University of Dayton

Isolating Antibiotic Producing *Pseudomonas* From Soil

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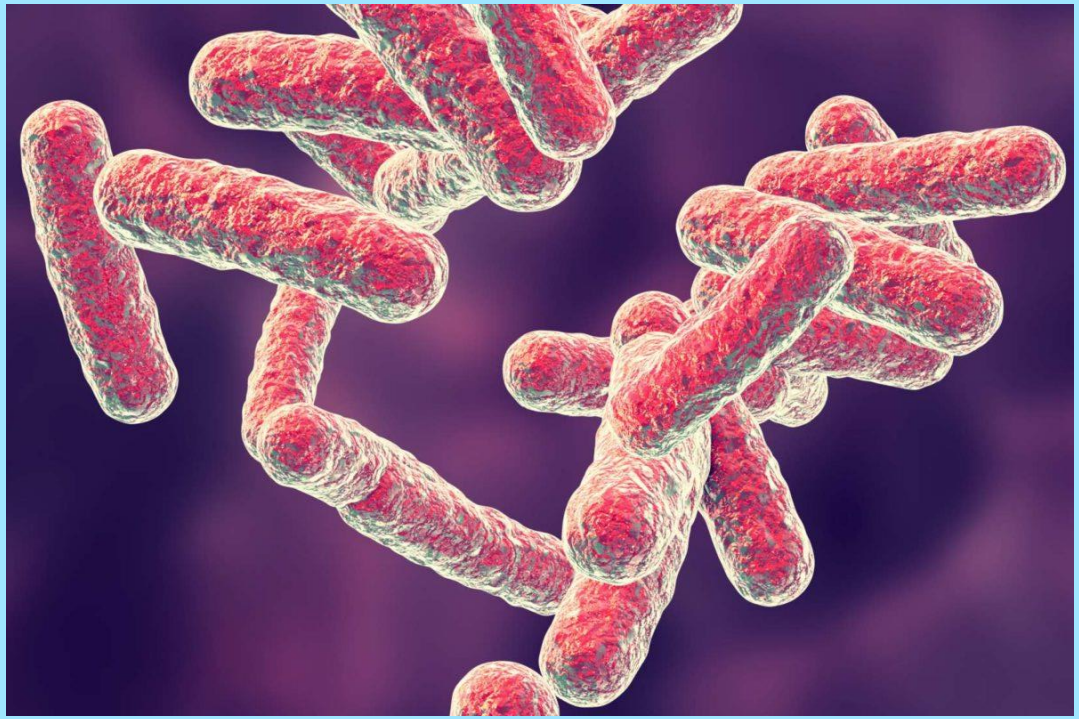


Introduction

- The Tiny Earth Network works to address the decreasing amount of effective antibiotics by testing soil bacteria for antibiotic production. Antibiotics are used in medicine to treat bacterial infections by killing or slowing the production of bacteria. A threat to the common treatment is antibiotic resistance which has resulted in a health crisis. To combat this, new antibiotics need to be discovered and through the Tiny Earth Initiative bacteria from soil samples are being used as a source. The isolated soil bacteria was tested for antibiotic production against clinical pathogens such as *E. coli* and *S. epidermidis*. Laboratory methods such as gram staining, biochemical testing, and extraction were used to identify the isolated soil bacteria. It is important to understand the isolate characterization to know if the isolate could be toxic to humans and therefore not an ideal source of antibiotic medication. A way to test for toxicity involved extracting the organic material of the isolate and letting it dry. Methanol was added to the dried extract and the solution was plated onto a water agar plate. Chia seeds were sprinkled onto the plate and left to grow or not grow. Chia seed growth indicated the antibiotic was not toxic to humans while no growth indicated toxicity. Discovery of antibiotic producing bacteria will help the ongoing battle of antibiotic resistance and its effect on bacterial infection treatment options.

Research Methods

Grow bacteria from soil sample

Pick colonies for testing

Test antibiotic producing activity

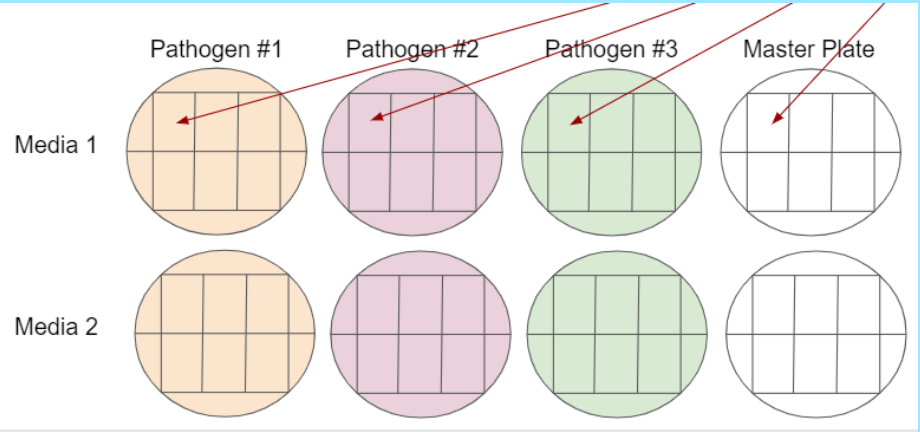
Isolate pure cultures

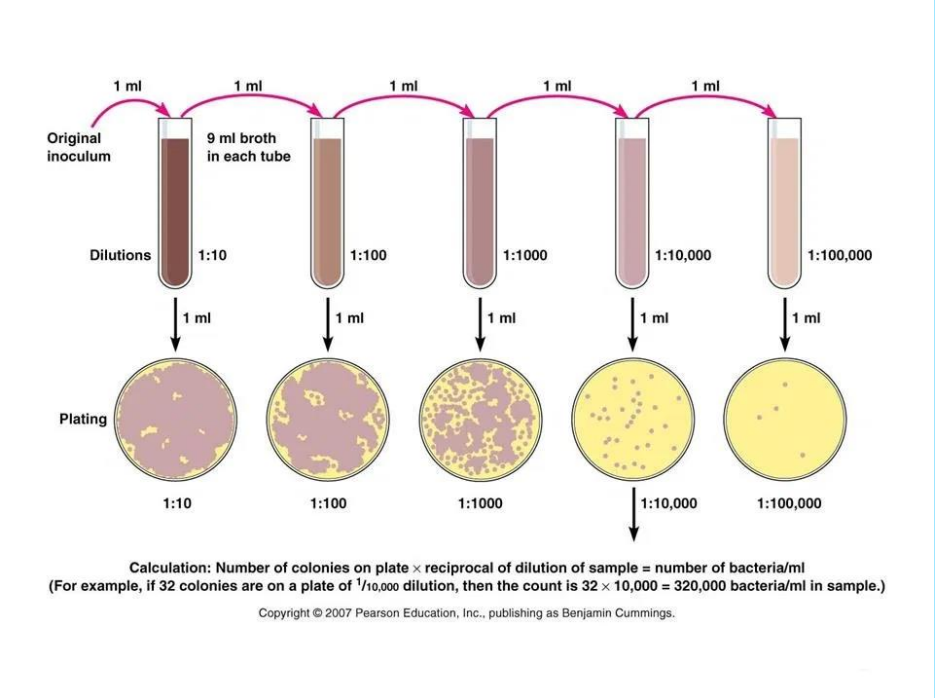
Identify isolates through biochemical testing and DNA sequencing

Extract active compounds and test isolates for toxicity

<u>Media used:</u>
Reasoner's 2A Agar (R2A)
Tryptic Soy Agar (TSA)

<u>Serial Dilution</u>: used to separate individual bacteria

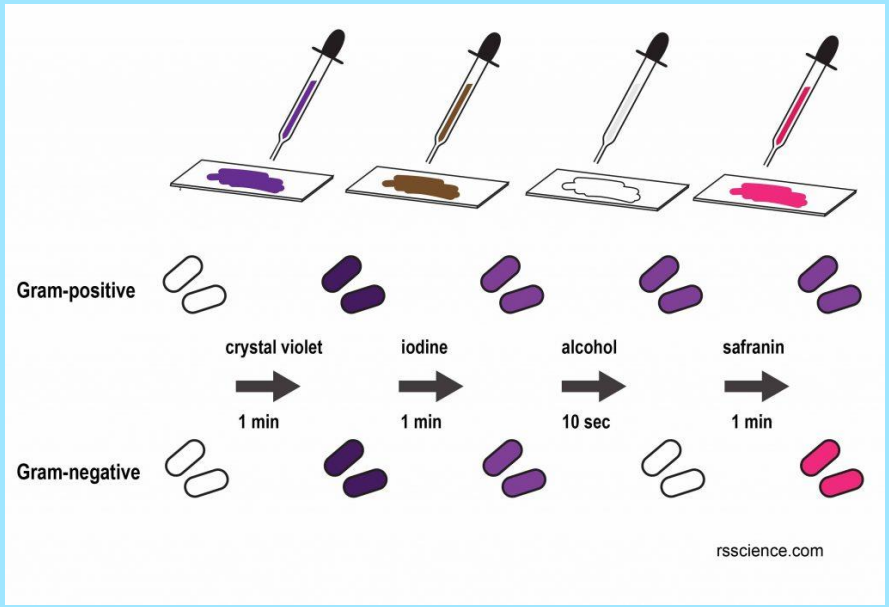




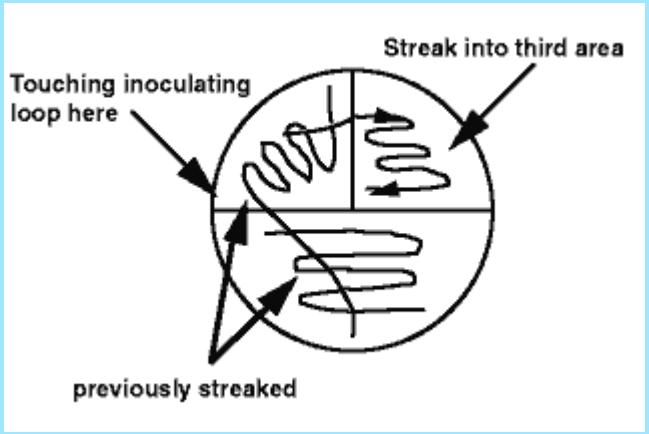
<u>Colony selection and master plate:</u> Two colonies from dilution plates that exhibit zones of inhibition are plated onto master plates

Research Methods

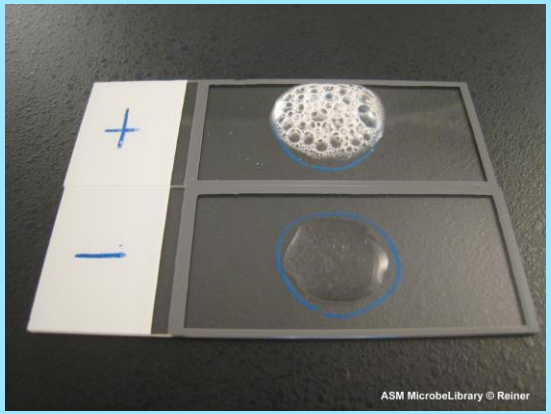
Gram staining: characterizes isolates as gram-positive or gram-negative



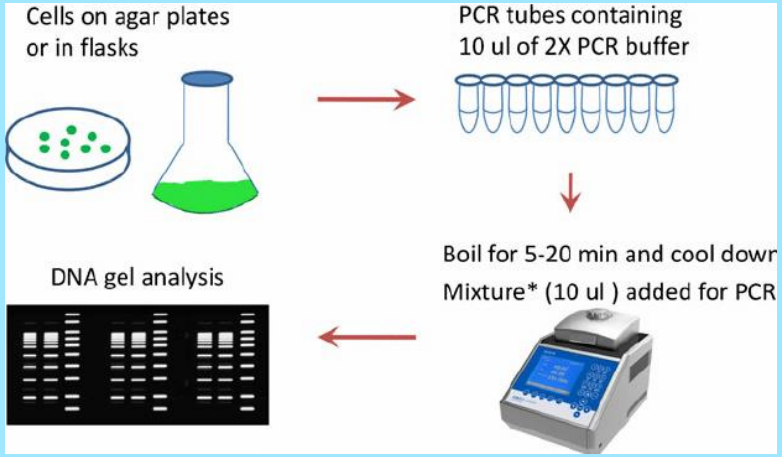
Streak plates: made to generate as many single colonies as possible



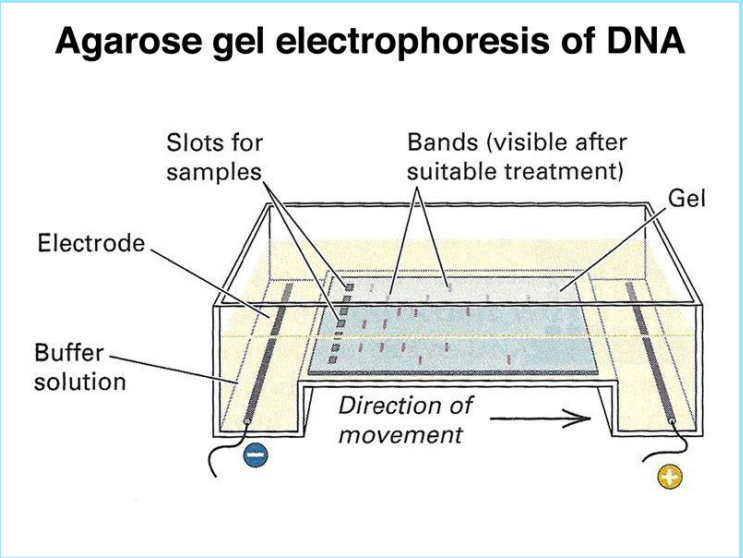
Catalase activity: using hydrogen peroxide, can test if isolate has enzymes to break down hydrogen peroxide



16s rRNA PCR: makes copies of the DNA templates

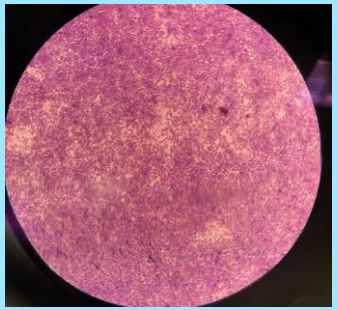


Gel electrophoresis: confirms the presence of PCR product to determine if can be DNA sequenced



Results

Gram staining:
R2A isolate: gram positive
TSA isolate: gram positive



Catalase test:
R2A isolate: positive
TSA isolate: positive

Blood agar:
R2A isolate: Alpha
TSA isolate: Alpha

MSA: no growth
R2A isolate: inconclusive
TSA isolate: inconclusive

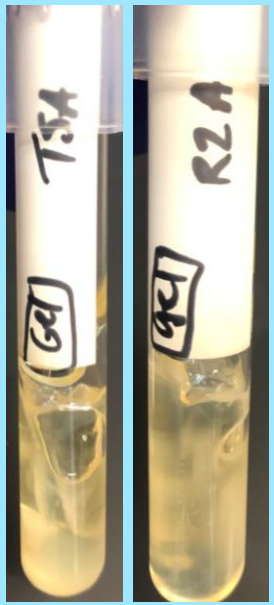
EMB: growth occurred on both sides of the agar plate
R2A: pink
TSA: pink

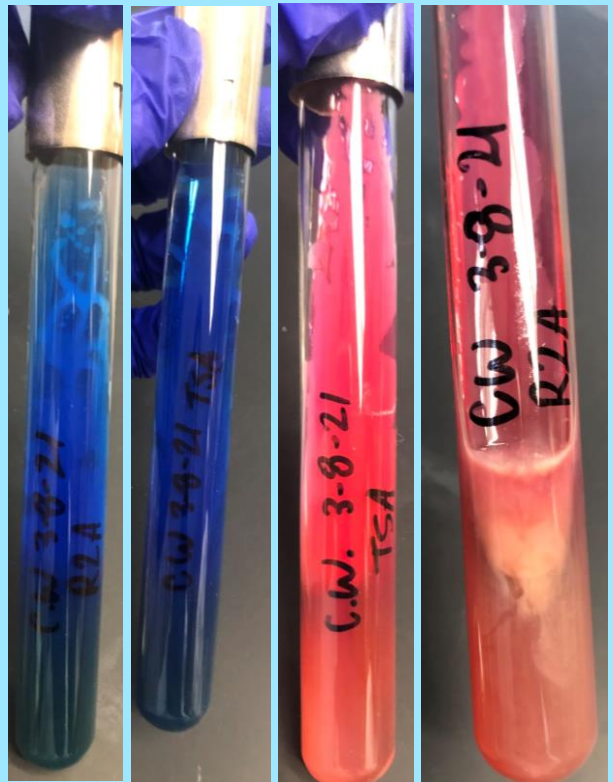
MC:
R2A: growth and red
TSA: growth and red

Simmons Citrate:
R2A: Blue
TSA: Blue

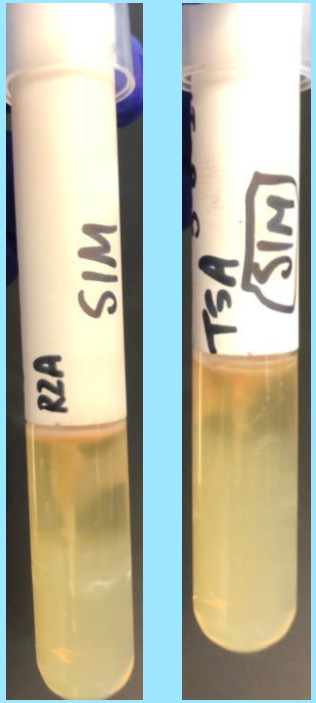
Triple Sugar Iron Slant:
R2A: K/K
TSA: K/K

Gel tubes:
R2A: Negative
TSA: Negative





SIM tubes:
R2A: negative
TSA: negative

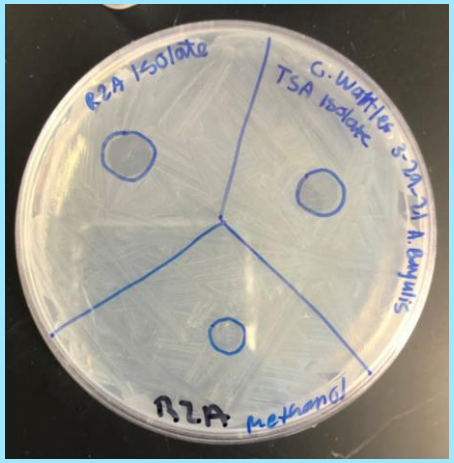


Cobac test:
R2A: negative
TSA: negative

Results

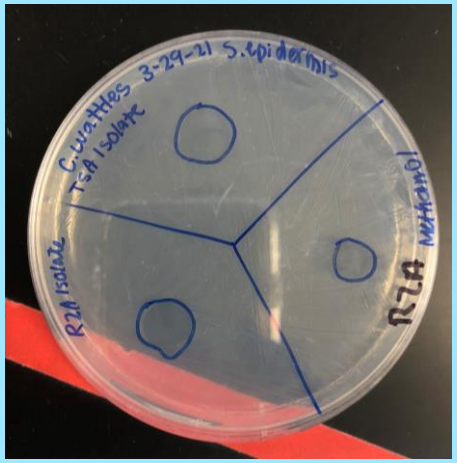
Gel electrophoresis: DNA present for sequencing

Efficacy: Anti-microbial activity



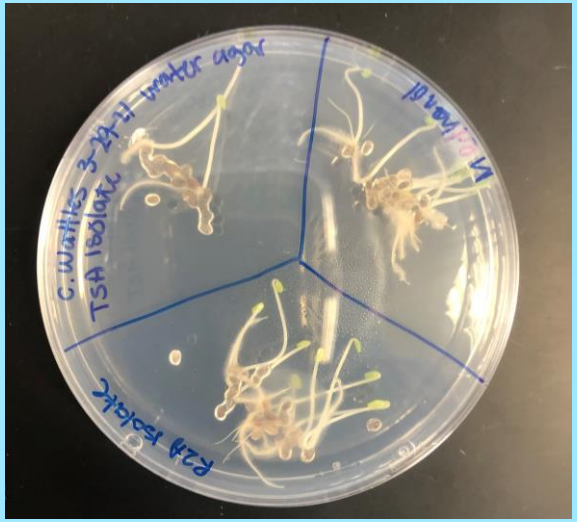
Positive

Negative



Positive

Toxicity: determines if harmful to eukaryotes
R2A isolate: not toxic
TSA isolate: not toxic



Discussion

- DNA sequencing determined both R2A isolate and TSA isolate are the genus ***Pseudomonas***
- Gram staining results indicated gram- positive, but *Pseudomonas* is typically gram- negative. This could be due to human error when using the gram-staining chemicals.
- The results of the biochemical testing specify that both isolates can secrete hemolysins, are low acidity, tolerate bile salt and crystal violet, performs lactose fermentation, and citrate is the carbon source.
- The isolates do not ferment glucose, sucrose, or lactose and there is no gelatinase production. They are not motile, no sulfur reduction takes place, and there is no indole production. The isolates are not toxic to Eukaryotes.
- Anti-microbial activity occurred with both isolates when plated with *S. Epidermidis* and *A. Baylyi* but no antibiotic production against *B. Subtilis.*

Sources

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