Antibiotic-producing Bacteria from Temperate Formicidae

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Abstract:

Recent studies on fungus-growing leaf cutter ants (tribe Attini) and the antibiotic/antifungal bacteria growing on their exoskeleton to protect their fungal farms suggests an important question: Could bacteria from temperate zone ants be a promising source for novel antibiotics? To answer this question, we collected six temperate zone ant species and plated whole body homogenate on six different kinds of agar media for the growth of bacteria. The bacterial isolates from each ant species were tested for the inhibition of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Tests indicate that a substantial portion (23.8%) of our isolates completely inhibit one or more of the aforementioned bacteria. The antibiotic-producing isolates that showed complete inhibition are in the process of tentative identification through 16s ribosomal RNA gene sequences.

Introduction:

Studies show that there are increasing numbers of multi-drug resistant (MDR) and antimicrobial resistant (AMR) pathogens. In 2013, the US alone had more than two million recorded illnesses due to MDR and AMR pathogens which caused over 23,000 deaths.[1] Because of this, antibiotic discovery has shown increasing importance as various antibiotics are becoming ineffective. With the rise of AMR and MDR bacteria strains, many bacterial infections that were treatable are now partially or completely resistant to current antibiotics. As a result, researchers have been frantically searching for new sources to find and develop novel antibiotics that can fight AMR and MDR bacteria. Current research on fungal-farming ants and other agricultural insects may pose a solution to this problem.

Tribe Attini, Genera *Atta* and *Acromyrmex*, are commonly known as the leafcutter ants. These ants are known for a mutualistic association with fungi (*Leucocoprini*) that they grow from the leaves they cut for food.[2] Not only do these ants have a symbiotic relationship with these fungi, but also with antibiotic and antifungal-producing bacteria that they cultivate on their exoskeletons.[3] These bacteria grow in cuticle foveae, which contain bacterial pockets called crypts.[4] The bacteria that the ants foster on their bodies are used to provide protection against an unwanted fungal parasite, *Escovopsis*, in their fungal gardens[5-6]; these ants can sense if the parasite is invading their fungal gardens and will rub their bodies against infected areas to apply the bacteria.

It has been estimated that these attine ants have been using these bacteria for approximately 50 million years and have made researchers question why the parasite that threatens their fungal farms has not become resistant to the bacteria that they use.[7] This question, along with the possibility of discovering novel antibiotics, has become the motive for further studies on mostly attine ants.

There are a total of 12,990 known species of ants in the family Formicidae.[8] With so many ant species that have not been surveyed, could there be more ants that use antibiotic-producing bacteria for their benefit other than the tribe Attini?

I hypothesized that there are ants that are not from the Attini tribe that have bacteria on their exoskeleton and/or in their bodies which will be antibiotically active.

We found various antibiotic -producing bacteria from six temperate zone ant species. Our results suggest that other ant species outside tribe Attini should also be considered as a reliable source for the discovery of novel antibiotics.

Methods:

Six ant species were collected, along with their nest material, from two areas near Powell, Wyoming. Three species have been identified thus far: *Pogonomyrmex occidentalis* (1), commonly known as the Western Harvester Ant, *Formica obscuripes* (2), commonly known as the Western Thatch Ant, and *Camponotus pennsylvanicus* (3), commonly known as the Black Carpenter Ant. The other three ant species were named based on size and color: Unknown Very Small Brown Ant (4), Unknown Small Black Ant (5), and Unknown Small Brown Ant (6).

All six species were homogenized separately in 7-8ml of sterile water. 10µl of the whole body homogenate was spread out using a sterile glass rod on seven different types of agar: Myxococcus Agar, Actinomycete Isolation Agar, Tomato Juice Agar, Trypticase Soy Agar, Half Trypticase Soy Agar, Sabouraud Dextrose Agar, and International Streptomyces Project Agar plates. All agar plates were placed in ambient air at room temperature (20-22°C) to promote the growth of bacterial colonies from each ant species. Individual colonies were streaked for isolation to ensure pure cultures. Once a pure culture was acquired, isolates were tested for antibiotic activity on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* lawns grown in ambient air at room temperature (20-22°C). The lawns were examined for areas of inhibition or zones of clearing after 1-2 days. Results were measured by complete, partial, or no inhibition of the aforementioned pathogens. The antibiotic-producing isolates that showed complete inhibition are being tentatively identified by 16s ribosomal RNA gene sequences. DNA from each antibiotic-producing isolate was extracted, the 16s ribosomal RNA gene was amplified using pcr, and the sequences were compared to NCBI's nucleotide BLAST database.

Results:

All six ant species combined provided 143 isolates for antibiotic testing: 17 from *Pogonomyrmex occidentalis* , 22 from Unknown Very Small Brown Ant , 14 from *Camponotus pennsylvanicus* , 23 from *Formica obscuripes* , 28 from Unknown Small Black Ant , and 39 from Unknown Small Brown Ant. Out of the 143 bacterial isolates, 39 showed complete and/or partial zones of inhibition or zones of clearing against *S.aureus*, *E. coli*, and/or *P. aeruginosa*. Complete inhibition was indicated by no bacterial growth around the isolate. Partial inhibition was indicated by a thin veil of growth within a noticeable zone of inhibition. 27.3% of the bacteria isolated for antibiotic testing showed antibiotic activity. Out of the 39 isolates that showed antibiotic activity, 34 (23.8%) showed complete inhibition against *S. aureus* and/or *E. coli*.

26 isolates completely inhibited only *S. aureus;* one isolate completely inhibited both *S. aureus* and *E. coli* and partially inhibited *P. aeruginosa;* two isolates completely inhibited *E. coli* and partially inhibited *S. aureus*; five isolates completely inhibited *S. aureus* and partially inhibited *E. coli;* three isolates partially inhibited only *S. aureus;* one isolate partially inhibited only *E.coli;* and one isolate partially inhibited both *S. aureus and E.coli.*

Individual species had different numbers of antibiotic-producing isolates against the three pathogens tested. See Table 1.

Table 1: Antibiotic Activity results by ant species.

Ant Species	Isolates Tested	Active Isolates	*E. coli:*	*E. coli:*	*S. aureus:*	*S. aureus:*	*P. aeruginosa:*	*P. aeruginosa:*
			Complete	Partial	Complete	Partial	Complete	Partial
Pogonomyrmex occidentalis	17	5	0	0	5	0	0	0
Camponotus pennsylvanicus	14	3	1	0	2	1	0	0
Formica obscuripes	23	7	0	2	7	0	0	0
Unknown Very Small Brown Ant	22	3	0	0	3	0	0	0
Unknown Small Black Ant	28	12	2	2	8	3	0	1
Unknown Small Brown Ant	39	9	0	2	7	2	0	0
TOTALS:	143	39	3	6	32	6	0	1

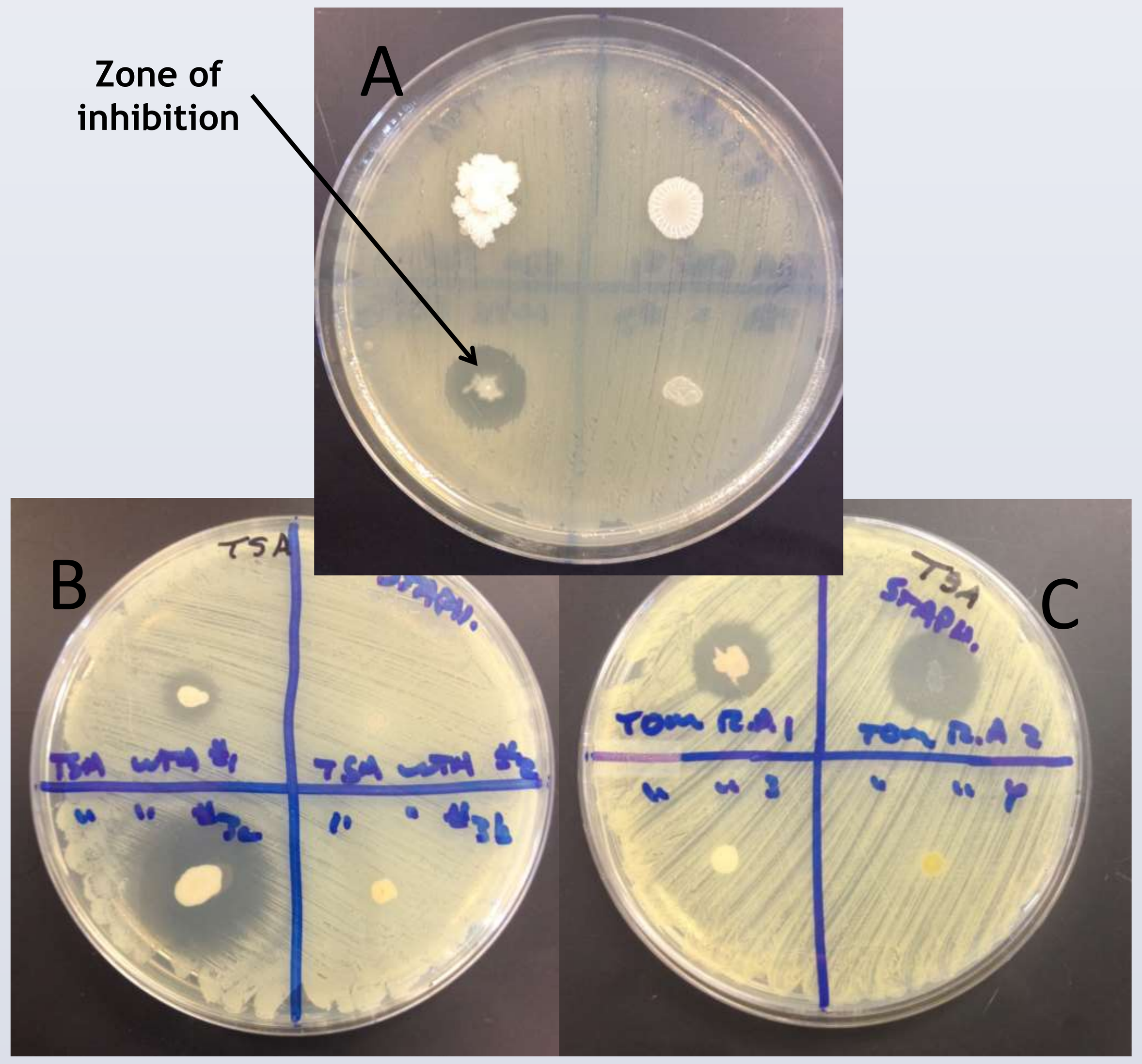
Out of the 34 isolates that showed complete inhibition against *S. aureus* and/or *E. coli*, I successfully sequenced 23 isolates for their 16s ribosomal RNA gene and made tentative identifications through NCBI's nucleotide BLAST search. Genera identified include *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Weissella*, and *Serratia*.

Discussion and Conclusion:

Because a considerable percentage of isolates were antibiotic-producing, my hypothesis is supported. Results show that not only attine ants harbor antibiotic-producing bacteria but also the ants of other tribes that I tested. Because 39 out of 143 bacteria have antibiotic properties, results suggest that these six ant species, and possibly other ants that are not part of tribe Attini, should be considered as a source for antibiotics.

Future work on these bacteria would include isolating and identifying the molecules which are responsible for the zones of inhibition present on the pathogenic lawns tested. If one new antibiotic molecule is found from one of the 39 antibiotic-producing isolates, the discovery would be significant. This work would require analytical chemistry techniques and equipment.

Antibiotic Activity:



A- *E.coli* lawn. B-*S. aureous* lawn. C- *S. aureous* lawn.

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