

Development of diazotrophic biofilm on plant roots under changing climate fixes atmospheric nitrogen and restricts arsenic mobility, enhancing plant anatomy

DR. ARNAB MAJUMDAR, PH.D., MRSB, AMRSC, IOLY KOTTA-LOIZOU AND MARTIN BUCK

Imperial College London

Nitrogen use efficiency in crops is a global concern due to the low nitrogen availability to plants. Microbial nitrogen fixation is a potential solution to this problem; however, the stability of nitrogen fixers in natural soil profiles is unpredictable. Fixing atmospheric nitrogen in bacterial biofilm on the plant roots is not fully understood. Also, hazardous soil elements like arsenic are a global threat to human health that is linked to soil-crop quality as well¹. Limited studies are available that indicate the possibility of diazotrophic biofilm development on plant roots with N-fixation in a natural environment². This study explored the optimum conditions for diazotrophic N-fixation and defined molecular aspects to enhance the process. Diazotrophic bacteria *Klebsiella oxytoca* was used to confront these dual issues by implementing its ability to form biofilms³. Wild-type *K. oxytoca* M5a1 and the Δ AmtB mutant were genetically modified to express biofilm-developing genes (BssR and BssS), cyanase (CynS, CynX) and vacuolar arsenic sequestration gene (ArsR). Variable temperature, precipitation, pH, and CO₂ concentrations were applied to mimic changing climatic effects. Cyanate, a naturally present transformative chemical, was also effectively used for additional ammonium production. These resulted in a higher translocation of available nitrogen from bacterial biofilm to the plant roots, detected using the N¹⁵ isotope in a secondary ion mass spectrometer. The biofilm also restricted the release of sequestered arsenic in vacuoles, as confirmed by differential cell elemental analysis. Plant vascular structures and root anatomy were closely monitored under these variable treatments and found to be structurally maintained compared to the control and stressed plants. Electron microscopy and energy-dispersive X-ray confirmed the structural rigidity and elemental distribution before and after the application of enhanced *K. oxytoca* M5a1 and Δ AmtB mutant, indicating their potential implementation in agro-environmental aspects as bioagents.

1. Majumdar, A., et al., 2024. J. Haz. Mater. 466, 133610.
2. Danhorn, T. and Fuqua, C., 2007. Annu. Rev. Microbiol., 61(1), 401-422.
3. Waite, C.J., et al., 2021. Front. Microbiol. 12, 718487

