. (2003). Bacterial diversity and sulfur cycling in a mesophilic sulfide-rich spring. Appl. Environ. Microbiol. *69*, 5609–5621.
4. Utami, Y.D., Kuwahara, H., Igai, K., Murakami, T., Sugaya, K., Morikawa, T., Nagura, Y., Yuki, M., Deevong, P., Inoue, T., *et al.* (2019). Genome analyses of uncultured TG2/ZB3 bacteria in ‘Margulisbacteria’ specifically attached to ectosymbiotic spirochetes of protists in the termite gut. ISME J. *13*, 455–467.
5. Matheus Carnevali, P.B., Schulz, F., Castelle, C.J., Kantor, R.S., Shih, P.M., Sharon, I., Santini, J.M., Olm, M.R., Amano, Y., Thomas, B.C., *et al.* (2019). Hydrogen-based metabolism as an ancestral trait in lineages sibling to the Cyanobacteria. Nat. Commun. *10*, 463.
6. Gruber-Vodicka, H.R., Leisch, N., Kleiner, M., Hinzke, T., Liebeke, M., McFall-Ngai, M., Hadfield, M.G., and Dubilier, N. (2019). Two intracellular and cell type-specific bacterial symbionts in the placozoan *Trichoplax* H2. Nat. Microbiol. *4*, 1465–1474.
7. King, W.L., Siboni, N., Kahlke, T., Dove, M., O’Connor, W., Mahbub, K.R., Jenkins, C., Seymour, J.R., and Labbate, M. (2020). Regional and oyster microenvironmental scale heterogeneity in the Pacific oyster bacterial community. FEMS Microbiol. Ecol. *96*, fiaa054.
8. Wilken, S., Yung, C.C.M., Poirier, C., Massana, R., Jimenez, V., and Worden, A.Z. (2023). Choanoflagellates alongside diverse uncultured predatory protists consume the abundant open-ocean cyanobacterium *Prochlorococcus*. Proc. Natl. Acad. Sci. USA *120*, e2302388120.
9. Needham, D.M., Poirier, C., Bachy, C., George, E.E., Wilken, S., Yung, C.C.M., Limardo, A.J., Morando, M., Sudek, L., Malmstrom, R.R., *et al.* (2022). The microbiome of a bacterivorous marine choanoflagellate contains a resource-demanding obligate bacterial associate. Nat. Microbiol. *7*, 1466–1479.
10. Ponce-Toledo, R.I., Deschamps, P., López-García, P., Zivanovic, Y., Benzerara, K., and Moreira, D. (2017). An early-branching freshwater cyanobacterium at the origin of plastids. Curr. Biol. *27*, 386–391.

[1]Marine Biological Laboratory, Woods Hole, MA 02543, USA. [2]Ocean EcoSystems Biology Unit, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel 24148, Germany. [3]Marine Science Institute, University of California, Santa Barbara, CA 93117, USA. [4]DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.
*E-mail: azworden@mbl.edu