Exploring the interplay of nitrogen fixation and photosynthesis in *Trichodesmium* at the single-cell level using NanoSIMS

Rebeca López-Adams[1] and Meri Eichner[1]

[1]*Institute of Microbiology, Centre Algatech, Czech Academy of Sciences*

Trichodesmium spp. is a marine filamentous non-heterocystous cyanobacterium, and one of the most abundant nitrogen (N_2) fixers in the ocean, with an estimated contribution of up to 50 % of the biologically fixed N_2. This cyanobacterium lacks specialized N_2-fixing cells (heterocysts), and the mechanism used to protect their nitrogenase (the enzyme that catalyzes N_2 fixation to ammonia) from deleterious oxygen (evolved during photosynthesis) is not fully elucidated yet. Previous works show that *Trichodesmium* controls these processes through a diel cycle, where N_2 fixation and photosynthesis are temporally segregated in filaments, while other studies indicate the spatial separation of the process through the so-called "diazocytes", or regions of cells dedicated to fixing N_2. However, *Trichodesmium* cells might be able to switch between both processes at shorter timescales than what has been studied before, implying that the spatial segregation of nitrogenase into diazocytes may be reversible.

In this work, we aimed to spatiotemporally resolve the N_2 and C fixation processes in *Trichodesmium* filaments by analyzing ^{15}N and ^{13}C uptake dynamics using stable isotope probing (SIP) combined with Nanoscale secondary ion mass spectrometry (NanoSIMS), a surface analysis technique with the capability to probe elements and isotopes at high spatial resolution (~50 nm). *Trichodesmium erythraeum* strains IMS101 and NIBB1067 were incubated in YBCII medium with added ^{13}C-$NaHCO_3$ and $^{15\text{-}15}N_2$ for 30 minutes during the peak N_2-fixing period. Afterwards, the cells were collected on a polycarbonate membrane, chemically fixed (glutaraldehyde) and air-dried prior to NanoSIMS analysis.

Our NanoSIMS results show that both *Trichodesmium* strains follow distinct ^{15}N and ^{13}C fixation strategies. The pattern in strain NIBB1067 is generally more consistent, showing either ^{15}N or ^{13}C fixation along most cells in single filaments, whereas strain IMS101 shows a more heterogeneous pattern, with some sections of continuous cells fixing ^{15}N or ^{13}C in the same filament, but in some cases, both isotopes are present in the same cells. The latter could suggest fast switching between the C and N_2 fixation processes, or rapid nutrient transportation between contiguous cells, which has not been observed before. These results agree with previous light microscopy analyses using Lugol's iodine (a carbohydrate dye); however, they challenge the role and prevalence of "diazocytes" as postulated previously, suggesting a more dynamic interplay of both processes in *Trichodesmium erythraeum* sp.