Discovery of a new host for rhizobial symbiont in the ocean

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Nitrogen is a vital element found in many biomolecules essential for sustaining life. Although nitrogen gas (N_2) is abundant in Earth's atmosphere, most organisms are incapable of utilizing N_2 due to its relative inertness. Biological nitrogen fixation by nitrogenase, which catalyzes the conversion of atmospheric N_2 to ammonia (NH_3), provides Earth with 50% of the biologically available nitrogen. This natural process enhances soil fertility and reduces the need for chemical fertilizers. In addition to being a crucial process in sustainable agriculture, biological nitrogen fixation in oligotrophic surface waters also plays a vital role in shaping the marine ecosystem as a primary source of new nitrogen in the ocean. Diazotrophic cyanobacteria have long been believed to be the only major contributor for oceanic nitrogen fixation, even though a variety of diazotrophic bacteria and archaea are widely distributed in ocean. However, it remains unclear whether these non-cyanobacterial diazotrophs significantly contribute to the nitrogen input to the oligotrophic ocean. This commentary focuses on a pioneering discovery of rhizobia–diatom symbioses and their role in oceanic nitrogen fixation, as detailed in "Rhizobia–diatom symbiosis fixes missing nitrogen in the ocean".[1] The discovered symbiosis between *Candidatus* Tectiglobus diatomicola and

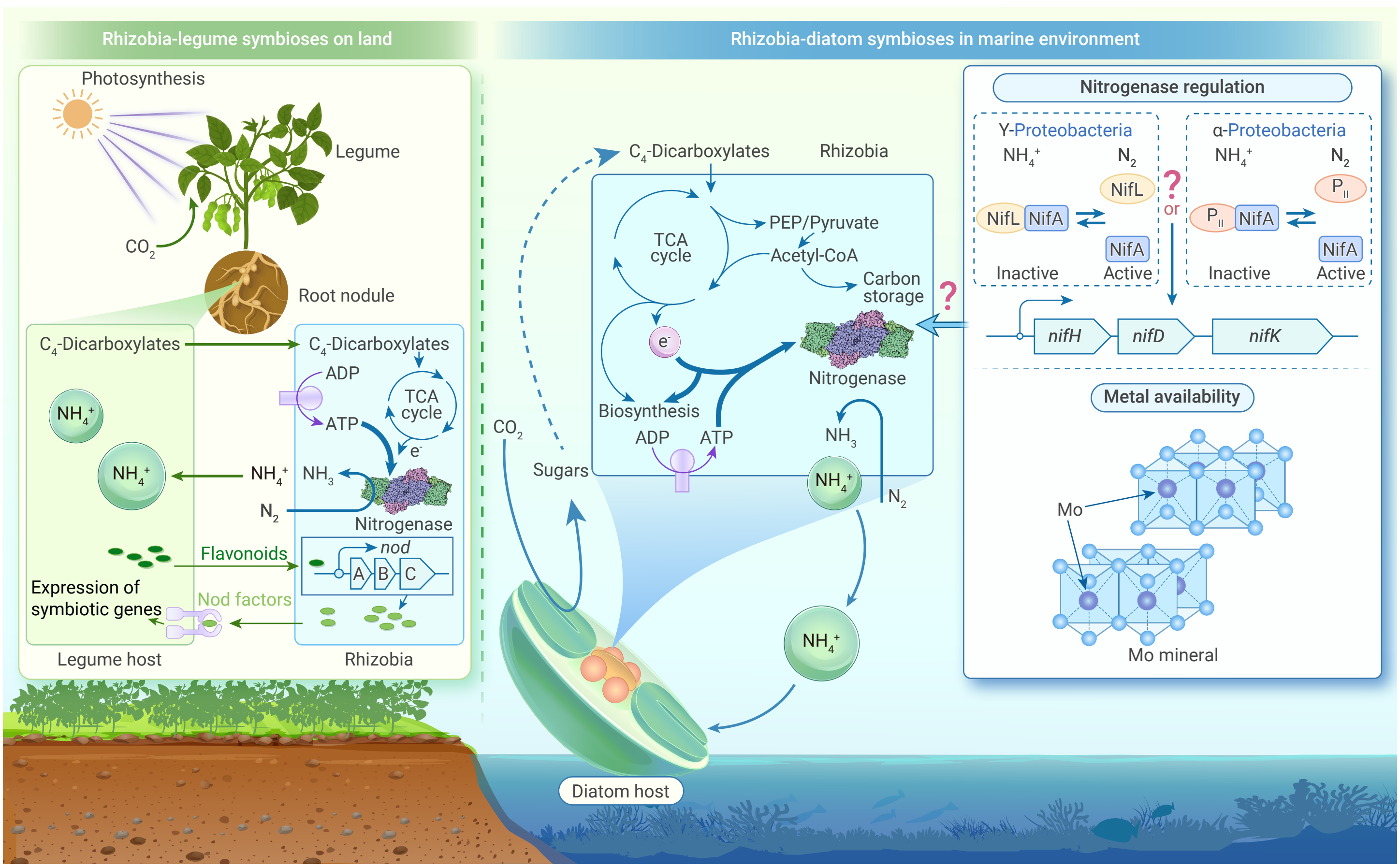

Figure 1. The nitrogen-fixing rhizobia form symbiotic relationships with diatoms in addition to the well-known legumes On land, the flavonoids excreted by legumes induce rhizobia to express nodulation genes such as *nodA*, *nodB* and *nodC*. The protein products of *nodABC* genes are responsible for the biosynthesis of Nod factors that stimulate the expression of symbiotic genes in legumes. Legumes fix CO_2 through photosynthesis and provide rhizobia with C_4-dicarboxylates as carbon sources. In exchange, rhizobia convert atmospheric N_2 to ammonia by nitrogenase, thereby providing fixed nitrogen to their legume hosts. In addition to the rhizobia-legume symbioses on land, the symbiosis between rhizobia and diatoms was newly observed in marine environments. The *Haslea* host provides the nitrogen-fixing *Ca.* T. diatomicola with C_4-dicarboxylates, and in return, *Ca.* T. diatomicola shares the fixed nitrogen with its diatom host. However, it remains unclear how nitrogenase synthesis is regulated and how the rhizobia-diatom symbioses acquire Mo for nitrogenase maturation in open marine environments. CO_2, carbon dioxide; N_2, nitrogen gas; NH_3, ammonia; NH_4^+, ammonium; TCA, tricarboxylic acid; PEP, phosphoenolpyruvate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Mo, molybdenum.

diatoms expands the known range of hosts for the order Rhizobiales beyond the rhizobia-legume symbioses (Figure 1), and also provides a reasonable explanation for the nitrogen fixation rates detected in oligotrophic ocean regions with sparse distribution of cyanobacteria, which firstly appeared about 3 billion years ago.[2]

The tropical North Atlantic is responsible for approximately 20% of oceanic nitrogen fixation, with only ~50% rates contributed by cyanobacteria. Therefore, Tschitschko et al. examined the contribution of non-cyanobacterial nitrogen fixation to this region, and confirmed the presence of heterotrophic diazotrophs gamma-A by metagenomic sequencing. This enigmatic bacterium is widely distributed in most of the world's oceans. The *nifH* genes of gamma-A were exclusively retrieved from the fractions larger than 3 μm, indicating that gamma-A likely exists as a symbiont. The *nifH* marker gene encodes iron (Fe) protein subunit of molybdenum (Mo) nitrogenase, which plays a major role in global nitrogen fixation. Additionally, a near-complete metagenome assembled genome (MAG) containing the gamma-A *nifH* gene was obtained. The gamma-A *nifH* gene was clustered within the Gammaproteobacteria. However, the MAG was clustered within the Alphaproteobacteria family Hyphomicrobiaceae in the order Rhizobiales, which is well-known for rhizobial symbionts of terrestrial legumes. Tschitschko et al. thus concluded that the gamma-A acquired its nitrogenase genes via horizontal gene transfer from a Gammaproteobacterial donor. It's worth mentioning that the process of nitrogen fixation is subject to regulation in response to the environmental nitrogen status. As the transcriptional activator of Mo nitrogenase genes, NifA directly controls the expression of Mo nitrogenase by interacting with the RNA polymerase sigma factor σ^{54} (RpoN) in diazotrophic proteobacteria. Most of the diazotrophic Gammaproteobacteria such as *Klebsiella pneumoniae* and *Azotobacter vinelandii* have genes encoding NifL, which inhibits the activity of NifA as a negative regulator. The inactivation of NifL results in the constitutive expression of nitrogenase even in the presence of ammonium. However, diazotrophs within Alphaproteobacteria usually lack genes for NifL. In these diazotrophs, P_{II} proteins play an essential role in nitrogenase regulation. Therefore, it would be highly interesting to investigate how NifA activity or nitrogen fixation is regulated in gamma-A, since the *nifL* gene from Gammaproteobacteria was not introduced into the gamma-A in the horizontal gene transfer event. In addition to regulation by nitrogen availability, the nitrogen fixation is also subject to regulation by the availability of metals such as Mo, which is essential for the activity of Mo nitrogenase. Besides the dissolved Mo, the mineral-bound Mo can also be utilized by diazotrophs for the maturation of Mo nitrogenase.[3] Therefore, it is of interest to investigate the influence of both dissolved and mineral-bound Mo on the nitrogen fixation in open oceans.

The newly discovered species was designated as *Candidatus* Tectiglobus diatomicola that belongs to a novel genus *Ca.* Tectiglobus. Another marine MAG from the North Pacific, designated as *Ca.* T. profundi, is also affiliated with this genus. Both species exhibit reduced genome sizes and GC content similar to those of UCYN-A (*Candidatus* Atelocyanobacterium thalassa), which was recently reported as a nitrogen-fixing organelle of the marine haptophyte algae *Braarudosphaera bigelowii*, the first nitrogen-fixing eukaryote ever discovered.[4] Tschitschko and coworkers also revealed that *Ca.* T. diatomicola resided exclusively inside diatom hosts, identified as likely belonging to the genus *Haslea*. This discovery expands the known range of diatom hosts for nitrogen-fixing symbionts, and marks the first known symbiosis between a diatom and a non-cyanobacterial nitrogen-fixing microorganism.

Tschitschko and coworkers used ^{15}N stable isotope tracing and nanoscale secondary ion mass spectrometry (nanoSIMS) to directly demonstrate that *Ca.* T. diatomicola could fix atmospheric nitrogen and transfer 99% of fixed nitrogen to its diatom host. Corresponding to the nitrogen source provided by nitrogen-fixing bacteria, symbiotic diatom transferred about 1% of the fixed carbon through photosynthesis to *Ca.* T. diatomicola. This tightly coupled symbiotic relationship ensures that the host diatom receives sufficient nitrogen source and the symbiont gets enough carbon source to maintain their growth and reproduction. In addition, both *Ca.* T. diatomicola and its *Haslea* host showed similar carbon-based growth rates. Compared to cyanobacteria-diatom symbioses, the growth rate of *Ca.* T. diatomicola-diatom symbioses was about 4-times faster, suggesting they may contribute more significantly to the input of new nitrogen into the oligotrophic tropical North Atlantic. This study reveals an intricate coordination between the *Ca.* T. diatomicola symbiont and the diatom host, ensuring efficient utilization of substances and energy within the symbiotic cells. The *Ca.* T. diatomicola likely play the same role in diatoms as UCYN-A does in haptophyte algae. In *B. bigelowii*, chloroplasts fix carbon dioxide (CO_2) into sugar through photosynthesis, providing UCYN-A and mitochondria with carbon sources. UCYN-A is surrounded by mitochondria, making it more convenient to access energy for nitrogen fixation. In addition, the size ratio relationship between UCYN-A and the marine phytoplankton *B. bigelowii* is governed by the exchange of resources between symbionts and hosts.[5] The sizes of UCYN-A and its host exhibit a nearly linear proportional relationship, highlighting the underlying mechanism of resource economy within the symbiotic system. This mechanism reflects the balanced interdependence among CO_2 fixation, respiration and nitrogen fixation. The evolutionary adaptation of *Ca.* T. diatomicola is similar to that of UCYN-A, which has evolved into a nitrogen-fixing organelle, suggesting that *Ca.* T. diatomicola may be in the early stages of becoming another nitrogen-fixing organelle. It is intriguing to explore whether such a nitrogen-fixing organelle derived from rhizobia has emerged in some diatoms. A better understanding of how nitrogen-fixing organelle works in eukaryotic cells will contribute to achieving nitrogen fixation in cereal crops.

In conclusion, the discovery of the symbiosis between the nitrogen-fixing Rhizobiales such as *Ca.* T. diatomicola and the diatom represents a breakthrough in extending the range of hosts for Rhizobiales beyond the well-known rhizobia-legume symbiosis. This study also reveals that *Ca.* T. diatomicola-diatom symbioses play a major role in oceanic nitrogen fixation, leading to a new understanding of the contribution of non-cyanobacterial diazotrophs to the nitrogen input in oceans.

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AUTHOR CONTRIBUTIONS

Mingyue Jiang was responsible for conceptualization and writing (original draft). Mengshuang Liu was responsible for writing (original draft). Yongqiang Gao was responsible for writing (review & editing). Yanning Zheng was responsible for conceptualization, funding acquisition and writing (review & editing). All authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.