

Investigation of Antibiotic Activity From Soil of *Dieffenbachia*

ABSTRACT

This semester-long research project was part of the Tiny Earth initiative, an organization that empowers students to address one of the most pressing global concerns – antibiotic resistance. Tiny Earth creates a network of students, professors, and researchers from several countries looking for novel antibiotic-producing bacteria. Student-sourced antibiotic-producing microbes were found in samples of soil bacteria recovered from a potted plant *Dieffenbachia*. Bacteria from the sample were successfully cultured on both LB and TSA agar plates to isolate single colonies. Three isolates were tested against safe relatives of ESKAPE pathogens, an acronym for six major virulent and multidrug-resistant pathogens. *Salvia* seeds served as a eukaryotic model for testing and found that 1 isolate showed promising antibiotic properties that can be investigated further.

INTRODUCTION

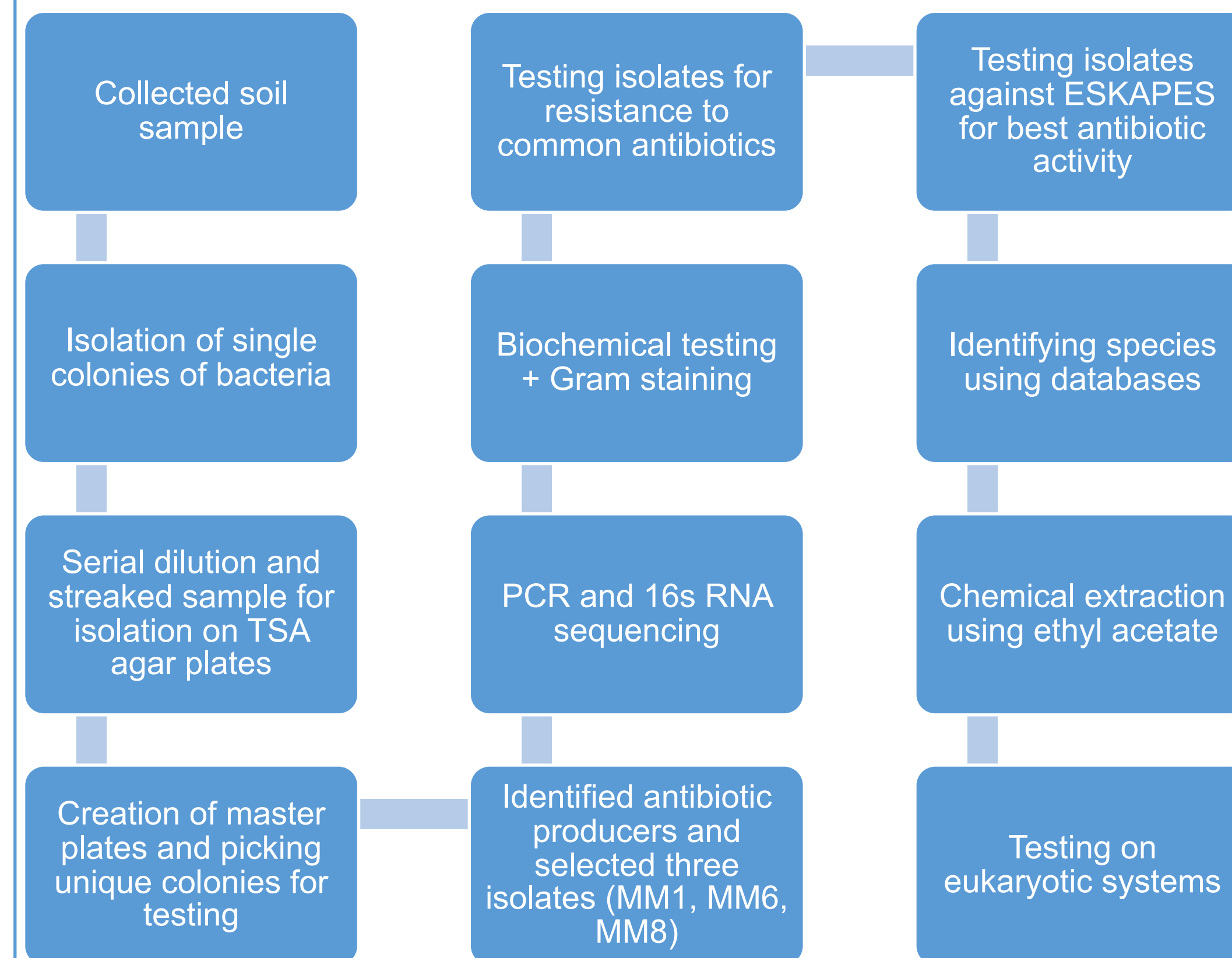
The CDC states antibiotic-resistant pathogenic bacteria are a growing public health threat, killing at least 1.27 million people worldwide and causing nearly three million antimicrobial-resistant infections occurring every year. Antibiotic-resistant bacteria are of great importance due to their increase in persistence, pathogenicity, and difficulty to treat, making them extremely dangerous to both immunocompromised and healthy immune system populations. Scientists are facing a need to rapidly develop new antibiotics, leading to the creation of the Tiny Earth initiative – a sustainable and educational opportunity for both students and researchers. Tiny Earth encourages students from all around the globe to cultivate, isolate, and test bacteria from a soil sample of their choosing.

SAMPLE

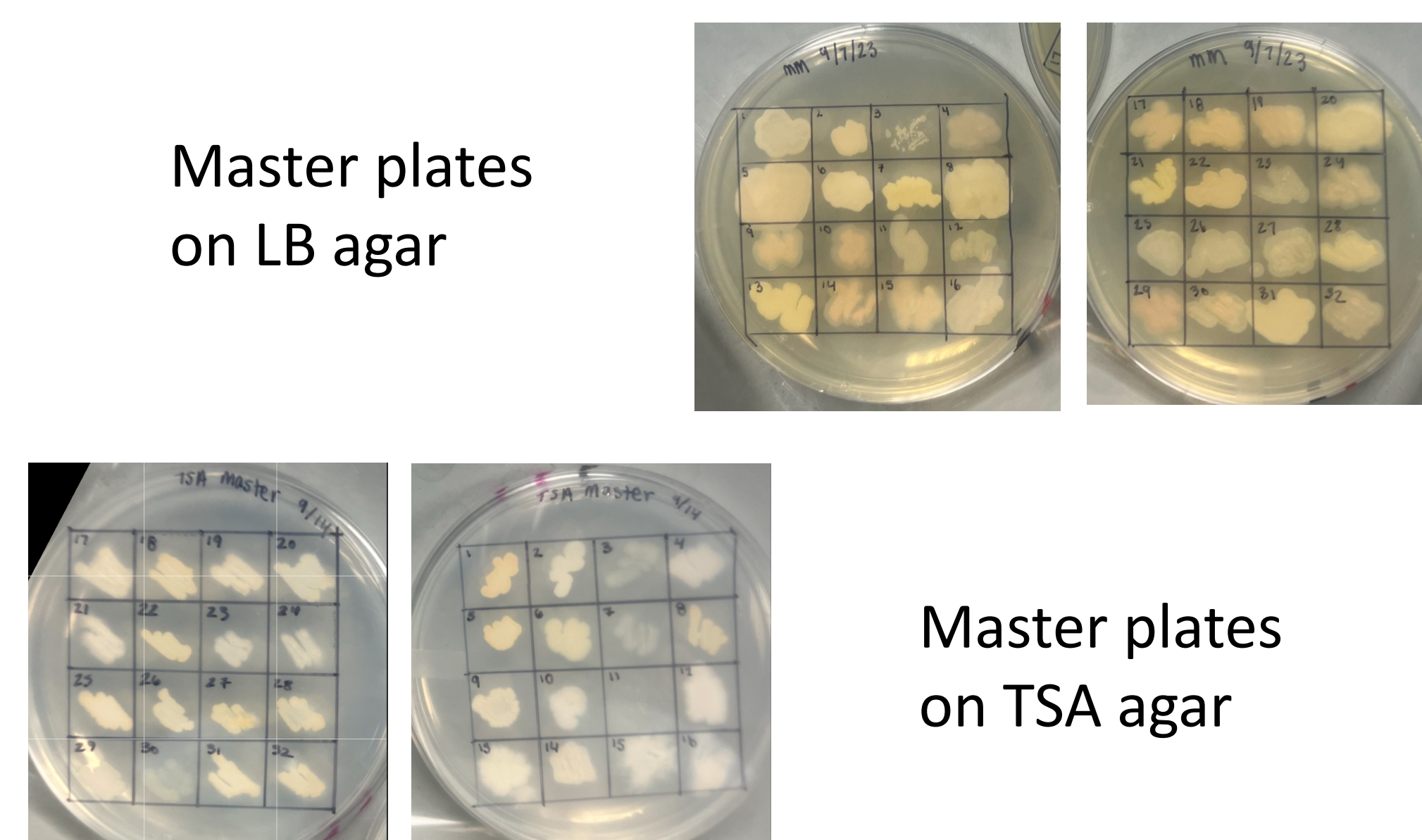
- Taken from indoor potted plant *Dieffenbachia* kept in a window
- Depth: 2-3 inches below the surface
- Indoor temperature was 76°F



METHODS

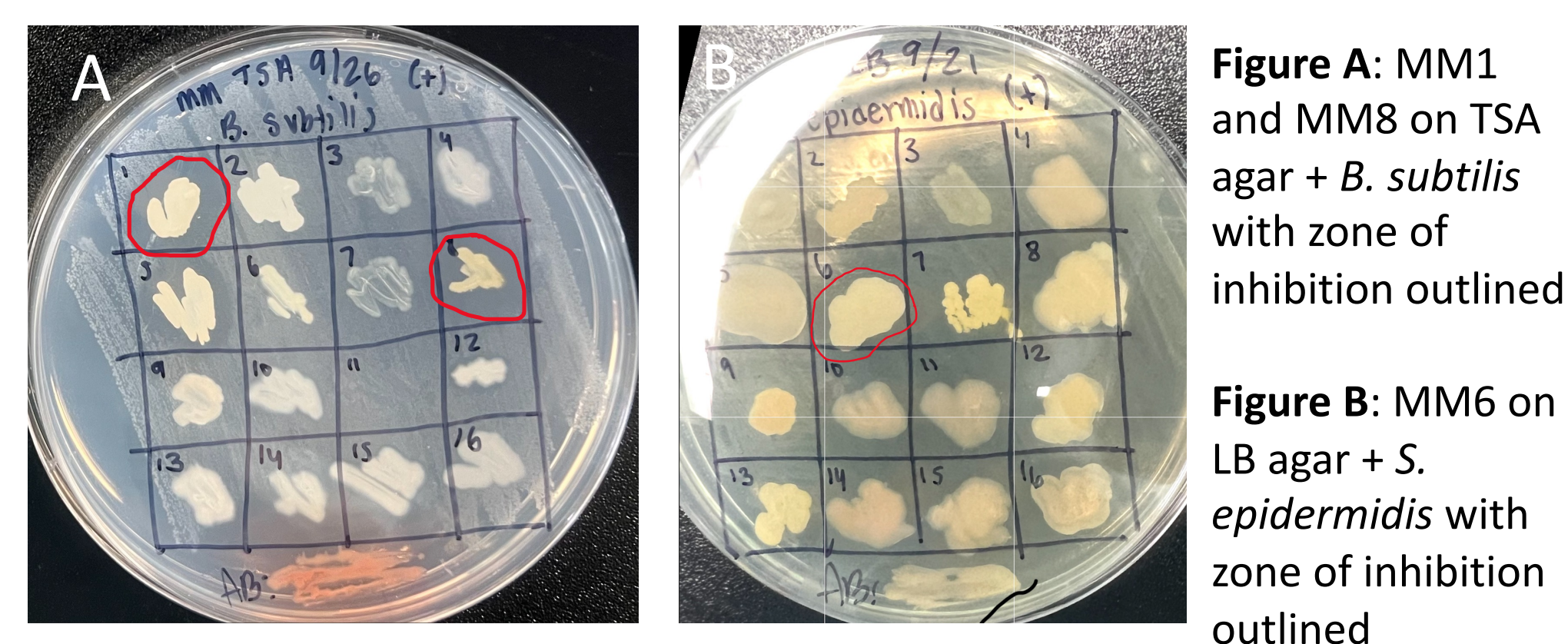


ISOLATES

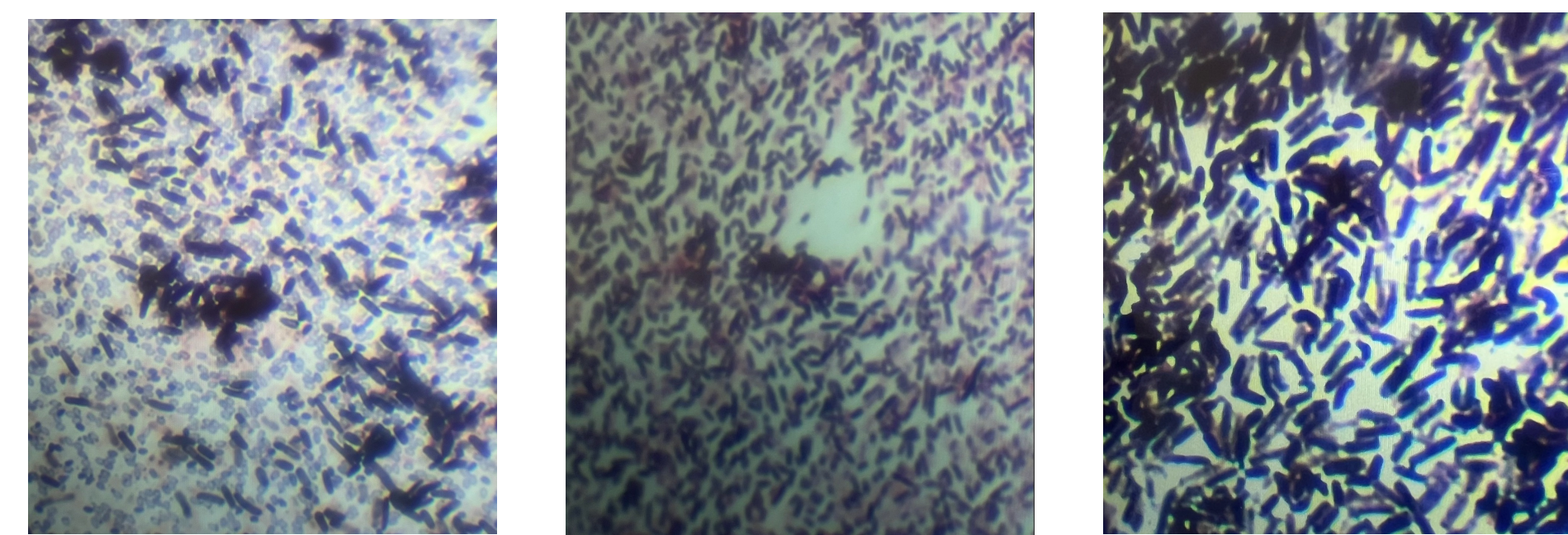


ESKAPE Relative Tested	Gram Stain	Zone of Inhibition (mm)
<i>Escherichia coli</i>	-	None
<i>Streptococcus salivarius</i>	+	None
<i>Erwinia carotovora</i>	-	None
<i>Staphylococcus epidermidis</i>	+	MM6: 16 mm
<i>Pseudomonas putida</i>	-	None
<i>Bacillus subtilis</i>	+	MM1: 14 mm MM8: 10 mm

- 3 isolates taken after initial ESKAPE testing:
 - MM1 from *B. subtilis* on TSA
 - MM6 from *S. epidermidis* on LB
 - MM8 from *B. subtilis* on TSA



IDENTIFICATION OF ISOLATES



- Left to Right: MM1, MM6, MM8
- Gram positive
- Rod-shaped

MM1 on TSA		MM8 on TSA	
Biochemical Test	Result	Biochemical Test	Result
Catalase	Positive	Catalase	Negative
Gelatin Hydrolysis	Negative	Gelatin Hydrolysis	Negative
Hemolysis	Alpha	Hemolysis	Gamma
MacConkey Agar	Gram-negative, NLF	MacConkey Agar	Gram positive
Starch	Negative	Starch	Negative
Glucose	Positive	Glucose	Positive
Fermentation		Fermentation	
Sucrose Fermentation	Negative	Sucrose Fermentation	Positive
Motility	Nonmotile	Motility	Nonmotile

Note: MM6 not tested due to limited resources
16S RNA SEQUENCING

MM1: Phylum: Proteobacteria Class: Gamma proteobacteria Order: Pseudomonadales Family: Pseudomonadaceae Genus: Pseudomonas • 98.72% identity to <i>Pseudomonas</i>	MM6: Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae Genus: Bacillus • 99.72% identity to <i>Bacillus</i>	MM8: Phylum: Pseudomonadota Class: Order: Xanthomonadales Family: Xanthomonadaceae Genus: Pseudoxanthomonas • 99.50% identity to <i>Pseudoxanthomonas mexicana</i>
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ANTIBIOTIC TESTING

Isolate	Resistance Against Common Antibiotics (Zone of Inhibition in mm)					
	Tetracycline	Erythromycin	Penicillin	Polymyxin B	Streptomycin	Chloramphenicol
MM1	8	None	None	8	12	None
MM6	22	24	None	10	18	24
MM8	22	28	None	10	22	34

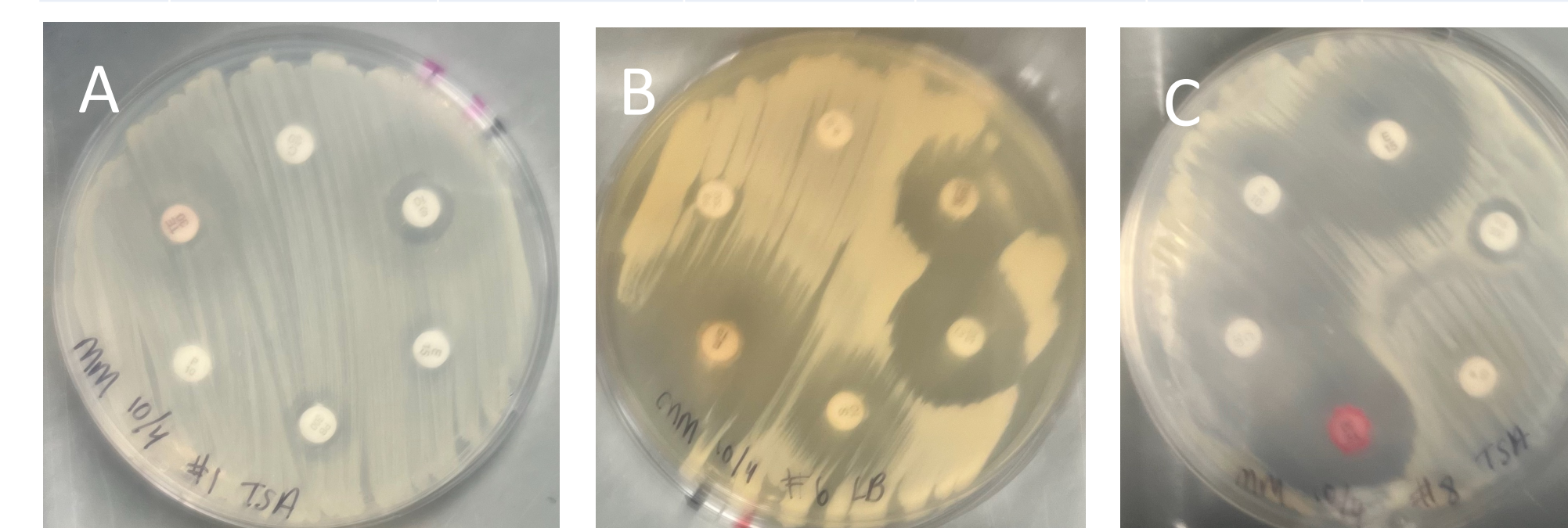
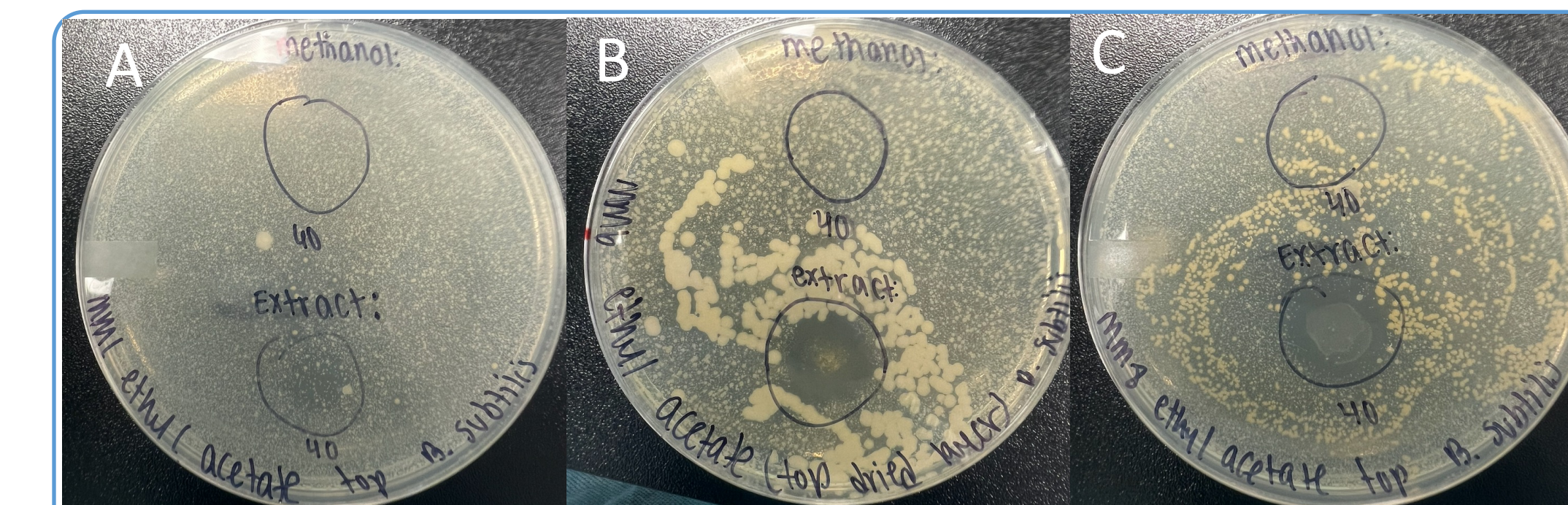


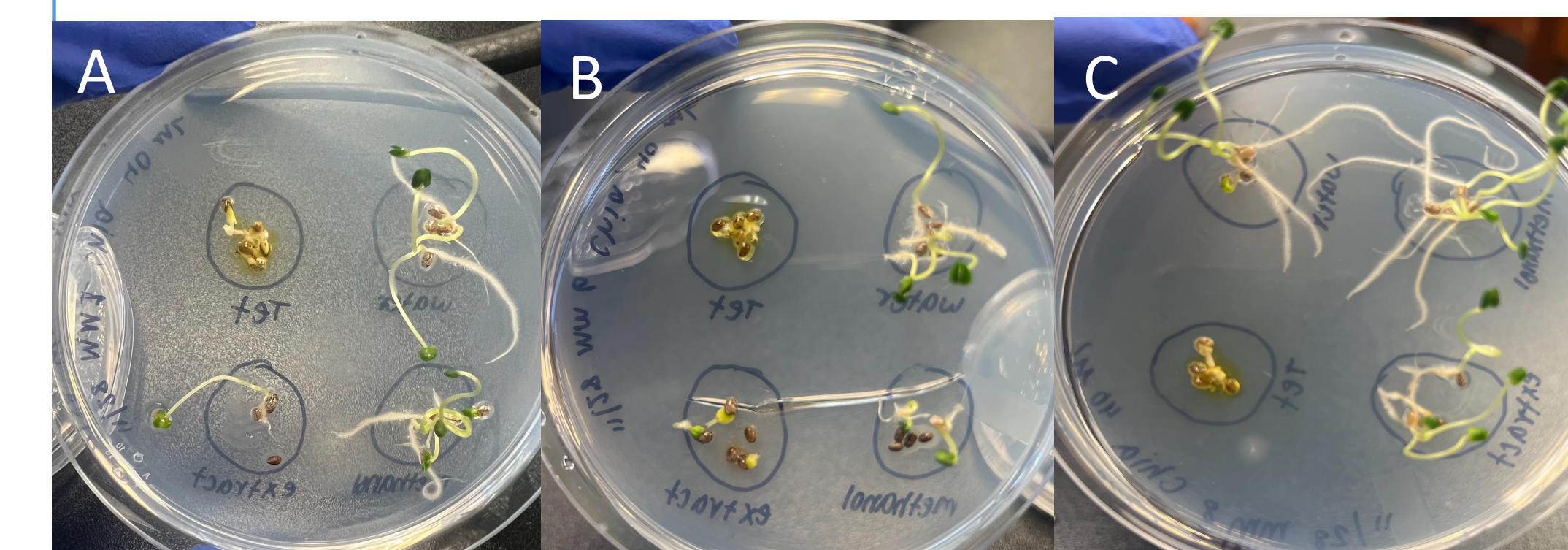
Figure (A) MM1 showed small ZOI (B) MM6 showed large ZOIs (C) MM8 showed large ZOIs

RESULTS

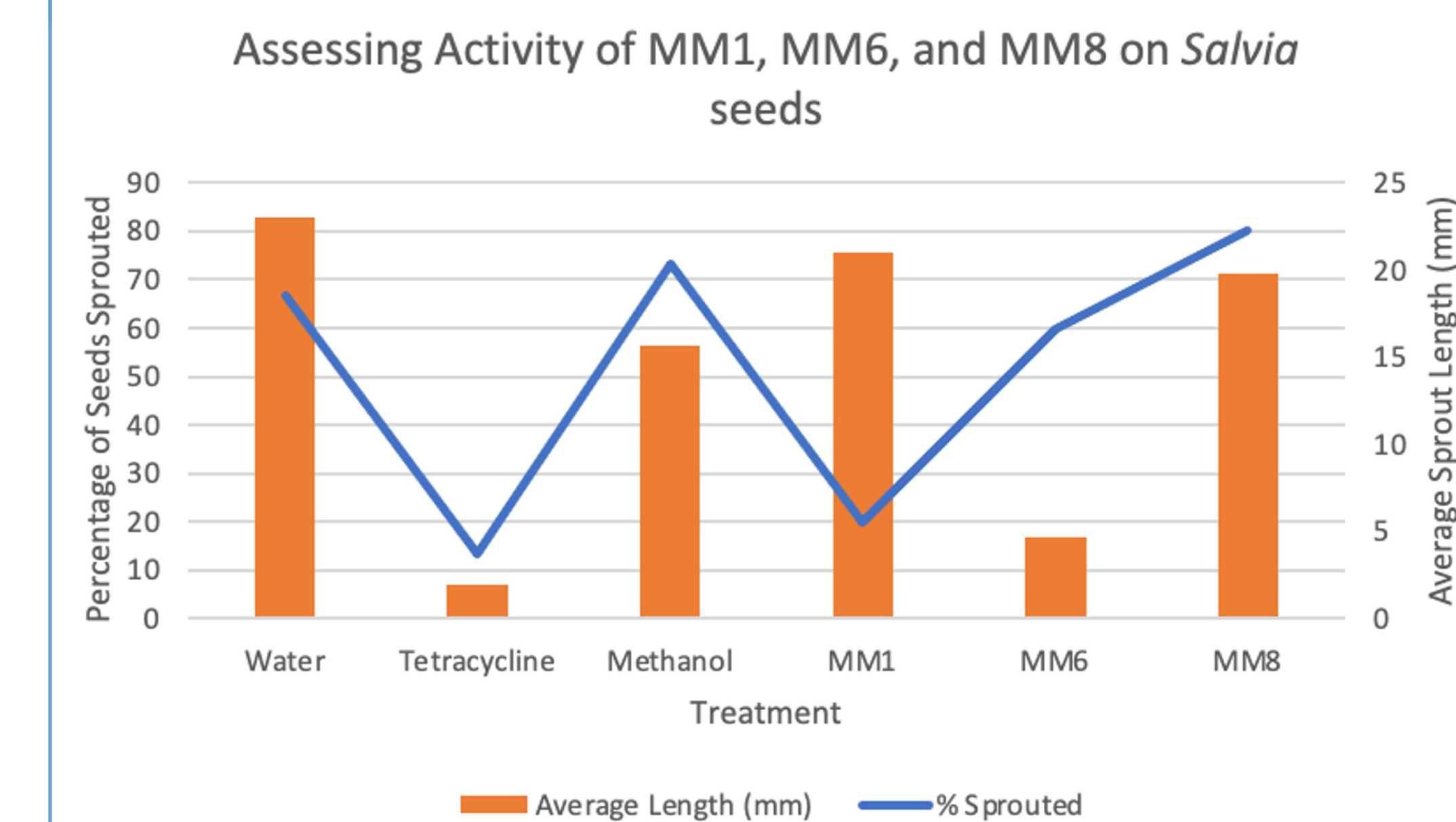
- Ethyl acetate, butanol, and methanol extractions: No inhibition
- Ethyl acetate redo: Successful



• Figure A-C: 40 microliters of MM1, MM6, and MM8 extract against *B. subtilis* respectively.



- Figure A: MM1 highly toxic to eukaryotes
- Figure B: MM6 somewhat toxic to eukaryotes
- Figure C: MM8 very low toxicity to eukaryotes



• Figure: Graph depicting the activity of isolates on chia seeds, illustrating percentage sprouted and average shoot length

CONCLUSION

- 2 isolates picked from TSA serial dilution and 1 from LB serial dilution for investigation
- Isolates MM1, MM6, and MM8 found to be *Pseudomonas*, *Bacillus*, and *Pseudoxanthomonas Mexicana* respectively
- Inhibition of chia seed growth seen in both MM1 and MM6 but not MM8, making it promising for study

ACKNOWLEDGEMENTS

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REFERENCES

"About Antimicrobial Resistance." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 5 Oct. 2022, www.cdc.gov/drugresistance/about.html.