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Bacterial Diversity of an Abandoned Mine Land Soil in Southeast Kansas

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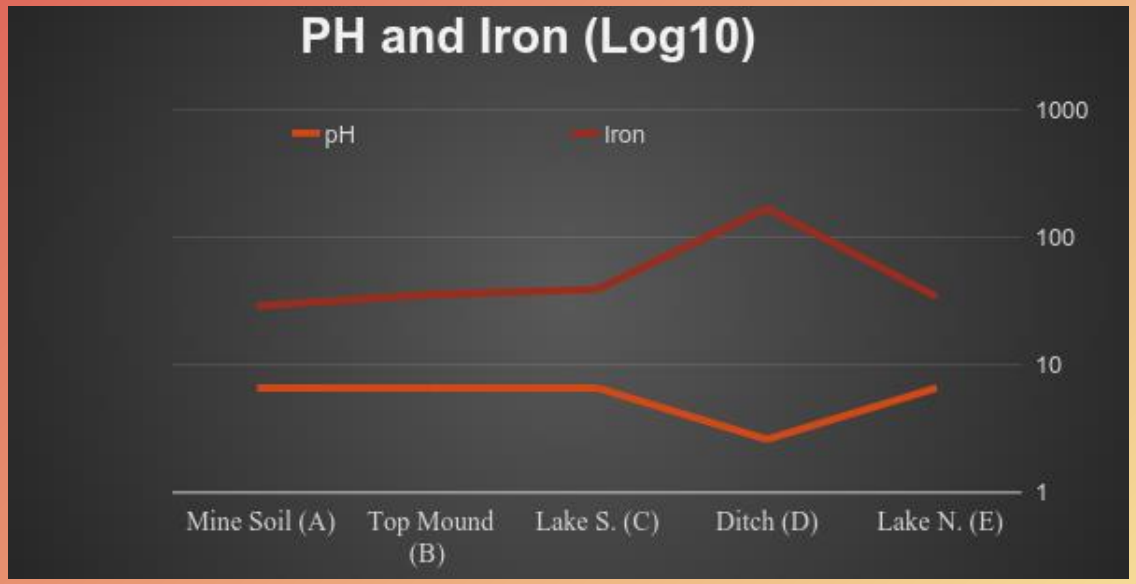
Background

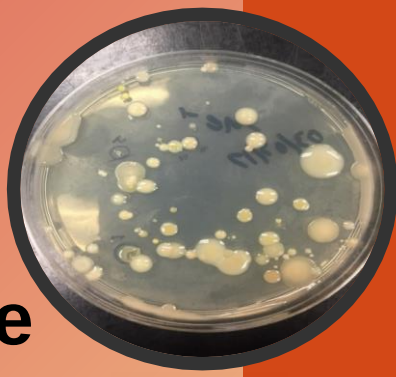
- Acid mine drainage (AMD) occurs from pyrite exposure to water and oxygen
- AMD infiltrates local soil and streams
- Bacterial diversity can indicate ecosystem health
- An ideal site for AMD study is Monahan Outdoor Education Center

Bioremediation

- **Sustainability** is economical, healthy, long-term planning
- Biotic techniques can be combined with abiotic, enhancing change
- Many environmental elements are interrelated, e.g. iron and pH



Why bacteria?



- Environmental conditions **change over time**
  - e.g. pH changes with weather fluctuations
- Additional data, such as a record of bacterial diversity, can make the study more **valid**
- Microbes **are definite indicators** of environmental change
- This **study is novel** in that only physicochemical characteristics have been measured previously

Ghosh et al. (2010). *Environmental Science and Technology* 44:1069-1077.
Chen et al. (2015). *The ISME journal* 9:1579-1592.
Johnson and Hallberg (2005). *Science of the Total Environment* 338:3-14.

Sulfate-reducing bacteria (SRB) and Bioremediatio

Chemistry of AMD

General equations for this process are:

- $2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$
- $4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$
- $4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_3 + 12H^+$
- $FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$

The net effect of these reactions is to release H^+, which lowers the pH, produces sulphate ions.

- H+ ions are released, ferrous iron becomes ferric iron
- At pH levels of 3.5, ferrous hydroxide (yellowboy) forms and H+ ions increase
- SRB could reverse the process of pyrite oxidation

Sheoran and Sheoran (2006). *Minerals Engineering* 19:105-116.
Kalin et al. (2006). *Science of the Total Environment* 366:395-408.

Hypothesis: Differences exist in the bacterial diversity of remediated vs. unremediated sites

Goals of the present study:

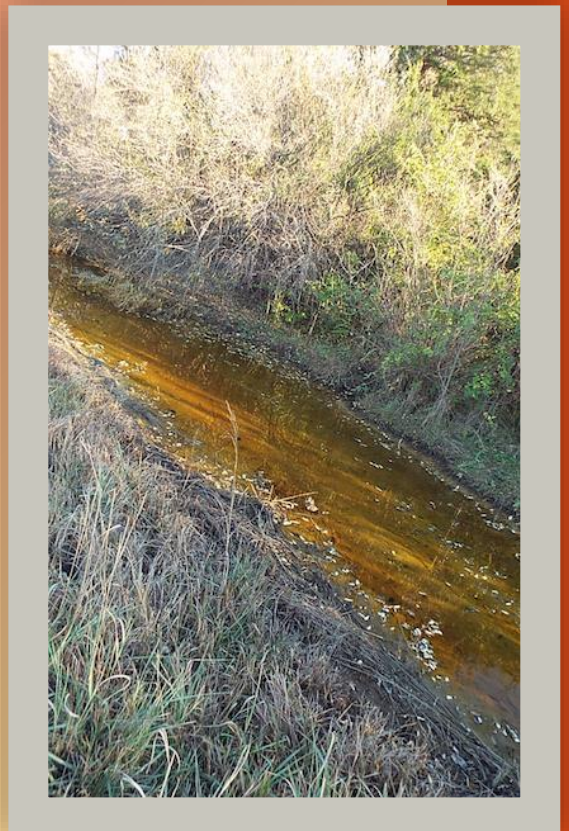
(i) To assess soil bacterial diversity and to evaluate physicochemical characteristics of soil

(ii) To isolate acidophiles

The findings of this study would also help in understanding the timeline of *ecosystem recovery paralleled with wetland development and/or bioremediation.*

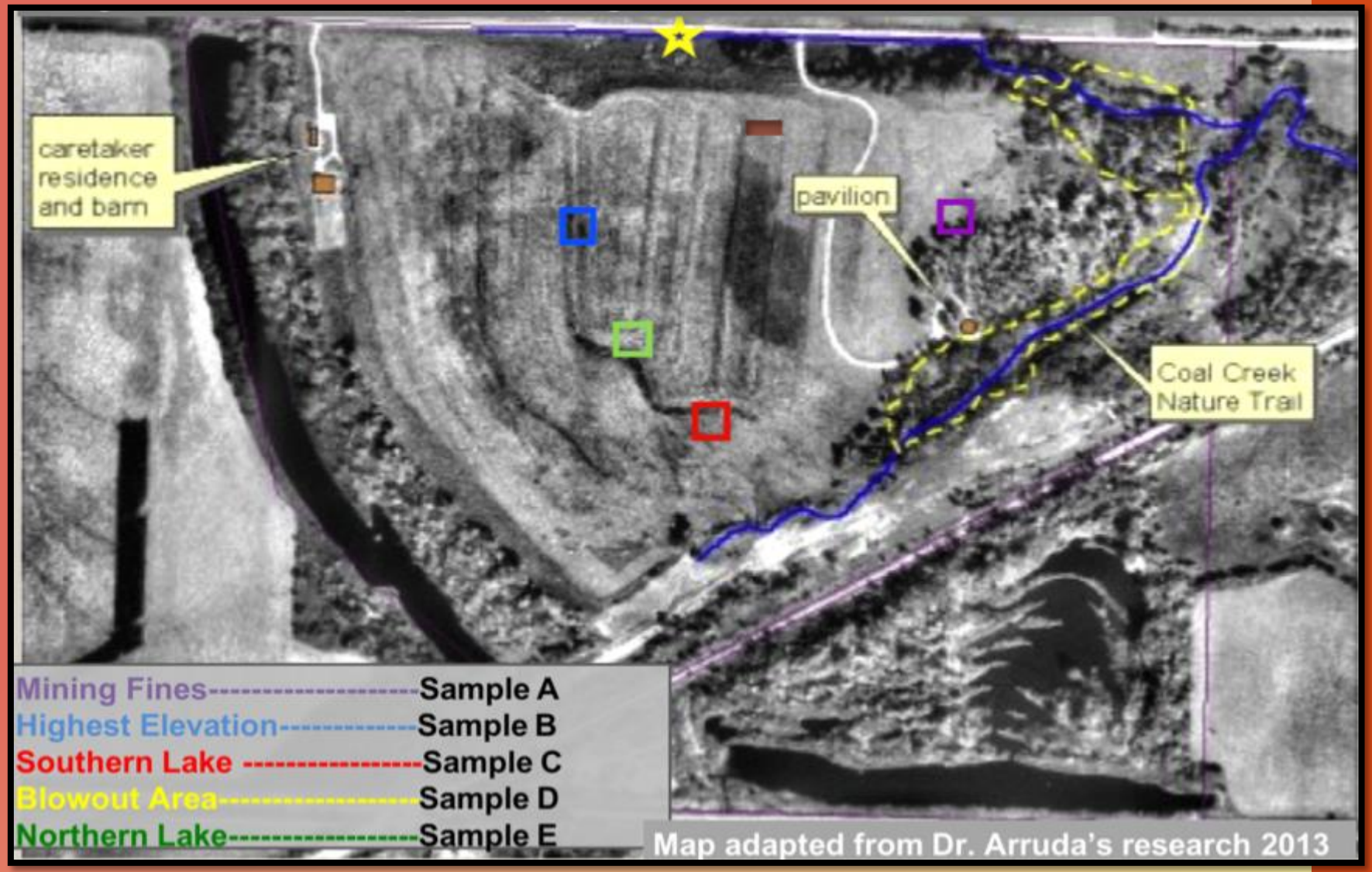
Methods

1. Sample collection
2. Physicochemical analysis
3. Bacterial concentration and identification by biochemical and molecular techniques
4. Isolation of acidophilic bacteria



1. Soil sampling in fall 2015 and summer 2016

Monahan Outdoor Education Center



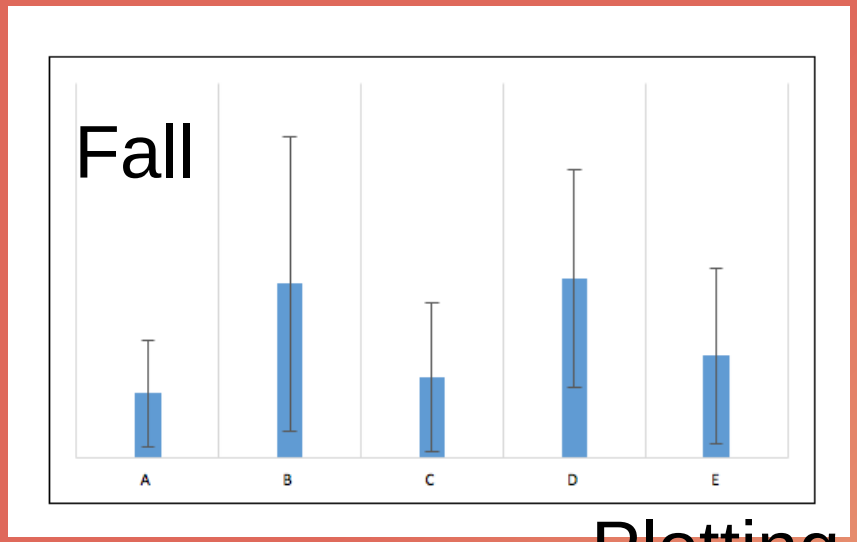
2. Physicochemical analysis

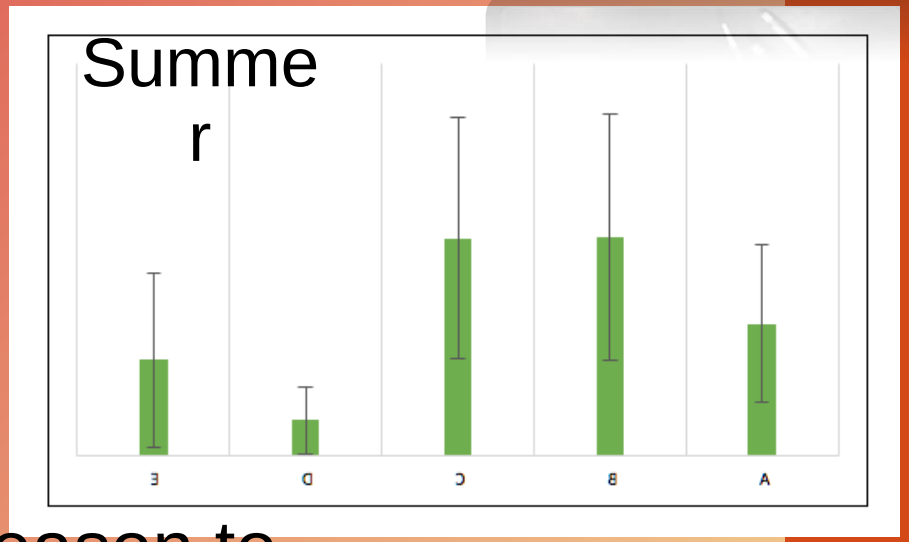
Soil samples digested and analyzed using an ICP OES (inductively-coupled plasma optical emission spectroscopy). [K-State Geology]

Location/ Sample ID	Texture	pH	As (mg/kg)	Mn (mg/kg)	Fe (g/kg)
Mine Soil (A)	Silt	6.6±0.0	10.11	614.69	28.72
Top Mound (B)	Sand/Clay	6.6±0.2	13.82	877.58	35.50
Lake S. (C)	Clay	6.6±0.0	15.52	932.66	38.93
Ditch (D)	Gravel	2.6±0.2	10.39	433.40	169.59
Lake N. (E)	Clay/Silt	6.6±0.0	8.52	997.96	34.13

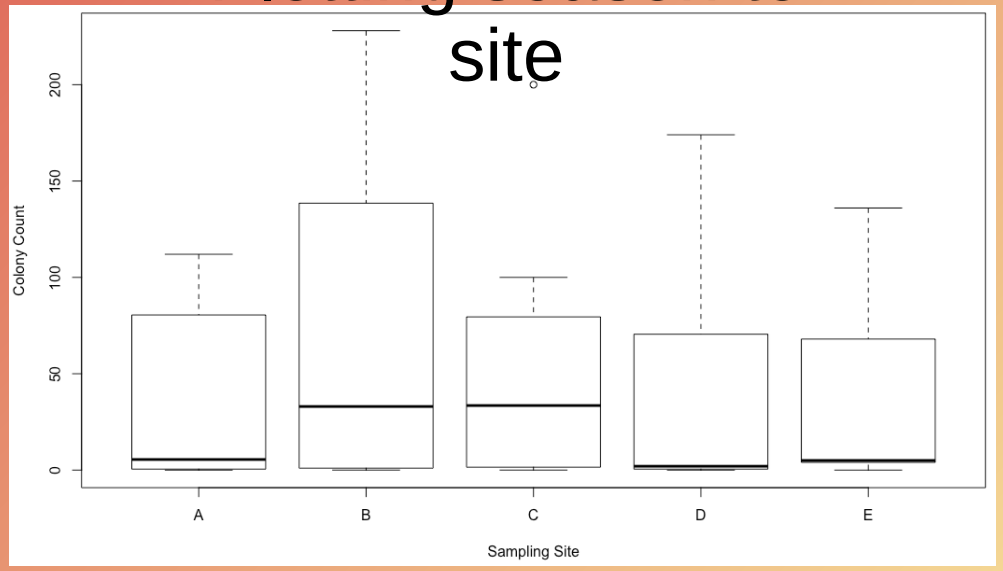
3. Bacterial concentration

Calculated CFU/g based on dilution plating



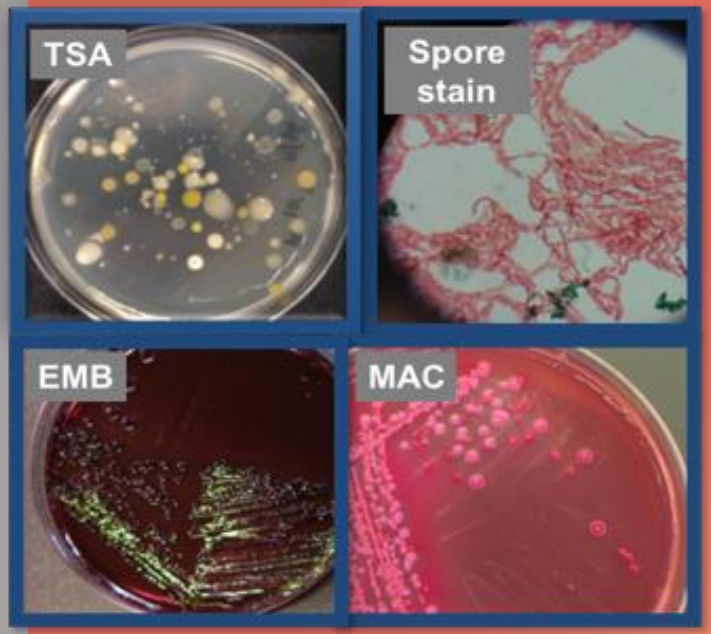


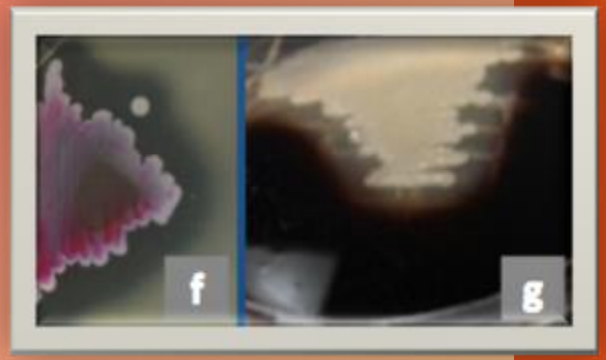
Plotting season to site

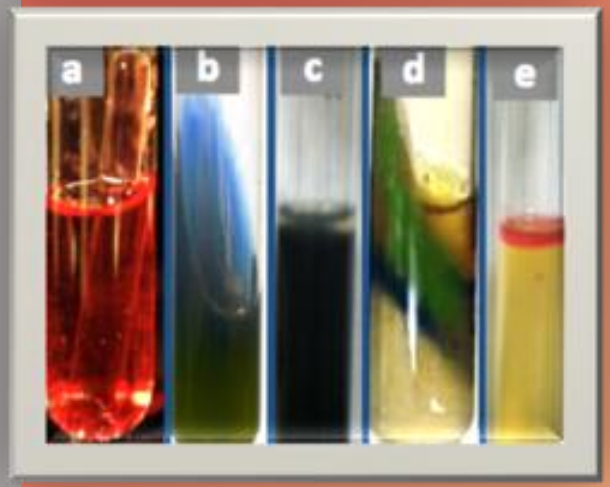


3. Identification of bacterial isolates

Selective-Differential Media and Biochemical Testing

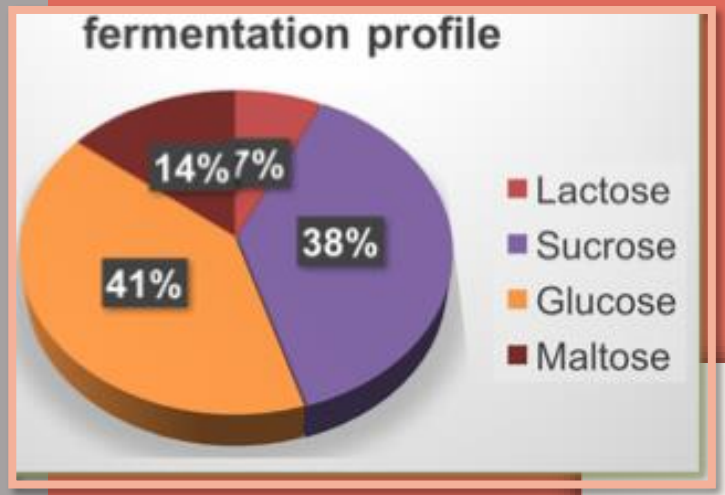




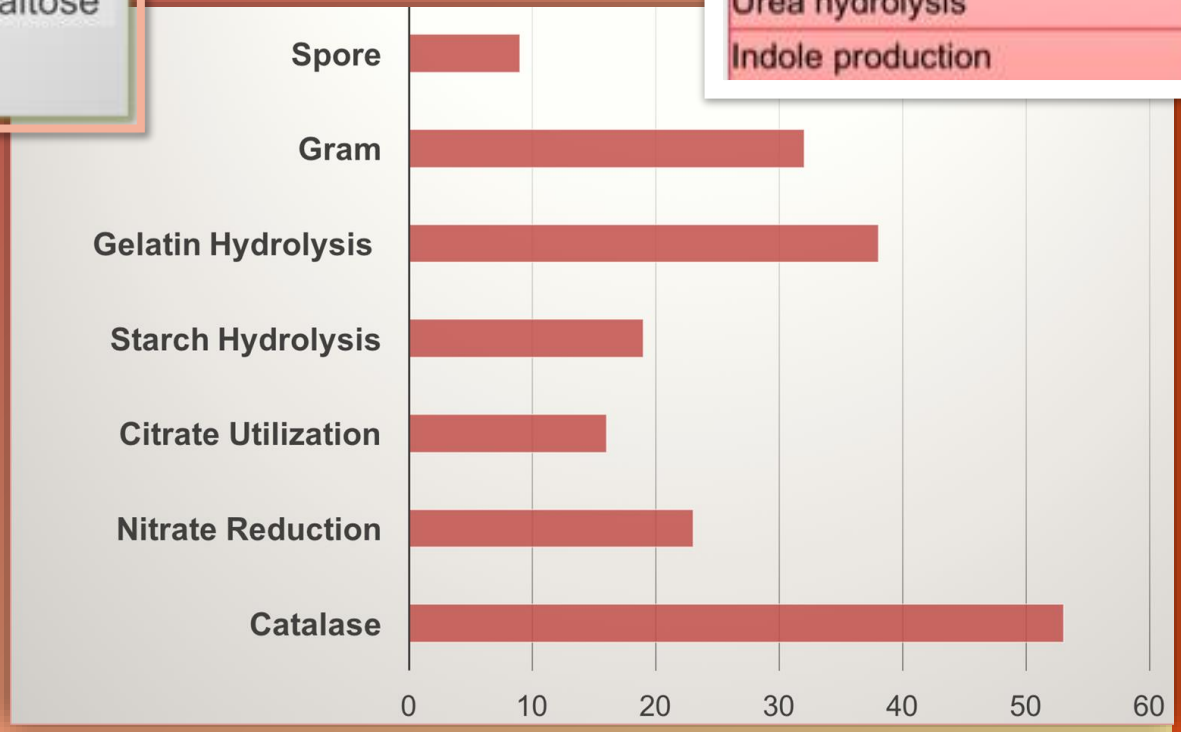


- Nitrate reduction
- Citrate utilization
- Cysteine desulfurization
- Phenylalanine deamination
- Indole production
- Gelatin & starch hydrolysis
- Catalase
- Oxidase

Results of carbohydrate fermentation and biochemical tests



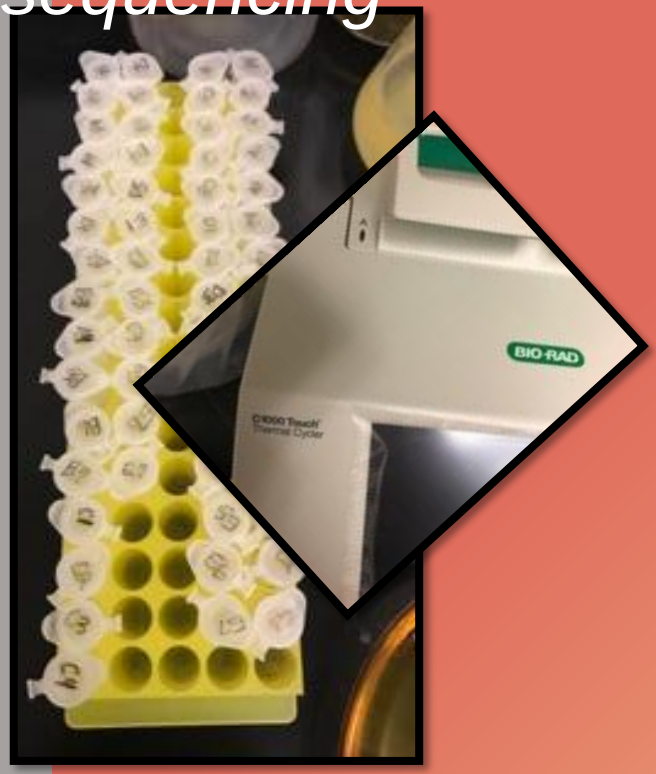
Biochemical tests	+ve rxn
Phenylalanine deaminase	2
Methyl Red	7
Vogues-Proskauer	6
Cysteine desulfurase	2
Litmus milk test (acidic)	5
Litmus milk test (alkaline)	4
Urea hydrolysis	5
Indole production	1



Metabolically, bacterial isolates were highly diverse

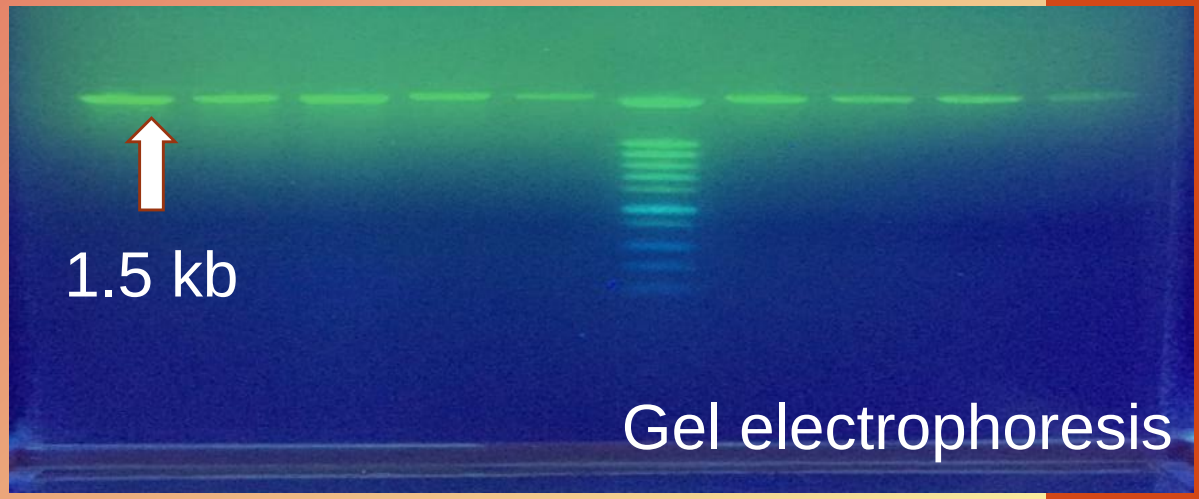
3. Identification of bacterial isolates

Using PCR amplification of 16S rRNA gene and sequencing

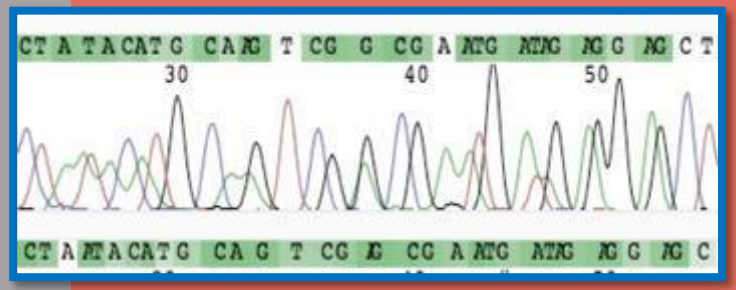


PCR

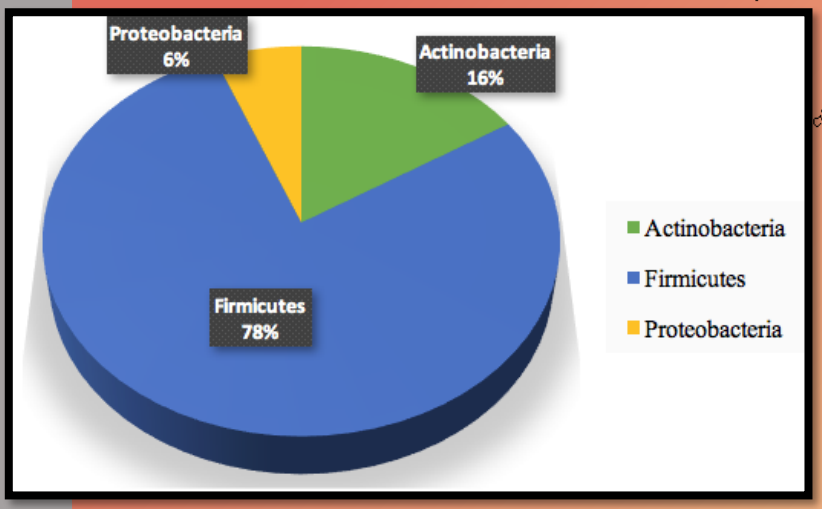
Purification and quantitation

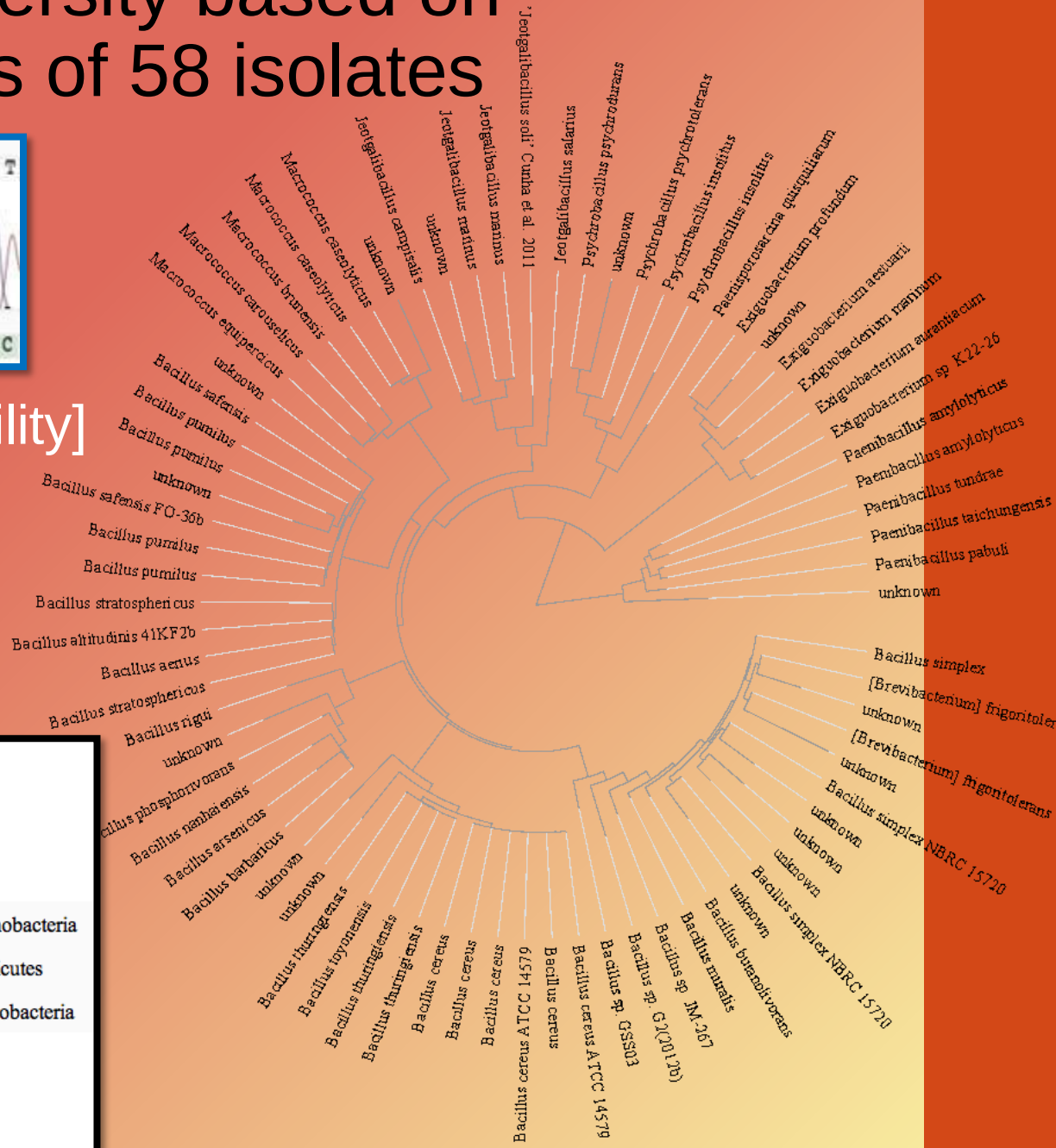


Total bacterial diversity based on sequence analysis of 58 isolates



[K State sequencing facility]





4. Screening for acidophilic bacteria

Citric acid buffer

A total of 17 acidophilic bacterial isolates identified

pH3 (N=5)*	pH4 (N=8)	pH5 (N=7)	pH6 (N=6)
Pantoea agglomerans	*Bacillus simplex*	*Paenibacillus alvei*	*Bacillus megaterium*
Pseudomonas syringae	*Frigoribacterium endophyticum*	*Curtobacterium flaccumfaciens*	*Microbacterium oleivorans*
Bacillus subterraneus	*Jeotgalibacillus campisalis*	*Pantoea agglomerans*	*Bacillus thuringiensis*
Bacillus pumilus	*Bacillus pumilus*		*Bacillus pumilus*
Terribacillus saccharophilus	*Frigobacterium endophyticum*		

Future study

- Physical remediation can be combined with biotic remediation, bench scale trials first
- Diverse metabolic profiling of the isolates can be improved upon with metagenomic data

Summary

1. Sampling and Laboratory Tests

2. DNA Sequencing and Acidophile Isolation

3. Bench Scale Lyophilization and Further Analysis

A baseline measurement of bacterial diversity and soil chemistry of AMD site is novel in its kind in southeast KS. Acidophilic bacterial strains from this study could be used along with anoxic limestone drains or wetlands to expedite the process of bioremediation and restoration of natural habitat for plants and animals.

Acknowledgements

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