Soil Bacteria Protecting People and The Earth

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ABSTRACT:

In spring 2022, two soil bacterium isolates were identified. The Gram + isolate was found to be an antibiotic producer. The Gram – isolate was found to be an environmental specimen that produced enzymes to break down waste. The isolation of these strains was done using microbiology techniques. Isolates were genetically tested using PCR, imaged using gel electrophoresis, and genome analysis were completed. The findings were compared to a database called BLAST and further information was gathered. Both discoveries support the hypothesis of the more diverse our garden soil is the better opportunities to discover beneficial microbial life. These discoveries can be used in all facets of life such as medicine and bioremediation.

INTRODUCTION:

- **Tiny Earth Project: Collect and biochemically test a local soil sample to identify specific bacterium for antibiotic activity. Samples are entered into a global database. It is hoped that future antibiotics and bioremediatory potential can be discovered and utilized from these microbes.**
- **Every bacteria has very specific metabolic attributes. Being able harness components of those attributes-might help us solve current and future antibiotic resistance and environmental concerns.**

Fig. 1 Soil Sample AKBB: **W122°13'39.19",N47°45'2.47"** Soil Sample YV: **47.6497939, -122.0422764**

MATERIALS and METHODS:

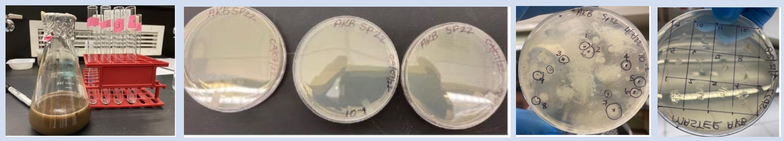


Fig 2. Process of selecting isolates

- **Serial Dilutions and Plating Microbes/master plates**
- **Picking interesting bacteria -> zone of inhibition**
- **Gram staining**
- **Microscopy**
- **PCR-BLAST**

Biochemical tests:

- **TSI; Urease**
- **Citrate; MOI**
- **Motility**
- **Selective and Differential media: EMB, XLD, MacConkey, Starch**

Fig 3: Gel Electrophoresis and PCR (Polymerase Chain Reaction)

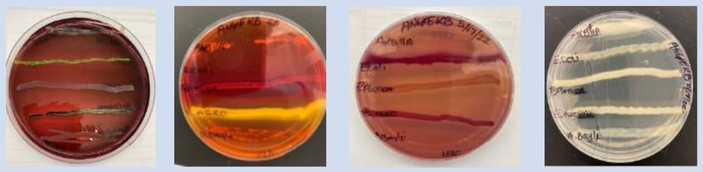
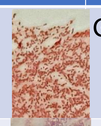
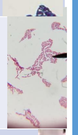
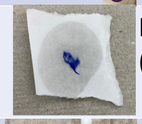


Fig 4: Selective and differential media plates

Biochemical tests	AKB-Hydrocarbon degrader	YV- Antibiotic producer
Gram Stain	Gram negative 	Gram positive 
Endospore Stain	None (vegetative) 	Endospore producer 
Triple Sugar Iron (TSI)	Negative for fermentation 	Positive for fermentation 
Citrate	Positive (uses sodium citrate as carbon source) 	Negative 
Motility	Positive (motile) 	Positive (motile) 
Urease	Negative for producing NH_3 and CO_2 from urea 	Negative for producing NH_3 and CO_2 from urea 
MIO + Kovax	Negative for indole production 	Negative for indole production 
Oxidase	Positive (Aerobe) 	Positive (Aerobe) 
Catalase	Positive (makes catalase enzyme) 	Negative 

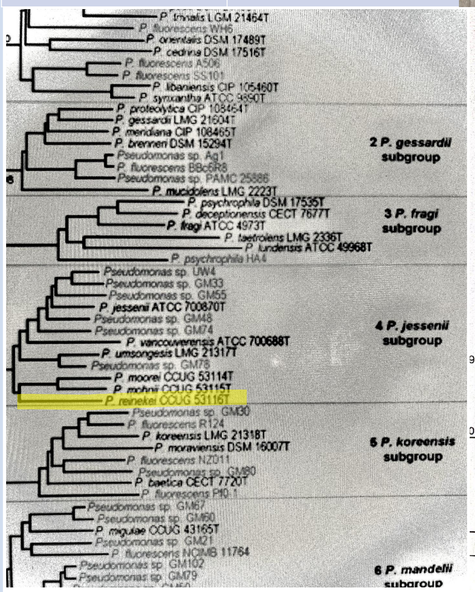


Fig 5: Phylogenetic trees of AKB and YV bacteria

The NCBI identified AKB#11A as~ *Pseudomonas reinekei*
Domain: Bacteria **Phylum:** Proteobacteria **Class:** Gamma proteobacteria
Order: Pseudomonadales **Family:** *Pseudomonas daceae*
Genus: *Pseudomonas* **Species:** *reinekei* (strain: PgBe 182)

The NCBI identified YV#7 as~ *Bacillus mobilis* **Domain:** Bacteria **Phylum:** Firmicutes **Class:** bacilli
Order: caryophanales **Family:** *Bacillaceae*
Genus: *Bacillus* **Species:** *mobilis*

DISCUSSION:

- **Antibiotics are important tools to treat and combat a large variety of microbial infections. Unfortunately, many infectious bacteria and microbes have developed resistance to the antibiotics that are currently used. It is important to discover and develop new antibiotics to combat emerging microbial resistances.**
- **Hydrocarbons are naturally occurring, organic compounds found in fossil fuels. Fossil fuel combustion is the major cause of greenhouse gases. Petroleum biproduct remediation is vital to combat global warming and environmental pollution.**
- **More research needed to further identify specific metabolites for the bacteria.**

CONCLUSION:

- **The entire process using microbiological lab protocols to discover our soil bacterial strains was fascinating!**
- ***Pseudomonas reineke* is a mesophilic bacteria capable of degrading chloroaromatics, chlorosalicylates and some resin acids. Its ability to break hydrocarbon makes it an especially interesting bacteria to further research. *P. reineke's* lipase enzymes are of particular interest to scientists working to remediate petroleum/plastic contamination in the environment.**
- ***Bacillus mobilis* is a highly motile member of the *Bacillus cereus* group. *Bacilli* are found in soil and work in symphony with mycorrhiza to facilitate plant growth in heavy metal contaminated soil. *Bacilli* use biotransformation and bioaccumulation to help plants increase nitrogen, phosphorus and potassium uptake. *Bacilli* strains have also been identified that aid in the bioremediation of aquatic ecosystems by breaking down nitrogen and phosphorus. Along with these bioremediatory capabilities, *Bacillus* species are capable of the production of structurally diverse, secondary metabolites that exhibit wide spectrum antibiotic activity.**

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