Winter 1-28-2020

Pseudomonas and Bacillus as Potential Sources of Novel Antibiotics

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Bacillus and *Pseudomonas* Genera as a Potential Source for Novel Antibiotics

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INTRODUCTION

The need for new antibiotics is an ever-growing issue in the medical field due to a multitude of issues, most notably the over-prescription of antibiotics and the non-cooperation of patients in following the advised prescription time period.[1] This has led to multiple drugs becoming resistant to the various drugs used to treat them. The ESKAPE bacteria are a group of six bacteria that are considered in critical or high need of finding new antibiotics (Figure 1). The six bacteria are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp. Through collaboration with the Tiny Earth Project Initiative, a worldwide database focused on finding novel antibiotics for the treatment of the ESKAPE strains, we hope to discover antibiotics through bacteria found in soil.[2]

Fig 1. The ESKAPE strains are becoming more resistant to the antibiotics used to treat them.[5] The graph shows common antibiotics and what percentage of just one strain, *Enterococcus faecium,* is resistant to them.

With the rise of methicillin-resistant *Staphylococcus aureus* (MRSA), among various other bacteria, hospital-acquired infections (HAI) are becoming a more significant issue.[1] *Staphylococcus aureus* and different other strains of the *Staphylococcus* genus are commonly found in surgical site infections (SSI).[3] A study done by Pal et al. showed that 20.8% of patients in a rural hospital in India obtained either a primary or secondary SSI after their procedure. A primary SSI is an infection typically occurring within seven days of the operation, generally while still in the hospital. A secondary SSI is an infection acquired after leaving the hospital. Of the patients who acquired an SSI post-surgery, 64.8% had the bacteria *Staphylococcus* in the infection 69.9% of which was MRSA, or methicillin-sensitive *Staphylococcus aureus* (MSSA). The discovery of new antibiotics to treat *Staphylococcus aureus* is in dire need. Similarly, the genus *Bacillus* can cause a wide range of issues, including infections, anthrax, and endocarditis.[4]

Through the use of the safe test strains *Staphylococcus epidermidis* and *Bacillus subtilis*, we can use a variety of molecular and biochemical procedures to find and isolate new antibiotic-producing bacteria. These strains, which are evolutionarily similar to the ESKAPE strains, allow for the safe testing and potential discovery of novel antibiotics against *Staphylococcus aureus* and *Bacillus anthracis.* Identification and isolation of antibiotic-producing bacteria will be reported back into the global network of the Tiny Earth Project Initiative.

METHODS

In collaboration with the Tiny Earth Project Initiative we were able to use many of their procedures described.[2] Unless otherwise noted, all of the procedures were followed exactly.

- Collect soil sample
- Serial Dilution of soil samples and isolation of microbes
- Media and selective media for isolation (10% TSA, LB, PDA)
- Formation of master plates
- Testing microbial isolates for the production of secondary metabolites with antibiotics (*Staphylococcus epidermidis, Bacillus subtilis,* and *Erwinia carotovora*)
- Formation of streak plates to allow for further testing
- Differential media for potential identification of isolates (MacConkey, Simmons' citrate, MSA, TSI slants, and blood agar)
- PCR of any antibiotic producing microbes (16S rRNA gene primers are 27F and 1492R)
- Gel electrophoresis of PCR products (1% agarose gel)
- Sequencing of the DNA of 16S rRNA gene (Iowa Institute of Human Genetics, University of Iowa)

RESULTS

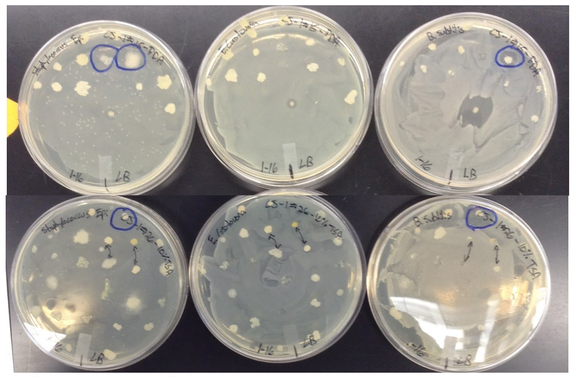

Figure 2. Evidence of antibiotic producing isolates present against both of my Gram-positive strains, *Staphylococcus epidermidis* and *Bacillus subtilis.* Halos were present in the first and third columns, *Staphylococcus epidermidis* and *Bacillus subtilis* respectively. The middle column was a Gram-negative bacteria, *Erwinia carotovora*. The plates were grown on LB at 28°C for 24 hours.

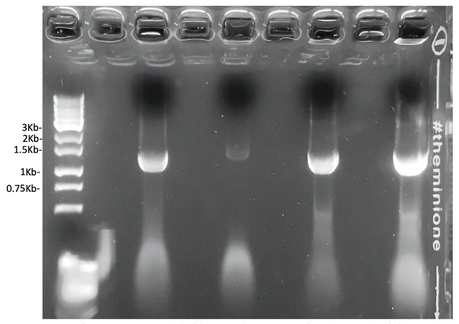

Figure 3. Successful amplification of the 16S rRNA gene from PCR. We were able to amplify the DNA from the bacteria in lanes 3,5,7, and 9. In these lanes were isolates CJ-1-TSA, CJ-2-TSA, CJ-4-PDA, and CJ-5-PDA, respectively. With the primers we chose, the DNA bands we expected, and produced, were around 1.4Kb. The PCR product was run for 25 minutes on a 1% agarose gel.

RESULTS (CONTINUED)

Description	Total Score	Query Cover	E value	Per. Ident
Pseudomonas silesiensis strain A3 16S ribosomal RNA, complete sequence	1633	98%	0.0	99.44%
Pseudomonas mandelii strain NBRC 103147 16S ribosomal RNA, partial sequence	1633	98%	0.0	99.44%
Pseudomonas mandelii strain CIP 105273 16S ribosomal RNA, partial sequence	1633	98%	0.0	99.44%
Pseudomonas tremae strain TO1 16S ribosomal RNA, partial sequence	1628	98%	0.0	99.33%
Pseudomonas caspiana strain FBF102 16S ribosomal RNA, partial sequence	1624	98%	0.0	99.12%
Pseudomonas savastanoi strain CFBP 1670 16S ribosomal RNA, partial sequence	1624	97%	0.0	99.33%

Description	Total Score	Query Cover	E value	Per. Ident
Bacillus subtilis strain IAM 12118 16S ribosomal RNA, complete sequence	1690	98%	0.0	99.68%
Bacillus tequilensis strain 10b 16S ribosomal RNA, partial sequence	1690	98%	0.0	99.68%
Bacillus subtilis strain JCM 1465 16S ribosomal RNA, partial sequence	1690	98%	0.0	99.68%
Bacillus subtilis strain NBRC 13719 16S ribosomal RNA, partial sequence	1690	98%	0.0	99.68%
Bacillus subtilis strain BCRC 10255 16S ribosomal RNA, partial sequence	1690	98%	0.0	99.68%
Bacillus subtilis strain DSM 10 16S ribosomal RNA, partial sequence	1690	98%	0.0	99.68%

Figure 4. 16S rRNA gene sequence data, showing that isolate CJ-1-TSA, and CJ-4-PDA are from the genus *Pseudomonas* and *Bacillus* respectively.[6] The description section lists the possible strains that our unknown isolates were. Query cover is the percentage of the actual gene that our sequenced data matches up with, in this case we did not amplify the entire gene, but instead about 98%. The E value is the likelihood that we just randomly obtained these strains. Finally, the percent identity is the percent of nucleotides from our isolates that line up exactly with the known strains in the description.

DISCUSSION

We were able to successful identify the genus of isolates CJ-1-TSA and CJ-4-PDA as *Pseudomonas* and *Bacillus*. For these two isolates we were able to conclude that they have the potential to be broad spectrum antibiotics against both *Staphylococcus epidermidis* and *Bacillus subtilis.*

Future directions of this project would include the isolation of the actual secondary metabolite. This would involve organic and biochemical separation techniques. Further directions would include full genome sequencing through the Tiny Earth Project Initiative and the potential identification of the structure of the secondary metabolite.

LITERATURE CITED

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