ISOLATION OF ANTIBIOTIC PRODUCING BACTERIA FROM SOIL

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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 31 years (1). During the spring of 2018, two soil samples were collected in an attempt to find antibiotic-producing bacteria. The soil isolates were patched onto 4 plates to determine if they could inhibit one or more tester strains (*Salmonella enteritidis*, *Enterococcus faecalis*, *Escherichia coli*, and *Staphylococcus aureus*). Two antibiotic-producing soil isolates were originally identified, but later were determined to be the same bacterium. The antibiotic-producing soil isolate was then subjected to a variety of physiological tests to further characterize the strain.

METHODS

Soil Collection: Samples were collected from two locations in Eau Claire, WI. Each soil sample was then suspended in 10mL of sterile water. Serial dilutions were performed and inoculated onto Potato Dextrose Agar (PDA) plates with a pH of 7 (2).

Patching: The bacterial colonies found on the 10^{-3} dilution plates were patched onto PDA plates (with a pH of 7), and then again on four PDA plates, each inoculated with a tester strain (2).

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

Gram Stain: The identified antibiotic-producer 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.

Blood Agar Plate: The isolate was streaked on blood agar to test for hemolysis. α hemolysis is characterized by a green zone surrounding the colony, β hemolysis is characterized by a clear zone, and γ hemolysis is no hemolysis.

Catalase Test: A drop of hydrogen peroxide was mixed with the isolate, along with positive and negative controls. Bubbling indicates a positive result for the catalase enzyme.

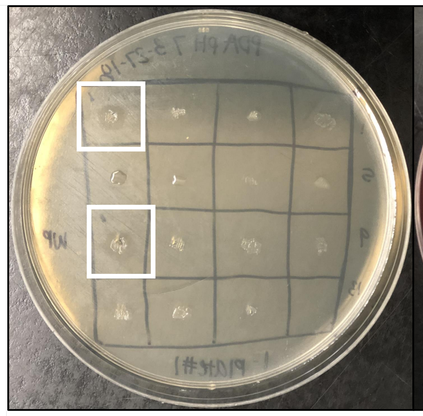

Figure 1. Tester strain plate (*S. enteritidis*) patched with bacterial isolates. Note the zone of inhibition surrounding the two outlined colonies- this shows antibiotic production.

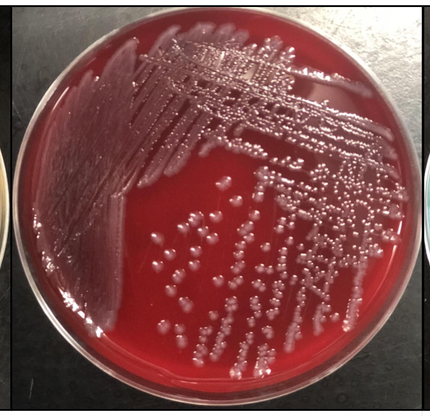

Figure 2. Blood agar streak plate of the antibiotic-producing bacterial isolate incubated at 37C overnight. No hemolysis was observed.

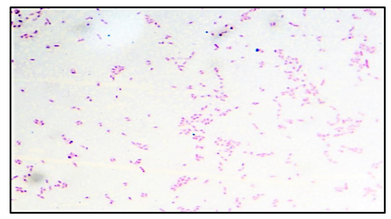

Figure 3. Gram stain of the antibiotic-producing isolate. Gram negative bacilli were observed.

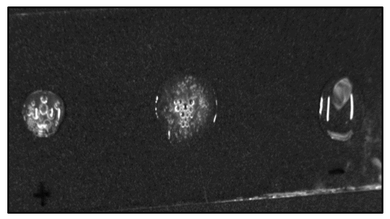

Figure 4. Catalase test of *S. aureus* (left; positive control), the antibiotic-producing isolate (middle), and *E. faecalis* (right; negative control). The bubbling shows catalase activity.

DNase Test: The antibiotic-producing isolate was tested for its ability to degrade DNA by performing a DNase test. It was streaked onto a DNase plate along with a positive control. A clear zone surrounding the streak indicates a positive result for the DNase enzyme.

Eosin Methylene Blue Agar Plate: EMB plates were utilized in order to ensure the isolate was Gram negative, along with testing for lactose fermentation. Any growth indicates Gram negative bacteria, with purple colonies indicating lactose fermentation.

Methyl Red/Voges Proskauer: MR/VP broth was inoculated to determine the types of products produced as a result of glucose fermentation. The MR test detects acidic end-products, whereas the VP detects neutral end-products of glucose fermentation.

Sulfide/Indole/Motility Test: The SIM media was used to detect H_2S and indole production, as well as motility. H_2S producers turn the media black, the development of a red color after the addition of Kovac's reagent indicates a positive indole test, and movement away from the stab line indicates motility.

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

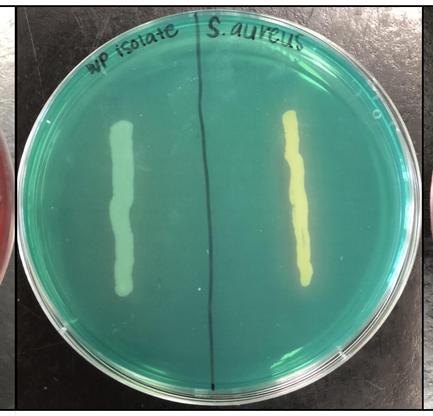

Figure 5. DNase tester plate of the isolate (left) along with a positive control, *S. aureus* (right). The test shows no DNase activity.

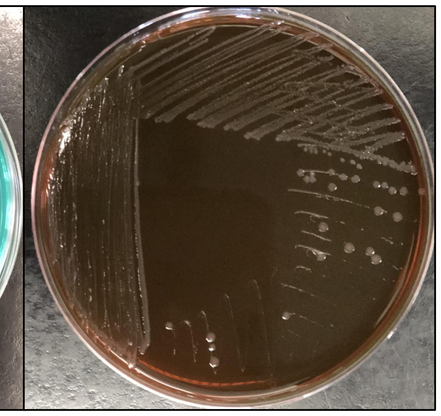

Figure 6. EMB streak plate with the antibiotic-producing isolate. No lactose fermentation was observed.

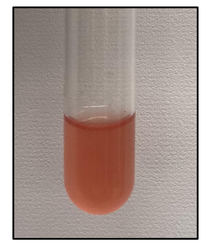

Figure 7. Positive MR test of the isolate. The reaction showed mixed acid fermentation.

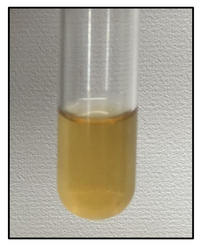

Figure 8. Negative Voges-Proskauer test.

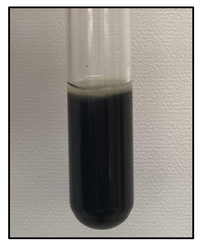

Figure 9. SIM test of the isolate showed motility and sulfide production, but no indole formation.

Figure 10. Negative Citrate test.

DISCUSSION

One antibiotic-producing soil isolate was identified on the tester plate inoculated with *S. enteritidis* (Figure 1). The Gram stain revealed the isolate to be a Gram negative bacillus (Figure 3), and the catalase test was positive (Figure 4). The soil isolate did not produce hemolysis when inoculated onto blood agar (Figure 2), and was a lactose non-fermenter (Figure 6). In addition, the soil isolate fermented glucose to produce mixed acids (Figure 7), and was able to reduce sulfur to form H_2S (Figure 9).
This antibiotic-producing isolate will be identified through 16S rRNA gene sequencing. The antibiotic will then be partially purified by extraction with an organic solvent with the extract being used to confirm the activity of the antibiotic.

REFERENCES

1. 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. Phisiol. Pharmacol.* 95: 595-603.
2. Hernandez S, Tsang T, Bascon-Slack C, Handelsman J. 2016. Small World Initiative; 4th Ed. Small World Initiative Press

ACKNOWLEDGEMENTS

Financial support of this project was provided by the UW-Eau Claire Office of Research and Sponsored Programs.

We thank the Office of Research and Sponsored Programs for supporting this research, and Learning & Technology Services for printing this poster.