



# Isolation and Characterization of Bacteria from Benzene-Impacted Iron Mat Communities

## Background:

- Greenville is home to one of the thousands of aquatic environments that are affected by failing infrastructure. Town Creek is victim to an underground storage tank that is slowly leaking benzene and other chemicals used in gasoline production into the ground water.
- Iron-oxidizing bacteria help aid these communities by being part of a cycle that degrades the benzene through the process of iron-oxidization.
- By isolating and characterizing the important players in the community, we can better understand their role and importance to the processes that reduce the amount of toxic benzene impacting the community.

## Guiding Question:

- What is the Identity and characteristics of the novel isolate from the iron mat community?



Figure 1: Town Creek Iron Mat, characterized by the orange color indicative of iron oxidation

## Sampling Design:

- Samples were taken from upstream and downstream of the source of the benzene leak on the 26<sup>th</sup> of September, 2019.
- 50 mL of iron mat was isolated from both locations.
- The environmental conditions at both locations were relatively similar except for dissolved oxygen percentages: (upstream sight, 80.4%, downstream sight, 5.6%)
  - The water temperature at both sights was approx. 23°C.
- The samples were plated by spreading, and isolated colonies were selected for streaking for isolation.
- Each isolate's 16s rDNA sequence was targeted via PCR for comparison using the Ribosomal Database Project Classifier and the Basic Local Alignment Search Tool.
- One isolate could not be uniquely identified by its 16s rDNA sequence and became the focus for characterization in this study.

## Results:

### Optimal Growth Temperature was found to be 20 °C

- Six temperature conditions were set and tested in triplicate to identify at which temperature the isolate grew optimally
- No growth at 4°C, 10 °C, or 45 °C
- The highest number of growing colonies found at 20 °C.
- The p-values from the Student's T-Test show that the number of cells growing at the three different temperatures were not significantly different statistically, but the difference is great enough to conclude that optimal growth occurred at 20 °C.

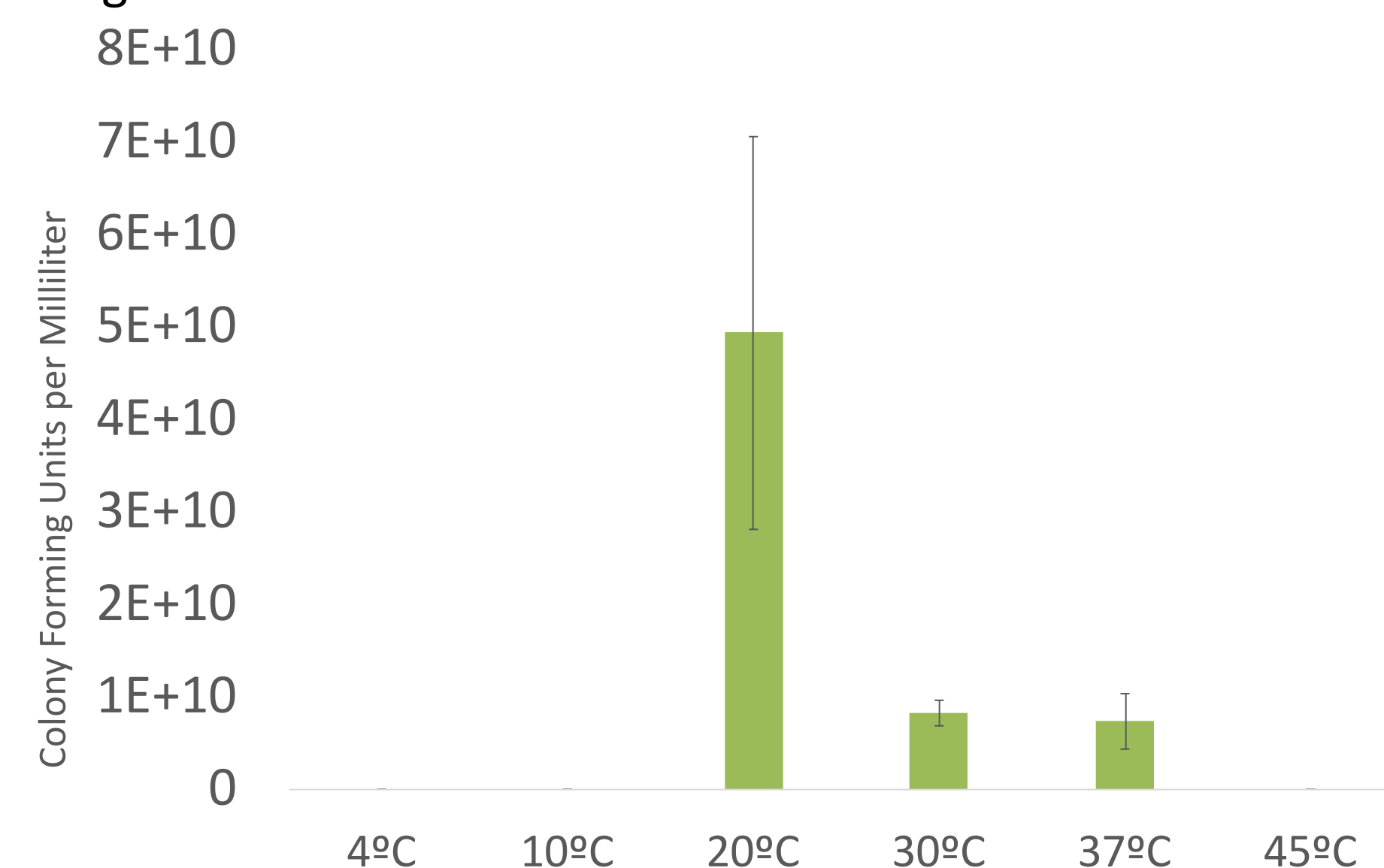


Figure 2: Spread plates were incubated at the six different temperature treatments in triplicate. The standard deviations were transformed using the same formula as the means. A Student's T-test showed p-values of: (20 C v 30 C) p = 0.107; (20 C v 37 C) p = 0.107; (30 C v 37 C) p = 0.338

### Triple Sugar Iron Agar: Negative for H<sub>2</sub>S production, Carbohydrate Fermentation

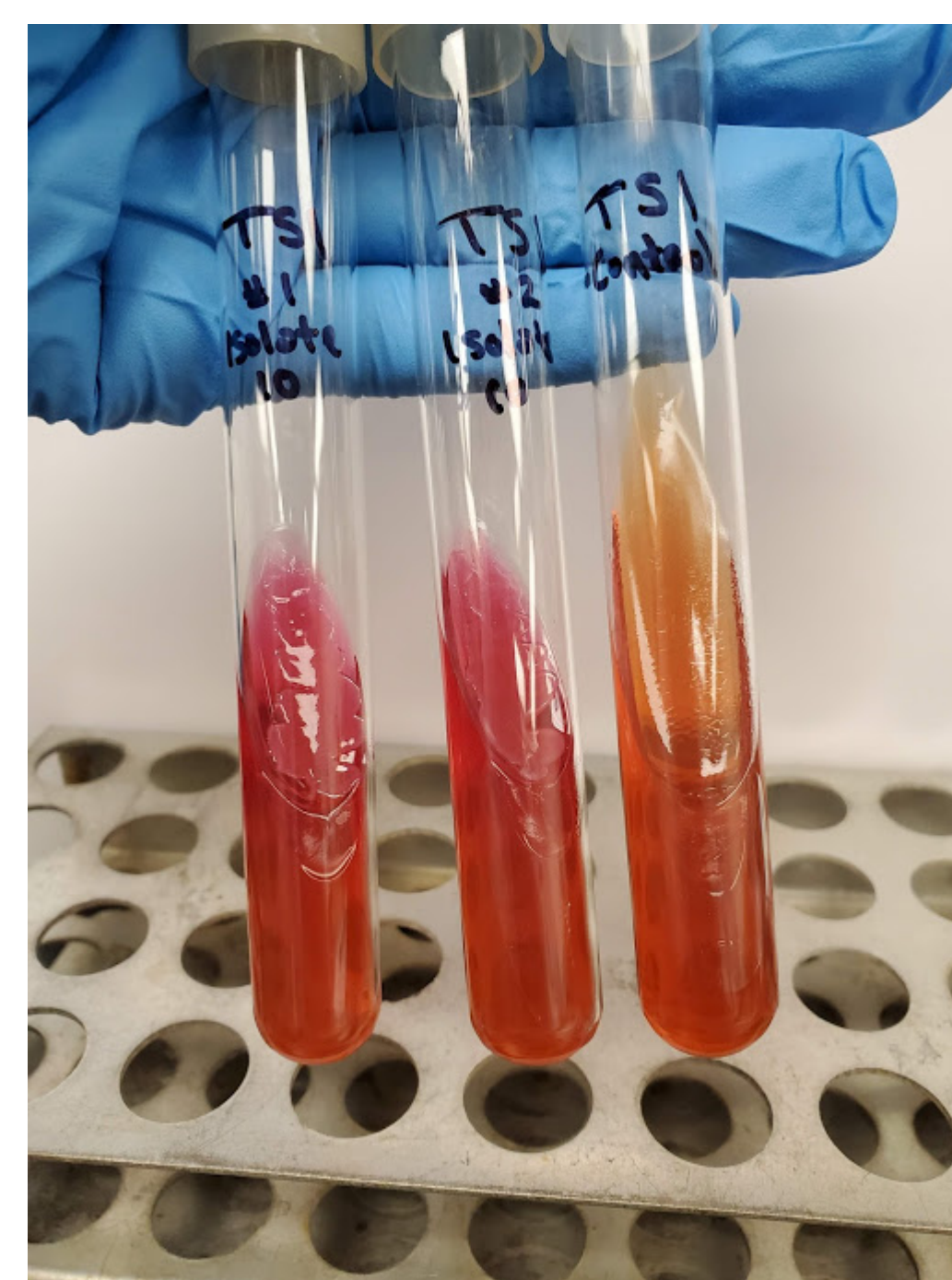


Figure 3: Triple Sugar Iron Agar incubated for 48 hours. The left and center tubes were inoculated and the right tube is a negative control

- Isolate does not produce hydrogen sulfide or ferment carbohydrates.
- Outcome suggests that the isolate is capable of aerobic deamination.
- This means the isolate is able to break down amino acids with an ammonia by product.
- Ammonia is a form of usable nitrogen that this isolate likely helps to maintain in the community.
- The isolate also had a negative result in a Simmons-citrate slant, meaning it cannot use ammonium as its sole nitrogen source.
- Possible that the isolate needs the ammonia by-product itself.

### Ornithine Decarboxylase Broth: Ornithine Decarboxylase Present

- Positive result for the presence of the enzyme Ornithine Decarboxylase.
- This indicates the isolate is a Gram-negative bacillus
- Confirms the results obtained by gram staining.

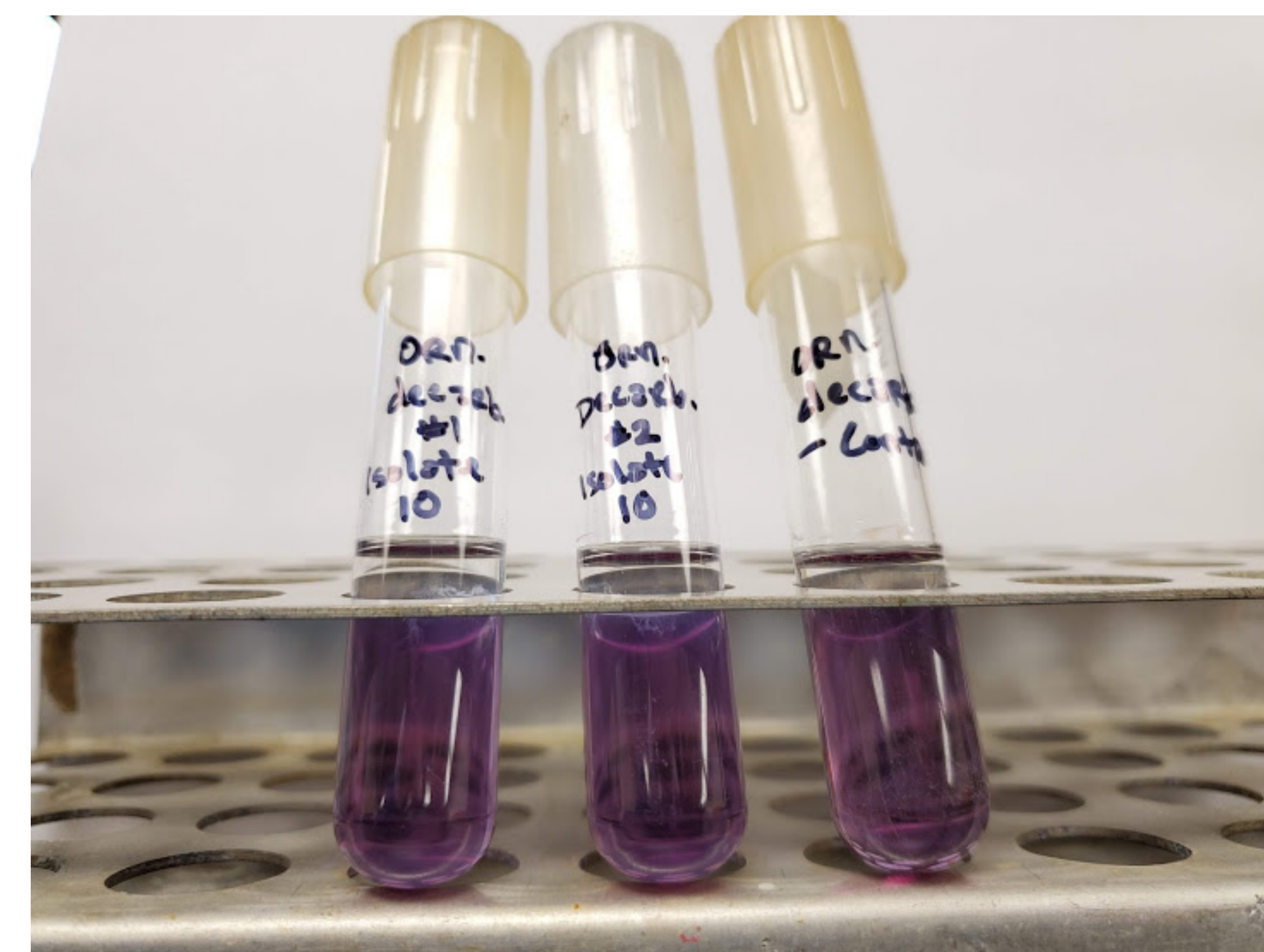


Figure 4: Ornithine Decarboxylase Broth is a differential assay for identifying Gram Negative Bacilli by testing for the presence of the enzyme Ornithine Decarboxylase. The two tubes on the left are the inoculated assays, and the tube on the right is a negative control.

## Conclusion:

- The isolate that was the focus of this study is a strictly aerobic, gram-negative bacillus organism.
- No evidence of it being able to ferment any type of carbohydrates.
- Growing optimally at 20 °C and only between the range of 20 °C - 37 °C narrowed down the possible identity to *Kinneretia asaccharophila* (t) KIN192 and *Mitsuaria sp.* KN1
  - 16s rDNA sequence over 99% similar in both the RDP and BLAST databases.
- In order to differentiate between these two organisms, further tests would be needed to determine if the enzyme catalase is present in the organism along with its ability to reduce nitrate.
- To better understand the bacteria's role in the iron mat communities, further research will be needed to determine if the bacteria can oxidize iron.
  - Also, more investigation into the isolate's ability of aerobic deamination could help provide more insight into its role in the complex cycles found in iron mats.

## Acknowledgments:

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