

- a. Research Project Title: Potential for combatting iron deficiency chlorosis with the soybean microbiome FY24.**

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- b. Research Overview and Objectives**

Iron deficiency chlorosis (IDC) is a wide-spread problem strongly affecting soybean production in North Dakota. The characteristic yellowing of plant leaves suffering from IDC is caused by a lack of chlorophyll formation due to poor function of iron-requiring enzymes involved in chlorophyll biosynthesis. North Dakota soils normally contain more than enough iron for plant function, however much of the iron is not in soluble form needed by the plant. A reduction in iron solubility at high soil pHs caused by high levels of CaCO_3 (lime) in the top-soil is the main cause of IDC; while the iron is there, it isn't available to the plant. High lime soil is common in North Dakota, and IDC is exacerbated by salinity which is also becoming more and more common.

In this study we aim to build on previous work to assess the potential of the soybean microbiome as a new tool to combat IDC. In FY22, in a study that analyzed four fields in Eastern ND with varying levels of IDC, we observed a significant correlation in the structure of the soybean root and rhizosphere microbiome with the IDC level of the soil (see FY22 report). We hypothesize that unique groups of microbes that are enriched under IDC conditions could help alleviate IDC in soybeans when cultured and used as inoculants along with root nodule forming rhizobia. We have already optimized a greenhouse assay that will be suitable for measuring growth potential of microbiome members, and a plate screening assay that can identify siderophore producers (microbe-produced iron solubilizing molecules that function like Fe-chelating fertilizers). With this study we aim to utilize these resources to attempt to identify individual microbes or groups of microbes, cultures from the IDC soybean microbiome that could have a beneficial effect to soybean plants grown under IDC conditions.

Objectives:

Objective 1) Culture a 100-member community of North Dakota microbes from the soybean microbiome.

Objective 2) Siderophore production screen from members of soybean microbial community.

Objective 3) Evaluate reduction of IDC from microbial inoculants with an optimized "Goos" Greenhouse assay.

- c. Completed Work:**

All objectives are still ongoing.

d. Progress of Work and Results to Date:

Objective 1) Culture a 100-member community of North Dakota microbes from the soybean microbiome.

We have successfully repeated field trials from FY22 and utilized them to perform high throughput culturomics from IDC and non-IDC fields. The Leonard location showed typical symptoms of high levels of IDC as observed in previous years and was used for the IDC field culturomics, whereas the Casselton field showed no IDC symptoms and was used as a non-IDC control. We made a slurry from roots of soybeans grown in each field, and cultured the microbiome through a dilution to extinction approach. We then used a barcoded next-generation sequencing strategy to identify the microbes that were cultured in this way. Next, identified microbes are purified and stocked in pure culture for future experimentation after a second, independent verification by sequencing their full-length 16S gene. We are currently in the process of this part of the culturing effort, and nearing completion (Table 1). In total we are aiming for 100 isolates for downstream screening and are on track to finalize such a collection. This collection will be used for Objectives 2 and 3 once finalized.

Table 1. Microbes in the process of culturing for Objective 1.

Genus	Status	Location
<i>Variovorax robiniae</i>	Stocked	Casselton (No IDC)
<i>Pseudomonas silesiensis</i>	Stocked	Casselton (No IDC)
<i>Pseudomonas cerasi</i>	Stocked	Casselton (No IDC)
<i>Pseudomonas koreensis</i>	Stocked	Casselton (No IDC)
<i>Phyllobacterium ifriqiense</i>	Stocked	Casselton (No IDC)
<i>Pseudomonas oryzihabitans</i>	Stocked	Casselton (No IDC)
<i>Lysobacter antibioticus</i>	Stocked	Casselton (No IDC)
<i>Pantoea agglomerans</i>	Stocked	Casselton (No IDC)
<i>Chryseobacterium gregarium</i>	Stocked	Casselton (No IDC)
<i>Paeniglutamicibacter sulfureus</i>	Stocked	Casselton (No IDC)
<i>Paenarthrobacter nitroguajacolicus</i>	Stocked	Casselton (No IDC)
<i>Cellulomonas cellasea</i>	Stocked	Casselton (No IDC)
<i>Variovorax paradoxus</i>	Stocked	Casselton (No IDC)
<i>Aeromicrobium ginsengisoli</i>	Stocked	Casselton (No IDC)
<i>Aeromicrobium ginsengisoli</i>	Stocked	Casselton (No IDC)
<i>Variovorax paradoxus</i>	Stocked	Casselton (No IDC)
<i>Variovorax paradoxus</i>	Stocked	Casselton (No IDC)
<i>Variovorax ureilyticus</i>	Stocked	Casselton (No IDC)
<i>Massilia agri</i>	Stocked	Casselton (No IDC)
<i>Rhodococcus qingshengii</i>	Stocked	Casselton (No IDC)
<i>Curtobacterium pusillum</i>	Stocked	Casselton (No IDC)
<i>Pseudorhodofera soli strain TBEA3</i>	Stocked	Casselton (No IDC)
<i>Polaromonas eurypsychrophila</i>	Stocked	Casselton (No IDC)
<i>Bacillus proteolyticus</i>	Stocked	Casselton (No IDC)
<i>Pseudarthrobacter sulfonivorans</i>	Stocked	Leonard (High IDC)
<i>Ensifer adhaerens</i>	Stocked	Leonard (High IDC)
<i>Pseudoxanthomonas japonensis</i>	Stocked	Leonard (High IDC)
<i>Cellvibrio ostraviensis</i>	Stocked	Leonard (High IDC)
<i>Pseudomonas brassicacearum</i>	Stocked	Leonard (High IDC)
<i>Hydrogenophaga intermedia</i>	Stocked	Leonard (High IDC)
<i>Ferrovibrio</i>	putatively culturable	Leonard (High IDC)
<i>Ferrovibrio</i>	putatively culturable	Leonard (High IDC)
<i>Ferrovibrio</i>	putatively culturable	Leonard (High IDC)
<i>Pseudomonas</i>	putatively culturable	Leonard (High IDC)
<i>Bosea</i>	putatively culturable	Leonard (High IDC)
<i>Bradyrhizobium</i>	putatively culturable	Leonard (High IDC)
<i>Asticcacaulis</i>	putatively culturable	Leonard (High IDC)
<i>Ensifer</i>	putatively culturable	Leonard (High IDC)

Genus	Status	Location
<i>Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium</i>	putatively culturable	Leonard (High IDC)
<i>Pseudoxanthomonas</i>	putatively culturable	Leonard (High IDC)
<i>Pseudoxanthomonas</i>	putatively culturable	Leonard (High IDC)
<i>Pseudoxanthomonas</i>	putatively culturable	Leonard (High IDC)
<i>Pseudoxanthomonas</i>	putatively culturable	Leonard (High IDC)
<i>Lysobacter</i>	putatively culturable	Leonard (High IDC)
<i>Lysobacter</i>	putatively culturable	Leonard (High IDC)
<i>Lysobacter</i>	putatively culturable	Leonard (High IDC)
<i>Sphingobium</i>	putatively culturable	Leonard (High IDC)
<i>Sphingopyxis</i>	putatively culturable	Leonard (High IDC)
<i>Novosphingobium</i>	putatively culturable	Leonard (High IDC)
<i>Sphingomonas</i>	putatively culturable	Leonard (High IDC)
<i>Chitinophaga</i>	putatively culturable	Leonard (High IDC)
<i>Pseudoflavitalea</i>	putatively culturable	Leonard (High IDC)
<i>Taibaiella</i>	putatively culturable	Leonard (High IDC)
<i>Chryseobacterium</i>	putatively culturable	Leonard (High IDC)
<i>Dyadobacter</i>	putatively culturable	Leonard (High IDC)
<i>Dyadobacter</i>	putatively culturable	Leonard (High IDC)
<i>Microbacterium</i>	putatively culturable	Leonard (High IDC)
<i>Pseudarthrobacter</i>	putatively culturable	Leonard (High IDC)
<i>Microbacterium</i>	putatively culturable	Leonard (High IDC)
<i>Microbacterium</i>	putatively culturable	Leonard (High IDC)
<i>Cellulomonas</i>	putatively culturable	Leonard (High IDC)
<i>Paenibacillus</i>	putatively culturable	Leonard (High IDC)
<i>Agromyces</i>	putatively culturable	Leonard (High IDC)
<i>Agromyces</i>	putatively culturable	Leonard (High IDC)
<i>Aeromicrobium</i>	putatively culturable	Leonard (High IDC)
<i>Rhizobacter</i>	putatively culturable	Leonard (High IDC)
<i>Rhizobacter</i>	putatively culturable	Leonard (High IDC)
<i>Methylibium</i>	putatively culturable	Leonard (High IDC)
<i>Variovorax</i>	putatively culturable	Leonard (High IDC)
<i>Variovorax</i>	putatively culturable	Leonard (High IDC)
<i>Methylibium</i>	putatively culturable	Leonard (High IDC)
<i>Ramlibacter</i>	putatively culturable	Leonard (High IDC)
<i>Variovorax</i>	putatively culturable	Leonard (High IDC)
<i>Xylophilus</i>	putatively culturable	Leonard (High IDC)
<i>Limnohabitans</i>	putatively culturable	Leonard (High IDC)
<i>Paucibacter</i>	putatively culturable	Leonard (High IDC)
<i>Acidovorax</i>	putatively culturable	Leonard (High IDC)
<i>Roseateles</i>	putatively culturable	Leonard (High IDC)
<i>Acidovorax</i>	putatively culturable	Leonard (High IDC)
<i>Aquicola</i>	putatively culturable	Leonard (High IDC)
<i>Hydrogenophaga</i>	putatively culturable	Leonard (High IDC)
<i>Massilia</i>	putatively culturable	Leonard (High IDC)
<i>Duganella</i>	putatively culturable	Leonard (High IDC)
<i>Microbacterium</i>	putatively culturable	Leonard (High IDC)
<i>Mycetocola</i>	putatively culturable	Leonard (High IDC)
<i>Pseudorhodofera</i>	putatively culturable	Leonard (High IDC)
<i>Hydrogenophaga</i>	putatively culturable	Leonard (High IDC)
<i>Polaromonas</i>	putatively culturable	Leonard (High IDC)
<i>Marmoricola</i>	putatively culturable	Leonard (High IDC)
<i>Streptomyces</i>	putatively culturable	Leonard (High IDC)
<i>Chitinophaga</i>	putatively culturable	Leonard (High IDC)

e. Work to be Completed:

Objectives 2 and 3 will be performed once the complete microbial culture collection is finalized, we are on pace to complete these by June 2024.

f. Other Relevant Information:

None to add to above progress.

g. Summary:

We have made good progress culturing microbes from the soybean root and rhizosphere microbiome. Based on our previous data that soybeans recruit unique microbial communities under IDC, we hypothesize we will identify Siderophore producers from these microbes once purified, and are hopeful to attain proof-of-concept that the microbes are capable of alleviating IDC when applied to soybeans in the greenhouse.