Finding Antibiotic Producing Streptomyces in Local Soil

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ABSTRACT

Streptomyces, a bacterium commonly found in different soils across the world, are some of the biggest antibiotic producers in the world. The objective of this study was to find and isolate an antibiotic producing strain of *Streptomyces* from soil samples collected from different areas, and to determine the optimal time to culture the bacteria prior to testing for antibiotic production. Three different soil samples were collected and plated, the colonies were isolated in order to test them for antibiotic production. Fifteen different colonies were tested from three different soil samples. One colony was identified as *Streptomyces* bacteria that produced an antibiotic. A five-day incubation seemed to be optimal for antibiotic production that inhibited the growth of *Staphylococcus aureus.* This antibiotic was isolated for further characterization using organic chemistry techniques.

INTRODUCTION

Streptomyces is a gram-positive bacterium from the Phylum Actinobacteria that can be found in different soils where the breakdown of organic matter is taking place. The soil environment is rich in many different species of bacteria with competition for nutrients occurring between the many different soil organisms. When microorganisms are put into low nutrient environments, they spread out in order to find nutrients and start producing secondary metabolites. Some secondary metabolites are substances that inhibit the growth of other organisms in order to reduce competition. These substances (antibiotics) can be isolated to treat bacterial infections in mammals.

METHODS

Three soil samples were collected from different areas with decaying organic matter. These soil samples originated from a forest with decaying leaves, a compost bin being made at the University of Indianapolis, and under a pine tree where dead needles were plentiful. From there, the soils were diluted 100-fold, streaked onto a nutrient agar plate and incubated for a total of 3-5 days. Colonies resembling a *Streptomyces* phenotype (dry, leathery, colorful colonies) were selected and isolated onto nutrient agar plates. Once they were grown in isolation, all the colonies were taken and spread on three different blood heart infusion agar plates in a straight line down the middle. The three plates were then incubated for 5, 14, and 21 days before adding two lines of *Escherichia coli* and *Staphylococcus aureus* to test for growth inhibition. Out of the all the different plates created, only one bacteria came back with antibiotic production that was effective against *S. aureus* but not *E. coli*. The five-day plate had the most inhibition with a total amount of 31mm between the two lines of *S. aureus*. The 14-day plate showed a total of 16mm of inhibition, and the 21-day plate showed only 10mm of inhibition. In order to isolate the antibiotic, the agar was first sliced and placed in a tube to freeze overnight. Next, 15mL of ethyl acetate and 10mL of water were added and the solution incubated with shaking overnight. Once thawed, the solution was left sitting for 2-3 minutes and then the top organic layer was transferred into a separate pre-weighed bottle. Once all of the organic layer was into the pre-weighed bottle, a rotovap was used to evaporate the ethyl acetate in order to leave the remaining antibiotic product at 20 micrograms.

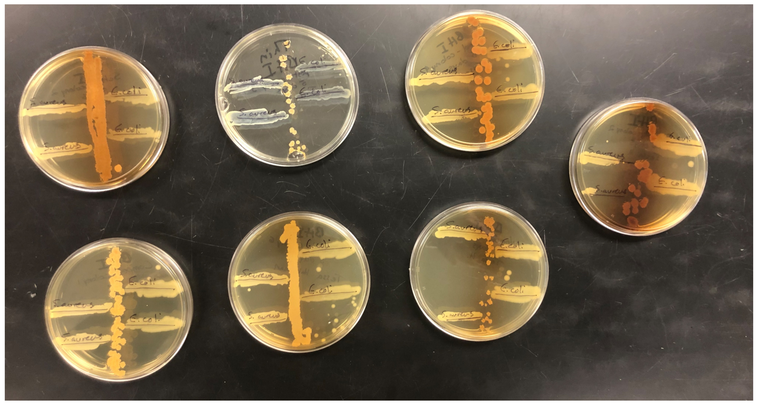

Figure 1: Above you see colonies that have both E.coli and S. aureus streaked in a perpendicular line to the *Streptomyces* colonies, but no inhibition can be seen on a majority of the plates present except for the plate on the very far right. The specific colonies above were School colonies 2 and 4, wooded colonies 1 and 6, and compost colonies 1, 2, and 3.

RESULTS

Figure 2: On the right shows a closer picture of compost colony 2, the plate found on the far right in figure 1. It has inhibition against the bacteria *S. aureus*. In the top line of 11mm (millimeters) and inhibition in the bottom line of 21mm. This data is also represented in Table 1 found below.

Table 1: Inhibition of S. aureus found on plates with Compost Colony 2

Compost Colony 2 (Inhibition Present)	Line 1 (S. aureus)	Line 2 (S.aureus)
5 Days	11mm	21mm
14 Days	5mm	11mm
21 Days	3mm	7mm

RESULTS & DISCUSSION

Five days was the optimal amount of days to grow streptomyces on brain-heart infusion agar plates before adding test bacteria. This time seemed to optimize the release of the antibiotics. Furthermore, the isolation resulted in 20 micrograms of product from that plate. The 20 micrograms of product that was received from the plate may have been slightly off as some of the aqueous layer may also have been taken from the tube. If the experiment was to be repeated one should pour the double layered ethyl acetate water solution with agar chunks into a separatory funnel using a utensil to keep the agar chunks from falling into the funnel. Then they should separate out the aqueous layer from the organic layer and put the organic layer into a flask usable on the rotovap. Although some of the aqueous layer was put into the end product, most of it was still able to be evaporated off to get the ending product.

Overall, when using a blood-heart infusion agar, the optimal time to allow *Streptomyces* to grow would be five days in order to allow proper growth and release of secondary metabolites from an antibiotic producing strain. Going beyond five days would allow too much time for the bacteria to grow causing the bacteria to dry out and stop producing secondary metabolites. Future studies should repeat this work with the same isolate and identify the antimicrobial produced. Figure 3 below shows the overall inhibition vs. the amount of days *Streptomyces* was cultured before *S. aureus* and *E.coli* were added.

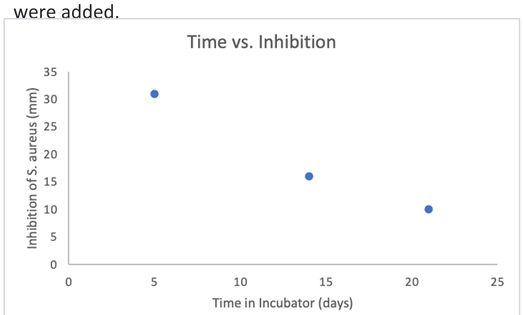

Fig. 3: Graph comparing time (in days) of incubation to the total amount of inhibition in millimeters (mm)

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LITERATURE CITED

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