Screening Environmental Soil Samples For Antibiotic Production

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Abstract:
Antibiotic resistance is a detrimental worldwide challenge, producing bacterial infections that are progressively more difficult to treat and cure. To attempt to help alleviate this issue, we screened soil samples for the presence of antibiotic-producing microorganisms. Soil samples were collected and diluted to 1:100 and 1:1000 ratios of soil and distilled water. These soil mixtures were then streaked onto tryptic soy agar (TSA) plates and incubated at 30℃ until colonies developed. These colonies were then selected and plated on a lawn of *Serratia marcescens*, which was utilized as the target organism.
Serratia marcescens was selected due to its known resistance to many widely prescribed antibiotics such as penicillin and ampicillin. Colonies that produced clear zones on the *Serratia marcescens* lawns, indicating antibiotic production, were then isolated into pure cultures by sequential rounds of streaking. Isolated organisms were submitted for partial 16S rDNA sequencing and preliminary identification. Using this approach, we isolated three bacterial strains with antibiotic activity. The results of sequencing verified that our organisms were *Bacillus mojavensis* and two individual strains of *Bacillus amyloliquefaciens* with 0.09%, 0.47%, and 0.28% genetic differences from known partial 16S rDNA sequences in the database, respectively. In the future, further characterization of each of these organisms and isolation of each compound of interest will be executed to further investigate each organism's antibacterial properties.

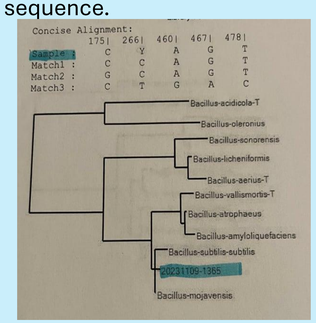
References:
"About Antibiotic Resistance." Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 5 Oct. 2022, www.cdc.gov/drugresistance/about.html.
"How Antibiotic Resistance Happens." Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 5 Oct. 2022, www.cdc.gov/drugresistance/about/how-resistance-happens.html.
Tamehiro, Norimasa, et al. "Bacilysocin, a Novel Phospholipid Antibiotic Produced by Bacillus Subtilis 168." Antimicrobial Agents and Chemotherapy, U.S. National Library of Medicine, Feb. 2002, www.ncbi.nlm.nih.gov/pmc/articles/PMC127064/.

Introduction:
Antimicrobial resistance is defined by the CDC as "when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them," essentially expressing the microbe's capacity to continue infecting the host despite an array of treatment methods. Worldwide this has become an increasingly substantial and hazardous issue with increasingly damaging effects. In 2019 alone it was associated with over 5 million deaths and directly caused 1.27 million of those. Although antimicrobial resistance is a naturally occurring process it can be accelerated by the recurrent utilization of remedies such as antibiotic or antifungal medication. It is most commonly established through the development of defense mechanisms in certain microbes undergoing treatments by such medication. Microbes with these specific mechanisms which allow for its survival are then yielded unscathed and can then reproduce and continue to infect the host. This new population, however, can now not be affected by the treatment used previously, as its survival and defense mechanism is reproduced in each new microbe of this population. This establishes a pattern of diseases and conditions that are perpetually more difficult to eliminate and cure, directly inducing the rise in infections and fatalities seen today.
In search of ways to alleviate this issue and attempt to generate new antibiotics, we honed in on antibiotic-resistant bacteria and elected to examine soil samples to locate organisms that are natural antibiotic producers. These were plated against *Serratia marcescens,* as it is resistant to many commonly utilized antibiotics.

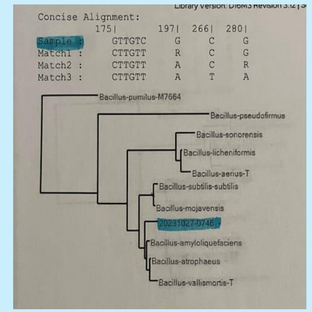
Methods:
Various soil samples were collected in sterile tubes from different locations on or around our university campus. Each selected sample was diluted to 1:100 and 1:1000 ratios of the soil and distilled water. These mixtures were then streaked onto two separate tryptic soy agar (TSA) plates and incubated at 30 ℃ until colonies developed. These colonies were then individually selected and plated on a lawn of *Serratia marcescens* which was utilized as the target organism. It was selected as such because it is known to be resistant to many widely prescribed antibiotics such as penicillin and ampicillin while also being safe to have contact with. These plates were then incubated at 30℃ until new colonies formed. Specific colonies with inhibition zones were then identified. This process was repeated until three colonies with clear zones on the *Serratia marcescens* lawn, indicating antibiotic production, were identified. These colonies were then isolated into pure cultures by three sequential rounds of streaking. Isolated organisms were submitted for partial 16S rDNA sequencing and preliminary identification. Through this process we were able to identify all three of our previously mentioned bacterial strains with antibiotic production. The sequencing confirmed that the organisms were *Bacillus mojavensis* and two individual strains of *Bacillus amyloliquefaciens* with 0.09%, 0.47%, and 0.28% genetic differences from known partial 16S rDNA sequences in the database, respectively.

Discussion:
As microbial resistance grows worldwide along with an aging population, the demand for new and novel antibiotics expands as well. The method of discovery we honed in on has been established as a reliable way to identify these components with many important discoveries from other soil organisms.
Each organism isolated and identified is classified in the genus *Bacillus.* In previous studies, many of those in this genus have been identified as natural antibiotic producers. For example, the antibiotics surfactin and bacilysocin produced from *Bacillus subtilis,* and bacteriocins and other peptides produced in *Bacillus sensulato.* This establishes our findings as neither unusual nor unanticipated, but reliable.
The analysis of the 16S rDNA however, was not completely conclusive. With great accuracy, it was able to identify the closest matching organisms known in the database, but not with certainty. These organisms could have unique mechanisms to naturally create this antibiotic which have not previously been studied or analyzed. Specifically, to further this study, the antibiotic components isolated will undergo more advanced research. In the future, we would like to dissect the specific chemical components through mass spectrometry and gas chromatography to precisely identify what is causing its antimicrobial effects. It will also be useful to compare these findings to other known antibiotics to establish whether they are novel and if not, their similarities, effectiveness, and method of production.

Results
Three organisms that were natural antibiotic producers for antibiotic resistant bacteria were identified through partial 16S rDNA sequencing.
The first organism was identified as *Bacillus mojavensis* with around 0.09% genetic differences from the known 16S rDNA sequence.



The second organism identified was a strain of *Bacillus amyloliquefaciens* with 0.47% genetic differences from the known 16S rDNA sequence.



The third and final organism identified was a second strain of *Bacillus amyloliquefaciens* with 0.28% genetic differences from the known 16S rDNA sequence.