N_2 fixation & microbial communities in the cyanobacterium *Trichodesmium*

Anxhela HANIA[1,2] & Meri EICHNER[1]

1. Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Třeboň 379 01, Czech Republic
2. University of South Bohemia in České Budějovice, České Budějovice 370 05, Czech Republic

N_2 fixation The filamentous cyanobacterial genus *Trichodesmium* is recognized as one of the most abundant N_2-fixers. Performing N_2 fixation while simultaneously producing O_2 is challenging for photosynthetic diazotrophs due to the irreversible inhibition of nitrogenase, the enzyme converting N_2 to NH_4^+, upon O_2 exposure. To overcome this challenge, several time- and space-separation strategies have been proposed to coordinate N_2 fixation and O_2 production in *Trichodesmium*, including down-regulation of photosynthesis during the peak of N_2 fixation, and confinement of nitrogenase into diazocytes, a subset of not fully differentiated cells within a filament. However, the overall mechanism of how *Trichodesmium* reconciles N_2 fixation and O_2 production remains unresolved, possibly due to the multitude of different protocols and/or strains employed across studies.

Since contradictory results derive from two laboratory strains of *T. erythraeum* (IMS101 and NIBB1067), herein, we directly compare their general physiology and spatial heterogeneity of N_2 fixation and photosynthesis at a single-cell level. We analysed specific growth rates, used Lugol's staining to characterize diazocytes lengths, employed mRNA Catalyzed Reported Deposition Fluorescence *in situ* Hybridization (mRNA CARD-FISH) to localize *nifH* expression, and performed qPCR analysis of genes related to photosynthesis, N_2 fixation, and O_2-lowering mechanisms. Additionally, techniques such as Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) and Fluorescence Lifetime Imaging Microscopy (FLIM) were performed to, respectively, estimate N_2 and CO_2 fixation and image photosynthetic pigments. Our findings revealed faster growth and, overall, larger cell-to-cell heterogeneity in IMS101 than in NIBB1067. The absence of spatial heterogeneity in the latter suggests that a temporal separation strategy might be more relevant, and that other O_2-lowering mechanisms are involved in the protection of nitrogenase. While these strain-specific differences can explain some of the discrepancies between previous laboratory studies, further investigation is needed to determine whether these laboratory results are also applicable to natural *Trichodesmium* colonies.

Microbial communities *Trichodesmium* can exist as both single filaments and puff- or tuft-shaped colonies. A large number of field studies have highlighted that these aggregated forms host a highly diverse microbial community. *Trichodesmium* colonies are believed to create a favourable environment for bacteria by providing sources of organic C and N. Additionally, recent work has suggested that bacteria and *Trichodesmium* may interact to enhance the availability of mineral Fe, which is bound in the dust particles captured by *Trichodesmium* and shuffled actively to the colony core. Within this microenvironment, bacteria can promote dust dissolution by producing Fe-complexing molecules, known as siderophores, thereby alleviating *Trichodesmium*'s high Fe requirements for N_2 fixation.

While taxonomic identification and functional traits of *Trichodesmium*'s associated bacteria have been explored by -omics approaches, the spatial distribution of these bacteria on *Trichodesmium* colonies have yet to be elucidated. Spatial distribution may vary among different bacteria, possibly reflecting differences in physical associations to dust particles, co-localization, and potential interactions. To address this gap, we collected *Trichodesmium* colonies in the Gulf of Eilat (Israel) and performed rRNA CARD-FISH. After selection, colonies were sorted for three different treatments: non incubated, incubated without- and with-particle addition for 24, 48 and 72 h. We then performed a double hybridization by targeting *Alphaproteobacteria* and their *Roseobacter* subgroup, and *Gammaproteobacteria* and their *Alteromonas* subgroup, both known to encode for siderophores. Our findings indicate that *Roseobacter* do not exhibit a preferred location on *Trichodesmium* colonies. Despite the potential for co-localization with Fe minerals, their abundance does not appear to be correlated with the presence of particles or the duration of incubation. In contrast, *Alteromonas* seem to preferentially locate in the centre of the colonies and to co-localized with Fe minerals, and their abundance to increase with incubation time. Overall, our CARD-FISH approach provides visual support that the role in mineral Fe dissolution, that was suggested based on -omics, is a key feature in the *Alteromonas*-*Trichodesmium* association.