Title: Can calcite dissolving bacteria promote peanut pod growth?

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2019 Brief & concise statement of objectives

1. Isolate calcite dissolving bacteria (CDB) from Georgia peanut field using a plate screening assay.
2. Test the efficacy of CDB strains in promoting peanut pod growth.
3. Determine the species of potential CDB strains.

2019 Procedures & plan of investigation

The following tasks were proposed in Year 1.

Collect bacterial population (completed). We have collected soil samples from the Blackshank Farm, Tifton. The soil samples were from fields with or without peanut-planting history.

Screening for calcite dissolving bacteria (completed). To isolate CDB strains, we tested four different media: a CDB media reported by Tamilselvi et al 2016, a calcite agar media reported by Li et al., 2011, a 1/10th LB media, and a 1/5th KB media. The calcite agar media gave us the most clear and robust results, and we isolated 65 CDB strains from fields with peanut-planting history in the Blackshank Farm. Representative CDB strains on calcite agar media are shown in the Figure 1. A clear zone surrounding bacterial colony indicates dissolving of calcite. We further quantified the CDB's ability to dissolve calcite using a solubility index.

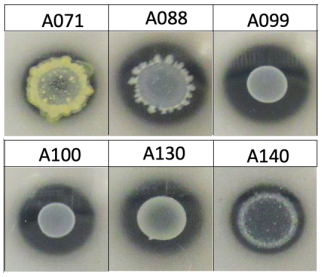

Figure 1: Representative CDB strains on calcite agar media.

Measure pod growth with calcite dissolving bacteria (In progress). We have established a growth-in-tube system to test pod-specific requirement of calcium. Pods developed in tubes are comparable to those grown in open soil. We will use this system to test the impact of CDB strains on pod and seed development.

Determine the species of CDB strains (In progress). We have established a method to extract DNA from CDB and amplified 16s RNA genes. We sequenced the 16s rRNA genes from 14 strains, and determined the bacteria genus. As expected, some CDB strains have identical 16s sequence, strongly indicating that they are the same species. This information will help us to narrow down the number of CDB strains to use in the soil test and growth promotion tests. A list of 14 CDB strains and their species are shown in the Figure 2.

CDB ID	Species
A017	*Bacillus manliponensis*
A018.2	*Megasphaera cerevisiae*
A027	*Bacillus manliponensis*
A062	*Paenibacillus borealis*
A070	*Enterobacter aerogenes*
A072	*Buttiauxella ferragutiae*
A073	*Buttiauxella ferragutiae*
A082	*Streptomyces rochei*
A083	*Bacillus circulans*
A095	*Paenibacillus macerans*
A099	*Buttiauxella ferragutiae*
A100	*Buttiauxella ferragutiae*
A130.2	*Cellulomonas massiliensis*
A133	*Bacillus fumarioli*

Figure 2: species of CDB strains

The following task was not proposed in Year 1.

Test the efficacy of CDB strains to increase calcium level (In progress). We measured calcium concentration from field soil (around 200ppm), potting mix (around 12,000ppm) and construction sand (around 40ppm). The construction sand has a low level of soluble calcium, which provides a sensitized background to test the efficacy of CDB strains to increase calcium levels. In the two strains we tested, one CDB strain (A99) increased soluble calcium level in sand from 42 ppm to 151 ppm, indicating its potential to promote calcium availability (Figure 3).

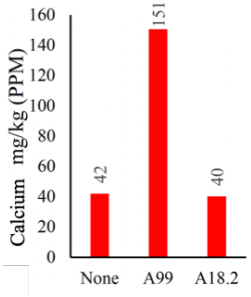

Figure 3: A99 increases calcium level in sand.