

# Extracting Antibiotic-Producing Bacteria

Jackie Buttafuoco, Vy Huyen, Mentor: Dr. Daniel Herman | Biology Department

## INTRODUCTION

Antibiotics are produced by microorganisms to inhibit or kill other microorganisms, and they can be modified to be used against human pathogens. The same few classes of antibiotics have been continuously modified, and bacteria are becoming resistant to the effects. One solution is to discover new antibiotic-producing microorganisms, which has only been done once in the last thirty-two years (1). Soil samples have been continuously collected in an attempt to find antibiotic-producing bacteria. The soil isolates were patched onto plates containing tester strains closely related to human pathogens (*Escherichia coli*, *Enterococcus faecalis*, *Salmonella enteritidis*, and *Staphylococcus aureus*). Seventeen antibiotic-producing soil isolates have been identified. A variety of physiological tests were performed to begin characterizing the antibiotic-producing isolates, as well as sequencing on a subset of the isolates.

## MATERIALS AND METHODS

**Soil Collection:** Samples were collected from multiple locations in Wisconsin and Iowa. Each soil sample was then suspended in 10mL of sterile water. Serial dilutions were performed and inoculated onto various media (Reasoner's 2A agar, 1/10-strength of Columbia Blood Agar, and 1/10-strength of Mueller Hinton Agar).

**Patching:** The bacterial colonies found on the dilution plates were patched onto R2A, 1/10 CBA, 1/10 MHI agar). They were then patched onto a lawn of tester strains, staying consistent with the original media used.

**Tester Strains:** Bacterial strains closely related to human pathogens (*E. coli*, *E. faecalis*, *S. enteritidis*, and *S. aureus*) were used to test the soil isolates for antibiotic-producing ability (2).

**Gram Stain:** The identified antibiotic-producers underwent Gram staining in order to learn about the cell wall, shape, and arrangement. Purple cells indicate Gram positive, and pink cells indicate Gram negative.

**Citrate Test:** A citrate test was performed to see if the isolates could use citrate as a sole carbon source and ammonium phosphate as a sole nitrogen source. A blue slant indicates a positive result.

**Eosin Methylene Blue Agar Plate:** EMB plates select for the growth of Gram-negative bacteria and differentiate based on lactose fermentation. Any growth indicates Gram negative bacteria, with purple colonies indicating lactose fermentation.

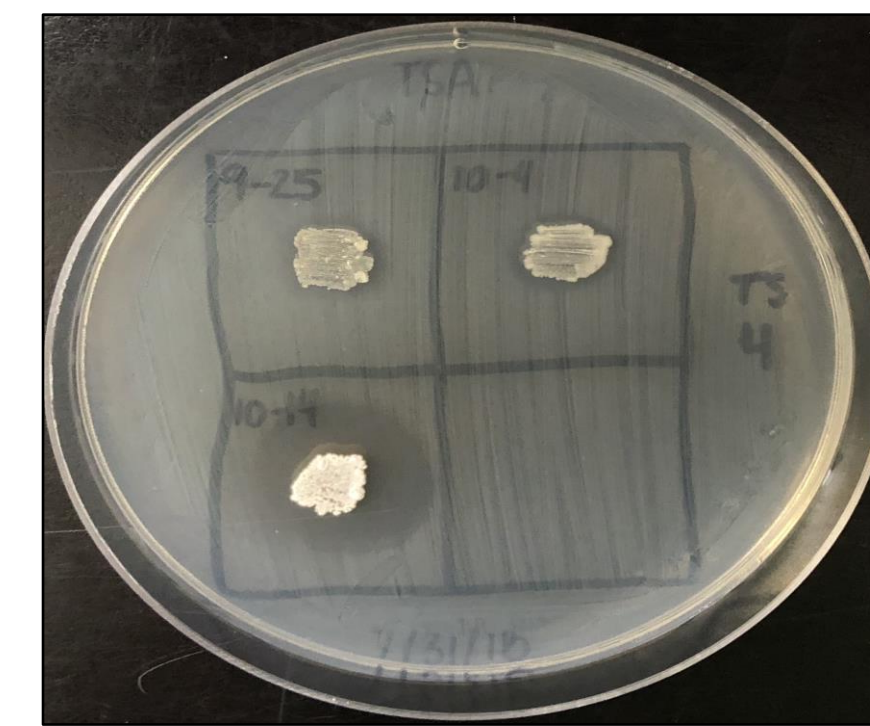
**Mannitol Salt Agar (MSA):** MSA plates were utilized to determine halotolerance and mannitol fermentation. Allow selection of isolates that tolerate 7.5% salt content and contains the pH indicator phenol red which turns yellow in the presence of acidic byproducts.

**Triple Sugar Iron (TSI) Test:** The TSI test was used to detect the ability to ferment different sugars (glucose, lactose, sucrose), catabolize peptone, produce H<sub>2</sub>S and gas.

**Methyl Red (MR) and Voges-Proskauer (VP):** Simple broth that contains peptone, buffers, and dextrose or glucose. It is used as differential tests as the medium in which both the Methyl Red and Voges-Proskauer tests can be performed.

**SIM (Sulfate, Indole, Motility) Medium:** Allow differentiation of Gram-negative enteric bacilli on the basis of sulfide production, indole formation, and motility.

## RESULTS



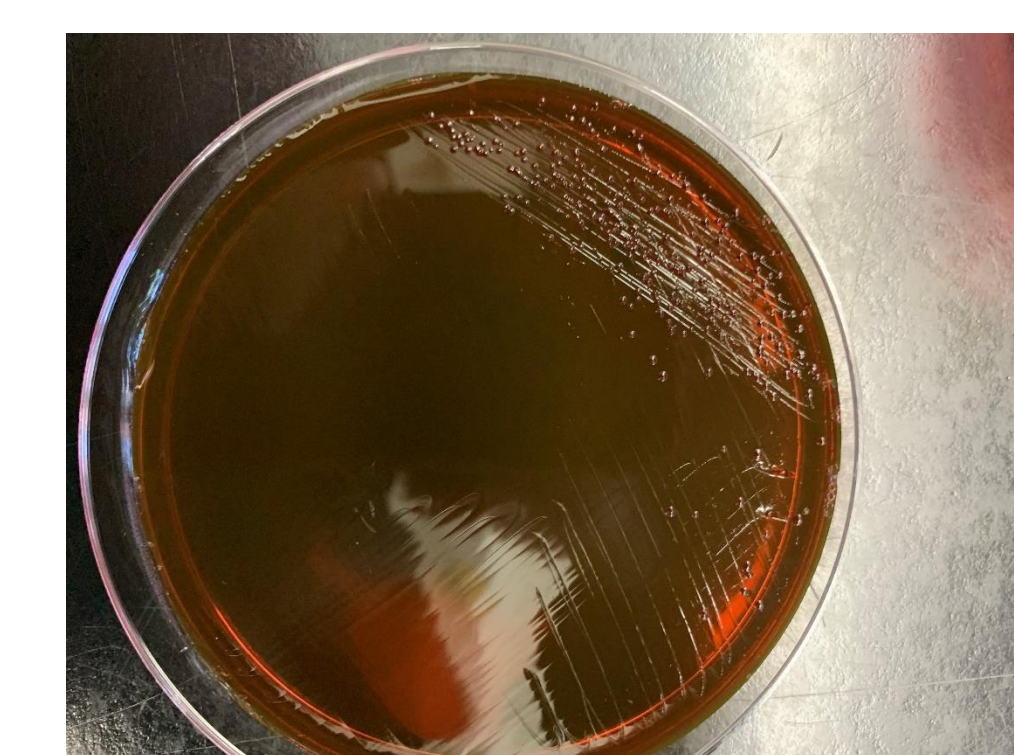
**Figure 1.** Tester strain plate (*S. aureus*) patched with bacterial isolates. Note the zones of inhibition surrounding the colonies - this shows antibiotic production.



**Figure 2.** Triple Sugar Iron Slant: results ranging from K/NC, A/A, A/NC, NC/A, NC/NC.



**Figure 3.** Mannitol Salt Agar plate streaked with an antibiotic-producing bacterial isolate. The test shows halotolerance and mannitol fermentation.



**Figure 4.** EMB streak plate with the antibiotic-producing isolate. Weak lactose fermentation was observed (purple colonies).

**Figure 5. Physiological testing results of antibiotic-producing isolates from 1/10 strength CBA and 1/10- strength MHI media**

	A21-1	B51-1	B51-3	B21-3	B21-4	B51-3	C42-3.1	D41-2	D41-1
inhibition	TS1, TS3, TS4	TS2, TS3, TS4	TS2, TS3, TS4	S1, TS3, TS4	TS4	TS4	TS4	TS4	TS4
TSI	K/NC	A/A	K/NC	K/A	AA	A/NC	A/NC	A/A	A/NC
citrate	-	-	+	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	+	+	-	+
EMB	Growth	G	G	NG	G	G	G	G	NG
	Color	-	+	N/A	+	-	+	+	N/A
MSA	Growth/No Growth	NG	NG	G	NG	G	G	NG	G
	Mannitol fermentation	N/A	N/A	+	N/A	+	+	N/A	+
SIM	sulfur utilization	-	-	-	-	-	-	-	-
	indole production	-	-	-	-	-	-	-	-
	motility	-	-	-	-	-	-	-	-

Labeled	Tester Strains
TS1	<i>Escherichia coli</i>
TS2	<i>Enterococcus faecalis</i>
TS3	<i>Salmonella enteritidis</i>
TS4	<i>Staphylococcus aureus</i>

Additional key
Triple Sugar Iron Slant. A : acid, K: alkaline, NC: No change. (Streak/stab : aerobic respiration/anaerobic respiration).
N/A: not applicable, + : positive results, - : negative results

**Figure 6. Physiological testing results of antibiotic-producing isolates**

	B11-10.1	B11-4.2	C21-1	C21-2	C42-4	D11-16	D22-6	A11-1
Inhibition	TS4, TS2	TS4, TS2	TS3, TS4	TS3	TS4	TS4	TS1	TS4
TSI	K/S	A/A	A/NC	K/A	A/A	K/NC	K/A	A/A
citrate	+	-	-	-	-	+	-	-
MR	-	+	-	-	-	+	-	+
VP	-	-	-	-	-	-	-	-
EMB	Growth	G	NG	G	G	G	G	NG
	Color	-	NG	++	-	++	-	NG
MSA	Growth/No Growth	+	+	-	-	-	-	+
	Mannitol fermentation	+	+	-	-	-	-	+
SIM	sulfur utilization	-	-	-	-	-	-	-
	indole production	-	-	-	-	-	-	-
	motility	-	-	-	-	-	-	+

## DISCUSSION

Isolation of bacteria from the soil samples has led to the unearthing of seven isolates that produce against bacterial strains closely related to human pathogens. The bacteria were characterized using various physiological tests. Due to the variety in the results, it is possible a novel antibiotic-producer was discovered.

The future of these 17 isolates will be identified through 16S rRNA gene sequencing. If a novel antibiotic producer is found, further testing will be done to identify the gene(s) responsible for antibiotic production through transposon mutagenesis. Antimicrobial substances will be isolated and identified.

## REFERENCES

- Dibrov, P., Dibrov, E., Maddaford, T.G., Kenneth, M., Nelson, J., Resch, C., Pierce, G.N. 2017. Development of a novel rationally designed antibiotic to inhibit a nontraditional bacterial target. *Can. J. Physiol. Pharmacol.* 95: 595-603.
- Hernandez S, Tsang T, Bascon-Slack C, Handelsman J. 2016. *Small World Initiative*; 4th Ed. Small World Initiative Press
- "Catalase Test with Negative and Positive Result on the Slide." Shutterstock, <https://www.shutterstock.com/image-photo/catalase-test-negative-positive-result-on-1020483955>.
- Malanicheva, I. A., et al. Antimicrobial Activity of Bacillus Megaterium Strains. *Microbiology*, vol. 81, no. 2, 2012, pp. 178-185.

## ACKNOWLEDGEMENT

- Funding support provided by:
- University of Wisconsin -Eau Claire Office of Research and Sponsored Programs
  - University of Wisconsin - Eau Claire Biology Department