Isolating Soil Bacteria in Order to Screen for New Antibiotic Producers

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ABSTRACT

Right now, human health, economics, and politics are being threatened due to an antibiotic crisis caused by antibiotic resistance. There are many diseases caused by bacteria that are becoming untreatable due to pathogens becoming resistant to treatment, and the current pace of antibiotics is no match for the rapid evolution of resistance within these organisms. Antibiotics are typically produced by microorganisms, and it has been found that soil has a high abundance of microorganisms. By collecting soil, students can contribute to helping solve this ongoing antibiotic crisis. Using serial dilutions, single colonies were selected and tested for antibiotic production through multiple laboratory experiments and tests. Ten isolates were screened against *Escherichia coli, Staphylococcus epidermidis*, and *Bacillus subtilis*. Using soil to screen for antibiotic producers serves as an important role in helping to solve this antibiotic crisis by creating the opportunity to find novel antibiotics.

OBJECTIVE

To find and isolate antibiotic producers from diluted soil. If positive producers were found, secondary screens were completed to further investigate the properties and characteristics of the bacterium.

METHODS

- Soil from California, PA was collected and diluted five times (up to a 1.0 x 10^{-5} dilution)
- Spread plates were created using the last three dilution plates
- From the spread plates, streak plates were created with 12 different bacteria types
- A master plate was created from the streak plates, and colon morphology was observed and recorded
- An antibiotic screen was run using safe relatives of pathogens *B. subtilis* on nutrient agar, *E. coli* on Lysogeny Broth, and *S. epidermis* on Lysogeny Broth. The bacteria was picked and patched from the master plate onto each of these three plates
- Two plates with Eosin Methylene Blue (EMB) 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 #3 and isolate #4 from the master plates
- Three biochemical tests were conducted. The tests consisted of a gelatin test on *B. subtilis* and isolate #4, an oxidase test on *B. subtilis, E. coli*, and isolate #4, and a catalase test with B. subtilis, E. coli, and isolate #4.

RESULTS

Antibiotic Production Screen Plates

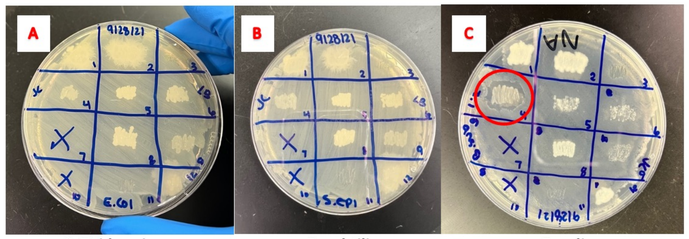
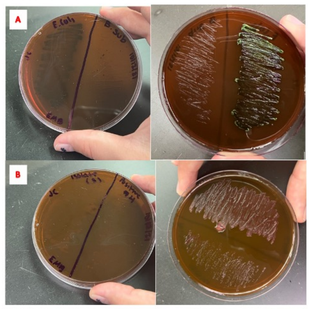


Figure 1: Each plate was screened against a different bacterium. The bacteria used were Escherichia coli (plate A), Staphylococcus epidermis (plate B), and Bacillus subtilis (plate C). Positive isolates were found when screened against B. subtilis on isolate number 4. This is indicated by the red circle.

Selective and Differential Media Plate

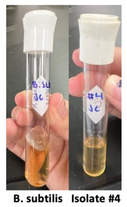


Control: B. subtilis (second picture on the left) and E. coli (second picture on the right)

Positive isolate #4 (second picture on the right) and isolate #3 (second picture on the left)

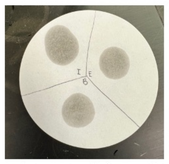
Figure 2: Eosin Methylene Blue (EMB) Agar was used to select for gram negative organisms and differentiate between lactose fermenting and non-lactose fermenting bacteria. Escherichia coli on plate A had growth and a metallic, green sheen. B. subtilis had no growth. On plate B, isolate number 3 had growth and a light purple color. On plate B, isolate number 4 had little to no growth and no color change.

Biochemical Tests



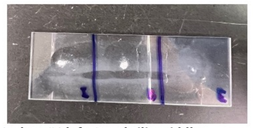
B. subtilis Isolate #4

Figure 3: A gelatin test was conduc on B. subtilis and isolate #4. The t with B. subtilis liquified and the tu with isolate #4 stayed as gelatin.



Isolate #4 top left, E. coli top right, B. subtilis bottom

Figure 4: An oxidase test was completed on B. subtilis, E. coli, and isolate #4. None of the bacteria tested produced any color change.



Isolate #4 left, B. subtilis middle, E. coli right

Figure 5: A catalase test was completed on B. subtilis, E. coli, and isolate #4 with hydrogen peroxide. All 3 bacteria produced bubbles. B. subtilis and isolate #4 had a lot of bubbles, and E. coli had little bubbles.

DISUSSION

Antibiotic Production Screen Plates. Out of all the bacteria plated and screened, there was one positive antibiotic producer on the plate that screened against *B. subtilis*. This was shown in figure 1C by the clearing around the bacteria plated. The clearing indicates a positive producer. This is important for the antibiotic crisis the world is currently in. If people can find and report antibiotic producers within soil, it could contribute to solving this crisis.

Selective and Differential Media Plates. For the plate containing *E. coli* and *B. subtilis, B. subtilis* had no growth on the EMB plate. There was also no color change. This indicates that *B. subtilis* is gram positive and non-lactose fermenting. *B. subtilis* is a gram-positive organism does not ferment lactose, and the results support this fact. The *E. coli* had blue/black growth and a metallic green sheen. These results indicate the organism is a gram-negative lactose fermenting organism. *E. coli* is gram negative and lactose fermenting, so the results support this fact. On the plate with isolate #3 and #4, isolate #3 showed a little growth, but no color change. This indicates that the organism is most likely gram-negative non-lactose fermenting organism. Isolate #4 had growth as well as a light purple color. This indicates it is a lactose-fermenting gram-negative organism. EMB agar plates allow for selection of gram-negative bacteria, as well as the differentiation of lactose fermenting and non-lactose fermenting bacteria. They allow for more information to be known regarding bacteria which is important for knowing how a certain bacteria will interact with humans.

Biochemical Tests. For the gelatin test, *B. subtilis* turned the gelatin into a liquid. This indicates the bacteria has the enzyme gelatinase. The gelatinase hydrolyzed the gelatin. The tube with isolate #4 still contained gelatin, so no gelatinase was detected. For the oxidase test, *E. coli, B. subtilis*, and isolate #4 did not have any color change. This indicates that there was no cytochrome oxidase present in the bacteria. For the catalase test *E. coli* produced little bubbles, and *B. subtilis* and isolate #4 produced significant bubbles. This indicates a positive result for catalase that breaks down hydrogen peroxide into oxygen and water. There may have been errors with the oxidase test. Enough bacteria may not have been transferred onto the filter paper. Biochemical tests are some of the most important tests for microbial identification. It also is a quick, cheaper way to identify unknown organisms.

REFERENCES

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