Isolation and Screening of Soil Bacteria as a Source of Antibiotics

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Abstract

Antibiotic resistance is becoming a prominent hardship when treating bacterial infections. The more resistant pathogens become, the more of a threat they are to public health. The overuse and irresponsible consumption of antibiotics is the leading cause of resistance development. Naturally occurring antibiotics are difficult to isolate which severely limits modern ability to counter resistance. The only way to steer this current crisis is to develop comprehensive strategies that combine antimicrobials with other agents to reverse the mechanisms of antibiotic resistance. The purpose of this experiment was to locate antibiotic producers within our environment from soil bacteria. Twelve bacterial colonies were isolated and screened against close relatives of known pathogens and included *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Escherichia coli*. One isolate was found to be an antibiotic producer when screened against *B. subtilis*. If enough positive producers are found, we may be able to combine a few to counter potential resistance mechanisms developed by harmful pathogens.

Objective

- To locate antibiotic producers within our environment from diluted soil bacteria. If producers were found, secondary screens were done to identify more characteristics of the bacteria.

Methods

- Soil was collected from California, PA and diluted five times up to a 1.0 x 10^{-5} dilution
- Spread plates were created from the dilution tubes
- Single colonies from the spread plates were used to create streak plates with 12 different bacteria types. A master plate was created from the streak plates using the pick and patch method
- Colony morphology was observed and recorded
- An antibiotic screen was run using safe relatives of pathogens *B. subtilis* on NA (nutrient agar), E. coli on LB (Lysogeny Broth), and *S. epidermidis* on LB agar. Each bacterial organism from the master plate was then patched onto each of the three pathogen plates.
- Two plates with MacConkey agar were used to plate four organisms using the streak plate method. One plate contained E. coli and B. subtilis, and the other contained isolate number 1 from the master plate and isolate 11.
- Biochemical tests including: a gelatin test with *B. subtilis* and the isolate number 11, an oxidase test with *B. subtilis*, *E. coli*, and the isolate 11, and a catalase test with *B. subtilis*, *E. coli*, and the isolate 11.

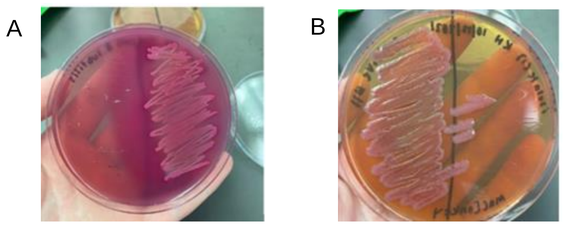
Results

Antibiotic Producer Screen

S. epidermidis *B. subtilis* *E. coli*

Figure 1: Antibiotic Producer Screen plates. Plate A was screened against S. epidermidis, plate B was screened against B. subtilis, and plate C was screened against E. coli. Positive isolates were found when screened against B. subtilis in numbers 9 and 11 as indicated by the circle in red. Both 9 and 11 appeared to be the same bacteria, so only isolate 11 was recorded.

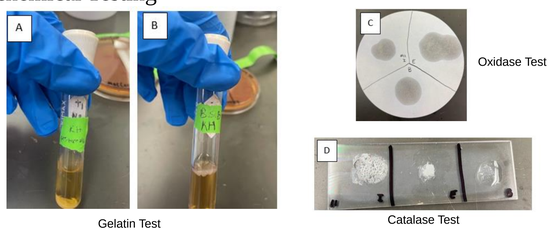
Differential and Selective Media



Control: *B. subtilis* (left) and *E. coli* (right) *Positive producer #11* (left) and *Isolate #1* (right)

Figure 2: Differential and Selective media plates using MacConkey Agar. Plate A tested for lactose fermenting bacteria in B. subtilis (left) and *E. coli (right). Agar remained purple and growth was observed for only E. coli. Plate B tested for lactose fermenting bacteria on the positive antibiotic producer number 11 (left) and the first isolate (right) from the master plate. Agar faded slightly to an opaque color. Growth was observed only for the positive antibiotic producer.*

Biochemical Testing



Gelatin Test Oxidase Test Catalase Test

Figure 5: Biochemical testing techniques. The gelatin test was conducted and observed in figure A for the antibiotic producing bacteria isolate 11, and in figure B for B. subtilis. Both gelatin samples were liquid after exposure. Clumps of bacteria were visible at the bottom of figure A and visible at the top of figure B. Figure C shows the oxidase test and contained patches of B. subtilis, E. coli, and the positive antibiotic isolate 11. No purple color observed. Figure D shows the catalase test and contained patches of B. subtilis, E. coli, and the positive antibiotic isolate. Hydrogen peroxide was added dropwise to each patch, and all three bubbled after exposure.

Discussion

- Antibiotic Producer Screen
- A positive result was found on the nutrient agar when screened against B. subtilis for isolates numbered 9 and 11. This positive result was identified by a clearing surrounding the patch of bacteria. However, both isolates had identical colony morphology descriptions, so it was assumed that both were the same organism. When streak plates were created, it was likely that the same bacteria was picked up twice from the spread plate. Only one positive result was found in all three plates.
- Differential and Selective Media
- Differential and selective media plates containing MacConkey agar were used to test for lactose-fermenting and gram-negative bacteria. The first plate containing E. coli and B. subtilis was considered the control plate. The E. coli grew on the agar, as well as kept it purple which indicates that this is a gram negative and lactose fermenting organism. The B. subtilis did not exhibit growth on the agar, and had no impact on the media which indicates that it is gram-positive organism, but it is unspecified whether it is a lactose fermenter or not. On the second plate containing the antibiotic producer isolate numbered 11 and the isolate numbered 1. Isolate 11 exhibited growth, indicating that it was gram-negative and caused the media to fade slightly to an opaque color meaning it was non-lactose fermenting. Isolate 1 did not appear to grow at all, indicating that it was gram-positive. The media also faded slightly to an opaque color, but because no growth was observed it is unknown whether or not this is a lactose fermenting bacteria.
- Biochemical Tests
- The biochemical gelatin test tested for the presence of the enzyme gelatinase in B. subtilis and the positive antibiotic producer isolate numbered 11. After exposure to the bacteria, both test tubes turned to liquid meaning they contained the enzyme gelatinase. The robust growth shown by the biofilm may have been formed from a continued growth of each bacterium once the gelatin liquefied. During the oxidase test, the filter paper with E. coli, B. subtilis, and the positive antibiotic producer isolate did not appear to have any color change indicating that no cytochrome oxidase was present in any of the three bacteria. The catalase test tested for the presence of catalase in E. coli, B. subtilis, and the positive antibiotic producer. The presence of bubbling directly correlated with the presence of catalase, and all three bacteria exposed to hydrogen peroxide experienced bubbling. Thus, we can conclude that the three bacterium tested for catalase were positive producers of the enzyme. The positive antibiotic producer was a Gram-negative organism that cannot ferment lactose but contains the enzymes gelatinase and catalase.

Literature Cited

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