Lake Washington Institute of Technology

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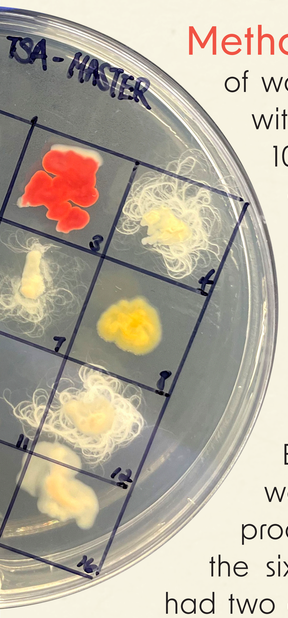
Discovering Rydalcat™

(the conclusively inconclusive bacteria)

Objective: To obtain knowledge and understanding about the production of antibiotics by making use of a variety of tests with bacteria from a soil isolate.

Research Question: Does our soil contain an undiscovered antibiotic producer that can fight against the increasing issue of drug-resistant infections?



Method: Serial Dilute 10g of soil with 90mL of water and repeat until there are plates with countable colonies. We used the 10-4 dilution plate for our countable colony [see figure 1]. We then picked and patched each of our bacteria to a new agar plate and incubated the bacteria for growth. After the growth of our master plate, we then picked and patched each bacterium to a new TSA media each with a different ESKAPE safe relative. After incubating, we screened the plates for antibiotic production with our single colonies. Of the sixteen different types of bacteria, we had two antibiotic producers [see figure 2] and a red bacteria that we chose to further analyze.

Procedures with Unknown #3:

PCR	Triple Sugar Iron Test	Urease Reaction
Indole Test	Oxidase Test	Gram Stain + KOH
Citrate Test	Mitility agar Test	Endospore Stain
Catalase Test	Gel Electrophoresis	

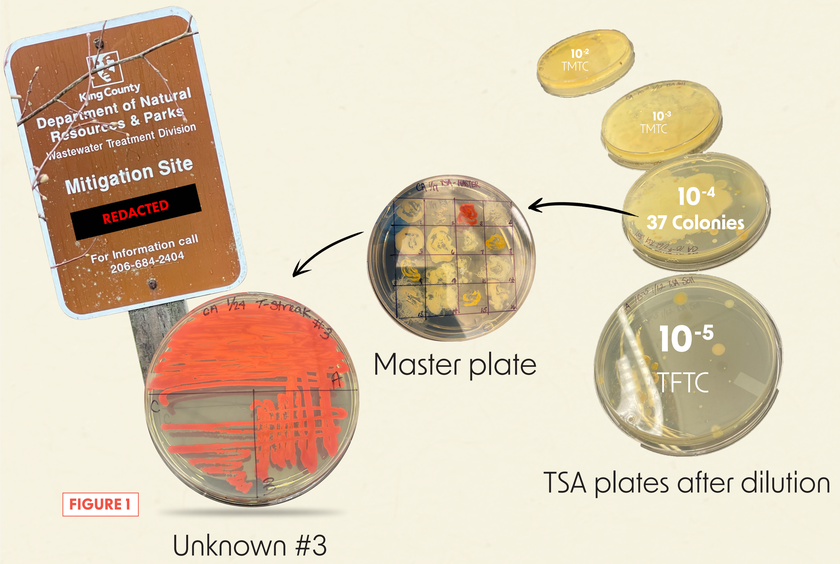


FIGURE 1

Antibiotic Results

FIGURE 2

Only unknown #1 and #5 were found to produce antibiotics when exposed to *E. raffinosus*, leaving faint zones of inhibition.

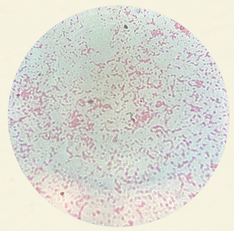
Key Results for Unknown #3

BLAST Analysis:
Serratia [inhibens]
E Value = 0.0

Gram Stain:
gram negative

Endospore Stain:
negative

KOH Test:
positive



Unknown #3 1000x

Biochemical Testing

-Catalse Test- Positive FIGURE 11

-Oxidase Test- Negative FIGURE 12

-Indole Test-	-Motility Agar-	-Citrate Test-	-Urease Reaction-	-TSI Test-

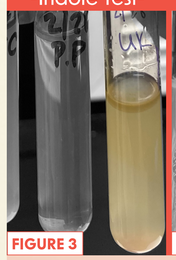
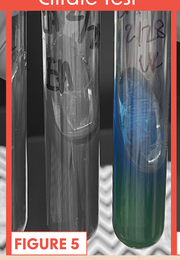
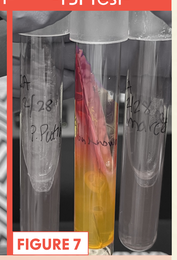

				

FIGURE 3	FIGURE 4	FIGURE 5	FIGURE 6	FIGURE 7
Negative: no signs of indole to breakdown tryptophan	Positive: is a motile bacteria due to flagella	Positive: uses citrate as a source of carbon	Positive: breaks down urea into ammonia with enzyme urease	A/K/+/-: can ferment glucose while making hydrogen gas

Differential and Selective Media Testing

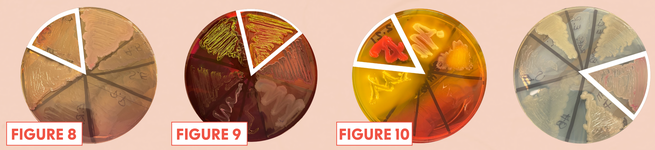


FIGURE 8 MAC | FIGURE 9 EMB | FIGURE 10 XLD | TSA

Growth +	Growth +	Growth +	Growth +
Colony Color: red/purple	Colony Color: metallic green	Colony Color: neon pink	Colony Color: pink
Medium Color: no change	Medium Color: no change	Medium Color: no change	Medium Color: no change
Conclusion: lactose fermenter	Conclusion: lactose fermenter	Conclusion: non-sugar ferm.	

Discussion: Our BLAST analysis revealed that our bacteria fell under the *Serratia* genus. The most common species of *Serratia* is *marcescens*: a gram-negative, facultatively-anaerobic bacterium and opportunistic pathogen that causes those pink rings around bath drains thanks to the red pigment prodigiosin (Haddix , 2018., Nazzaro, 2019).

The closest BLAST result was *Serratia inhibens*, a strain with anti-fungal properties that was discovered in 2020 (Hennessy, et al., 2020).

The Indole test indicated that our bacteria was unable to metabolize tryptophan [figure 3]. The urease reaction showed that the bacteria can break down urea [figure 6]. The catalase test indicated that our unknown can break down hydrogen peroxide [figure 11].

The TSI test shows that the bacteria creates hydrogen gas and ferments glucose [figure 7]. The oxidase test was negative meaning the unknown bacteria had no enzyme to break down the reagent [figure 12]. It can use citrate as a carbon source as revealed by the citrate test and uses flagella for movement as seen with the motility agar [figure 5, 4].

The MAC and EMB plates allowed us to determine that our bacteria is a lactose fermenter, which is rare for the genus (Enterobacteriaceae, 2020) [figures 8, 9].

Summary of Contradictions

The TSI test [figure 7] says it can't ferment lactose or sucrose. This contradicts the MAC and EMB while being supported by the XLD plate. Due to the strong results on the MAC and EMB, we believe the unknown *Serratia* strain can ferment lactose - but further testing is required to form a solid conclusion.

MAC and EMB show lactose fermentation, but the XLD plate shows no sugar fermentation – this contradicts the MAC, EMB, and the glucose fermentation claims of the TSI.

Conclusion: We found two antibiotic producers and a third non-antibiotic producer that shows us the importance of performing several different tests in order to possibly discover unique strain-specific capabilities.

REFERENCES

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