

Creating representative microbial communities to promote biological nitrogen fixation on sweet sorghum

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Project Goals: We aim to promote biological nitrogen fixation on sweet sorghum, a target crop for biofuel production, using a systems biology-based approach involving both the plant and associated microbes. On the microbial side, we plan to do this by 1) *isolating and characterizing microbes associated with sorghum aerial roots*, 2) *forming representative communities from these isolates and investigating community dynamics and functions by building computational models to predict their behaviors*, and 3) *testing the efficacy of these synthetic communities to promote biological nitrogen fixation, and, by extension, sorghum health, and growth.*

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Biological nitrogen fixation (BNF) on cereal crops to increase crop production sustainability and reduce environmental damage from synthetic fertilizers has long been an agronomical Holy Grail. Cereal crops cannot form symbiotic relationships with nitrogen-fixing rhizobia. They rely on synthetic fertilizer inputs and less efficient associative nitrogen fixation or free-living nitrogen fixation from soil microbes¹. Some indigenous maize accessions from Central America can obtain 29%-82% of their nitrogen from the air through BNF². This 'nitrogen-fixing corn' recruits environmental diazotrophic microbes to live around its aerial roots, where root border cells produce mucilage that provides a low oxygen, sugar-rich niche suitable for BNF. Similar aerial root production and BNF have also been noticed in sorghum accessions but have not been characterized like maize. Since sorghum is a crop of interest for biofuel production, breeding this aerial root trait into more sorghum lines and optimizing BNF on aerial roots are target areas for research and development. On the microbial side, we aim to 1) *isolate and characterize microbes associated with sorghum aerial roots*, 2) *form representative communities from these isolates and observe community dynamics through model building*, and 3) *test the efficacy of these synthetic communities on protecting sorghum health and promoting biomass accumulation*. Currently, we have a diverse environmental isolate collection of ~90 strains selectively isolated on artificial mucilage media from aerial root producing corn and sorghum lines. With additional mucilage microbiome data, we may add more strains if we feel our selection missed key community members. All environmental isolates will be screened for plant growth-promoting phenotypes, such as nitrogen fixation, auxin and siderophore production, and polysaccharide catabolism. Further, we will examine the conditions under which these environmental diazotrophs release ammonia or nitrate to the plant via the mucilage and the survival of these strains around aerial roots through wet/dry cycles. Using high-throughput, combinatorial, *in vitro* community assembly experiments, we will measure temporal changes in community composition and ammonia production. These data will be used to build computational models at different resolutions to elucidate keystone bacterial species in community growth, survival, and

BNF, and decipher the microbial and metabolic interactions driving ammonia production. Our experimental data, coupled to computational models, will guide the selection of defined communities that can be studied in the context of sorghum accessions. With these defined communities we will confirm survival, nitrogen fixation, and promotion of sorghum nutrition, growth, and health in the field.

Acknowledgments

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References

- 1 Pankievicz, V. C. S., Irving, T. B., Maia, L. G. S. & Ané, J.-M. Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops. *BMC Biol* **17**, 99, doi:10.1186/s12915-019-0710-0 (2019).
- 2 Van Deynze, A. *et al.* Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS Biol* **16**, e2006352, doi:10.1371/journal.pbio.2006352 (2018).