The Role of Calcite-Dissolving Bacteria in Promoting Sustainable Peanut (*Arachis hypogaea*) Production.

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Abstract

Calcium is an essential macronutrient for proper growth and development of peanut (Arachis hypogaea). Calcium plays a significant role in cell wall development, nutrient uptake, and embryo formation. Calcium deficiency in peanut development leads to embryo abortion, reduced yields, and increased susceptibility to pathogens. To mitigate calcium deficiency various strategies have been employed, including the use of plant growth-growth promoting rhizobacteria (PGPRs). PGPRS are a diverse group of beneficial bacteria that are known to enhance plant growth and provide various benefits to plants. One of these PGPRs are Calcite Dissolving bacteria (CDB), which are capable of dissolving calcite, a mineralized form of calcium, producing and facilitating the uptake of calcium by the peanut plant. CDB have been isolated from soil in southern Georgia peanut fields and identified through 16s rRNA Sanger and whole-genome Illumina sequencing. To date 117 CDB have been identified and morphologically characterized, including the rate of calcite dissolution. Recent findings have begun to elucidate the CDB dissolution function. The function of CDB's ability to dissolve calcite is unknown, however results indicated that CDB produces an organic acid able to significantly change pH and manipulate their environment. Further investigation into identifying dissolution mechanism will allow us to modify CDB to increase its applications to field production. Our results have shown that application of CDB to calcium deficient soil significantly increases the available calcium by an average of 30% compared to the control. Peanut seeds treated with CDB showed an increased germination rate, a reduction in disease severity caused by soilborne fungal pathogens and provide drought resistance. In conclusion, calcium deficiency can have severe implication for peanut growth and productivity. The application of CDB is a promising strategy to mitigate calcium deficiency and provide an alternative to traditional management strategies.

Objectives

1. Isolate and characterize calcite dissolving bacteria
2. Test efficacy of calcite dissolving bacteria in improving soil health.
3. Study the potential of CDB to limit soilborne fungal pathogens.

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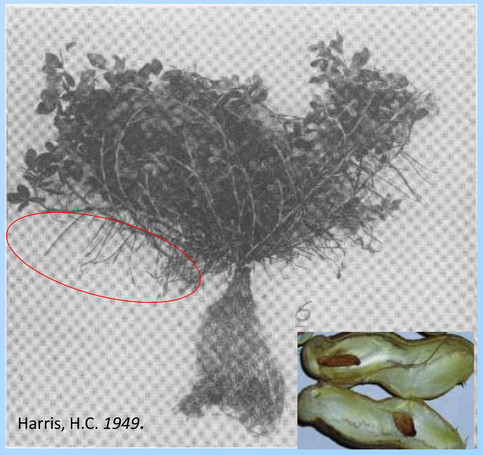
References

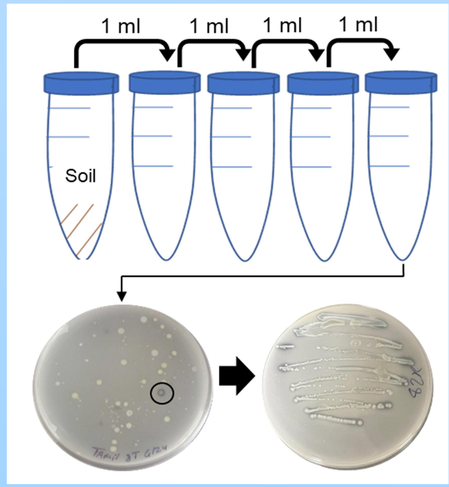
Narasimhulu et al., 1984. *Theor Appl Genet* 69: 87-91
Huang et al., 2017. *Environ Sci Pollut Res* 24:16458–16464
Kachonpadungkitti et al., 1992. *Bioscience, Biotechnology, and Biochemistry*, 56:4,543-546
Dey et al., 2004. *Microbiological Research,* 159, 371—394
Rana et al., 2015. Geomicrobiology Journal, 32:9, 846-852
Tamilselvi et al., 2016 Front Plant Sci, 7:1828

Calcium is Critical for Proper Peanut Development

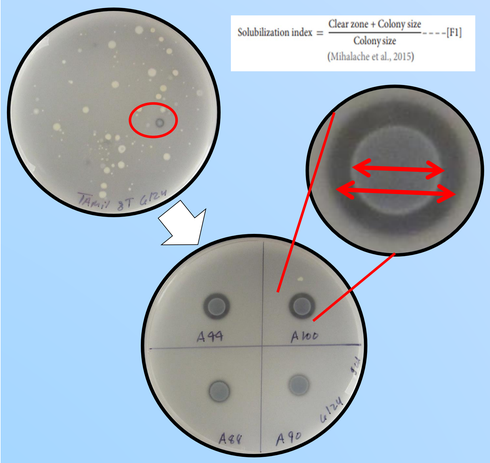


Without Calcium

Isolation and Identification of Calcite Dissolving Bacteria



Soil Isolation of Calcite Dissolving Bacteria

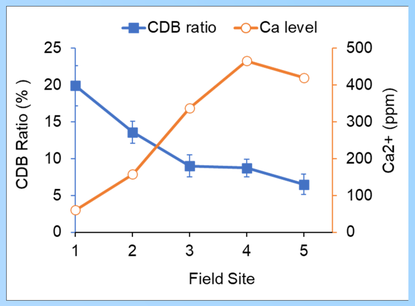


Calculation of Solubility Phenotype

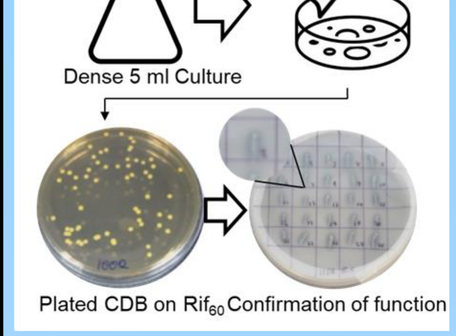
CDB Strain ID	Genus/Species	% Identity	Solubilization Index
17A	*Bacillus luciferensis*	98.57	2.82
18.2A	*Buttiauxella noackiae*	100.00	2.70
54A	*Paenibacillus xylanexedens*	100.00	5.08
62A	*Buttiauxella warmboldiae*	99.77	2.97
70A	*Paenibacillus timonensis*	98.47	3.45
72A	*Enterobacter soli*	99.31	3.38
93A	*Bacillus megaterium*	100.00	5.17
95A	*Cellulomonas hominis*	100.00	4.14
99A	*Lelliottia aquatilis*	99.55	4.77
100A	*Lelliottia amnegena*	96.90	3.60
115A	*Buttiauxella noackiae*	100.00	5.89
130.2A	*Bacillus circulans*	98.87	2.10
140A	*Paenibacillus etheri*	100.00	2.33
141A	*Paenibacillus phocaensis*	100.00	2.88
188A	*Staphylococcus pasteuri*	99.55	5.89

Identification of 117 CDB Through 16s rRNA Sequencing

CDB Population Response to Calcium in Field soil

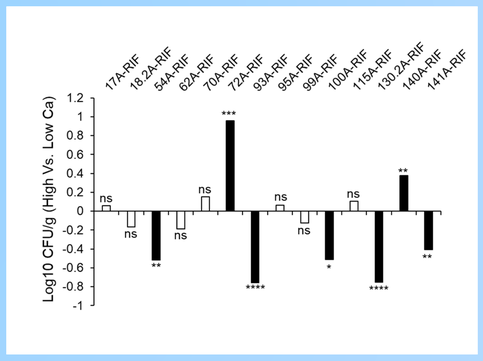


Mutagenesis of CDB: Rifampicin as a Selection Marker



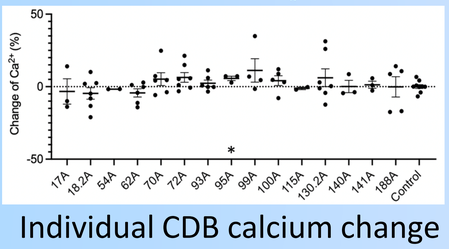
CDB Mutation Workflow

Correlation Between CDB Abundance and Soil Calcium Level

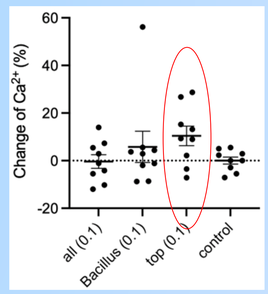


Individual CDB Response to Soil Calcium Level

CDB Alters Soil Calcium Availability

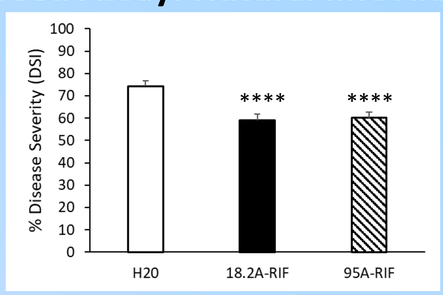


Individual CDB calcium change

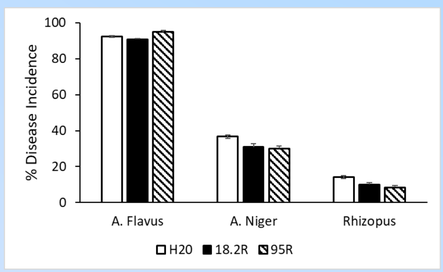


Synthetic CDB Communities Changes Soil Calcium Level

Seed Assay: Natural Infection

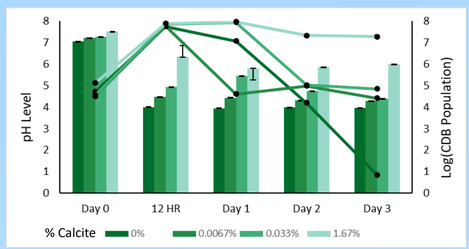


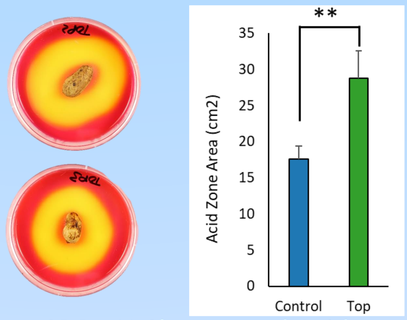
Inhibition of fungal infection with CDB



Incidence of 3 Major Fungi on Peanut Seed After Treatment of CDB

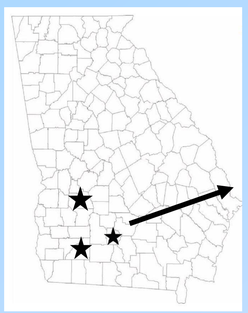
Preliminary Results on CDB Function





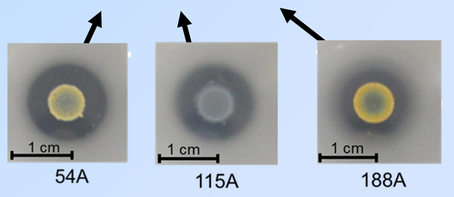
CDB pH Change and Acid Production

Future Work



Development of "Core" and Synthetic Microbiome

Identification of CDB Functional Genes



Application of Calcite Dissolving Bacteria into Peanut Production