Webinar Q&A
Engineering nitrogen-fixing microbial communities associated with maize and sorghum roots
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The webinar recording is available on the Phytobiomes Alliance YouTube channel at https://youtu.be/ici1k20pWJM?si=cMmMhODGyRutPcyS

Q: why is fitness affected in engineered bacteria with higher ammonia release?

This fitness decrease is probably due to the large amount of ATP the nitrogenase enzyme consumes.

Q: How is the behavior of these engineered strains in field conditions? Could more natural conditions affect their fitness negatively?

Their fitness decrease makes them less competitive, and their abundance decreases over the weeks and months after planting.

Q: if the engineered bacterium cannot establish itself well in the plant rhizosphere, then what is the relevance of its use in agricultural systems?

These genetically modified diazotrophs can grow on crop roots, but we aim to enhance their competitiveness in the rhizosphere to supply nitrogen for as long as the plants require.

Q: Hi Jean, Great talk! I was wondering about the nitrogen fixation by the gram positive diazotrophs such as Paenibacillus and what could be the target sites for engineering these bacteria? Do you agree if these bacteria still remain uncharacterized in terms of their nif gene clusters?

Yes, absolutely. Gram-positive diazotrophs are of great interest to us due to their longer shelf life, but they also present challenges.

Q: what was the basis of selecting strains for constructing community/syncom?

We aim to recreate the composition and functions of the broader community. Check out this excellent review by Julia Vorholt on the subject: https://www.cell.com/cell-host-microbe/fulltext/S1931-3128(17)30287-1

Q What are the key challenges in engineering associative diazotrophs for biological nitrogen fixation, and how can biorthogonal signaling address these challenges? appreciate your answer in advance

The goal is to trigger the release of ammonium only when the plant is present, and not in culture or away from the plant, in order to limit fitness defects when not necessary and potential environmental impacts.

Q: To what extent does the host plant influence nitrogenase activity in colonizing bacteria, and is enhancing carbon supply from the plant to the bacteria also necessary

Growth and nitrogenase activity can be influenced by the amount of carbon available to the diazotrophs. For example, this is why nitrogen fixation rates are high in the mucilage produced by aerial roots.

Q: can you tell us more about the observations you've had regarding the poor translation between lab and field?

Answered Live at 49:44

Q: any maybe speculate about why the translation is so poor?

Too many technologies and products have been primarily tested in the greenhouse and not under field conditions.

Q: Is there any plant derived signal that can also be used as a "helper" for enhanced N fixation? Have you explored that possibility?

Answered Live at 52:35

Q: You mentioned checking the results of N-fixing bacteria in the real world (field). How do you think this might affect the N cycling metabolic pathways of the rhizosphere? Additionally, is there any plan to synergistically retain the produced N to favor plant uptake?

Answered Live at 53:18

Q: You mentioned about diazotroph helper bacteria. Are there any bacterial helpers already known and how do they help diazotroph? do you have any hypothesis about mutual benefits between the helper and the fixer?

Other labs have already isolated helpers of diazotrophs. One proposed mechanism is the reduction of oxygen tension. This may be the case in some associations, but we have evidence that other mechanisms are also involved. See: https://www.nature.com/articles/s41467-022-31113-w

Q: how in microcosm experiments, the engineered microbe or synthetic community is behaving?

Answered Live at 54:25

Q: How long Klebsiella remain stable/effective throughout one farming season?

Answered Live at 56:26

Q: Thanks for the great talk. You mentioned the co-operation of bacteria. I was wondering how the interaction or communication of the bacteria is happening (mechanisms).

We believe that the exchange of metabolites between the bacteria plays a role in their cooperation. For example, DAPG produced by Pseudomonas enhances nif gene expression in Azospirillum. See: https://apsjournals.apsnet.org/doi/10.1094/MPMI-07-10-0148 . However, that may not be the only mechanism.

Q: What are the requirements for BNF in the phyllosphere?

Same as in the rhizosphere: low oxygen tension protects the nitrogenase while allowing aerobic respiration, carbon availability to the bacteria, and nitrogen transfer from diazotroph to the plant.

Q: I have a question regarding your diazotroph and AM fungi approach. You did not see any increase in N content taken up by the plant upon inoculation with your engineered strain. However, the addition of AM fungi essentially increased the N content if I understood it correctly. Do you think this is because the bacteria don't colonize the plant well and the AM hyphae networks enable the higher distance transport of the released fixed nitrogen, or is it that the hyphae serve as hub for the bacteria bringing them in closer proximity to the roots essentially bringing the released N closer to the root? Or is it another mechanisms you are thinking of?

It is possible that these mechanisms are at play. Another factor to consider is that diazotrophs release ammonium, and maize has a strong preference for nitrate over ammonium. We are speculating that maize may require more assistance from AM fungi to obtain ammonium compared to nitrate, but we have not yet conducted tests to confirm this.

Q: What do you think is the new direction or challenge in the research of synthetic communities?

I find the perspective of creating metabolic models for SynCom and plants to be really fascinating. It's also exciting to think about developing SynCom with both bacteria and fungi. I believe it's important for us to focus on enhancing models in order to accurately translate predictions from SynComs to native (field) communities.

Q: in your opinion, how much synthetic N replacement is acheivable with improved diazotrophs?

8-10% can be achieved in our current maize hybrids. It may be possible to reach 15-20% in the future, but we will likely need different plant cultivars for higher percentages.

Q: What is the best method in your opinion to evaluate nitrogen fixation in green house experiments?

It's important to use a combination of techniques when studying diazotroph fixation and benefits for your crops. In the greenhouse, you can use techniques such as 15N isotope dilution, 15N natural abundance, and nitrogen balance. For ureide legumes like soybeans, ureide quantification is a good option. If your plants are small enough, 15N gas enrichment and ARA are also viable techniques. It's crucial to use multiple techniques to draw accurate conclusions, as each technique has its own advantages and disadvantages. Additionally, it's important not to rely solely on greenhouse experiments, as their findings may not always apply to field conditions.

Q: What could be the best way to applied these modified bacteria into the soil, by adhering to the seed or inoculated directly in soils?

The use of seed treatments varies depending on the crop, but they are widely accepted as a simple and effective solution for many crops.

Q: Can adding started C be helpful?

There is evidence that addition of carbon to the soil boosts microbial activity including nitrogenase activity but it is not always a cost effective method and could have negative consequences too. See: https://www.frontiersin.org/articles/10.3389/fenvs.2018.00160/full

Q: as there is the phenomena of horizontal gene transfer quite evident in case of bacteria . How successful do u think engineering these bacteria will be?

Horizontal gene transfer from engineered strains to native strains may occur. However, the modifications we make (currently) significantly decrease the bacteria's fitness, so the recipient's fitness will decrease, too, and they are likely to be wiped out quickly. All the edits we make in diazotrophs benefit the plant but not the bacteria. Transfer from native strains to engineered ones may occur, but the engineered ones would be inefficient again for the plant. The potential risks of such transfers, and their implications, are areas that require careful consideration.