Isolation of antimicrobial compounds from insect-associated bacteria

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Introduction

Bacterial infections remain a pertinent threat to human health, causing over 20,000 deaths annually in the United States alone. Furthermore, due to increasing antibiotic resistance, many antimicrobial medications currently in use are becoming less effective. Therefore, new antimicrobial agents are urgently and critically needed in order to counter the problems posed by antibiotic-resistant microorganisms.

Bacteria that live near and on insects may potentially be a good source of novel antimicrobial compounds. Bacteria often have symbiotic relationships with insects, providing essential nutrients for their hosts or metabolizing the waste products of the insects. In addition, recent studies show that these symbiotic bacteria are directly associated with a host's resistance to pathogens and parasites. Therefore, if these pathogen-killing bacteria could be isolated, the antimicrobial compounds they produce could be extracted and used for antibiotic purposes.

Hypothesis

Antimicrobial compounds are produced by bacteria that are associated with insects, especially those in a symbiotic relationship with their host. If isolated, these antimicrobials may be developed into antibiotic therapeutic drugs.

Procedure

Collection: Experiments were performed using three different types of insects found and collected in various locations on the UCSD campus.

Growing Bacteria: Insect samples were pulverized and streaked across 3 different types of agar plates: MSGG, ISP2 and LB; and incubated at 3 different temperatures: room temperature (approx. 25°C,) 30°C and 37°C.

Isolation of Bacteria: Bacteria strains were purified by dilution, using the four way streak technique.

Streak Tests: Bacteria strains were streaked across a plate of weakened *E. coli* strain (*imp* mutant) in order to test if they could kill.

Lawn Tests: Bacteria strains that killed the weakened *E. coli* strain (*imp mutant)* in the streak tests were streaked out onto lawns in order to test for killing against wildtype *E. coli* strains 25922 and MG1665.

Making Extracts: Agar slabs with bacteria strains grown on them, (made the day before) were chopped up and soaked in ethanol and then concentrated in preparation for microscopy.

Microscopy: Grew up the strains to logarithmic phase, then added a 1:10 dilution of bacterial extracts. After they grew for two hours, they were stained with fluorescent dyes and imaged on LB agar pad slides.

Results

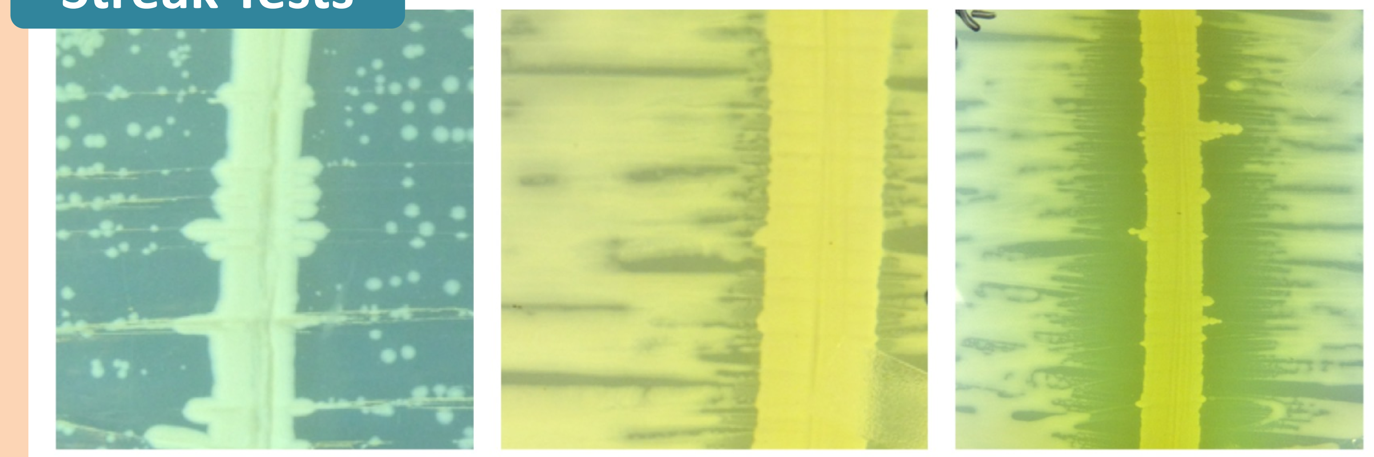
Collection

Insect 1 was a striped black and orange spider, approximately 1.25 cm in length and 0.75 cm in width. It was found in the bushes in front of Urey Hall on the UCSD campus.

Insect 2 was a 2.5 cm centipede that was orange at the head and tail and brown at the center. It was found just outside of the Natural Sciences Building on the UCSD campus.

Insect 3 was a dark grey colored pill bug, (or "roly poly"). It was approx. 1.4 cm in length and 0.6 cm in width. It was found on a tree near Urey Hall on the UCSD campus.

Streak Tests

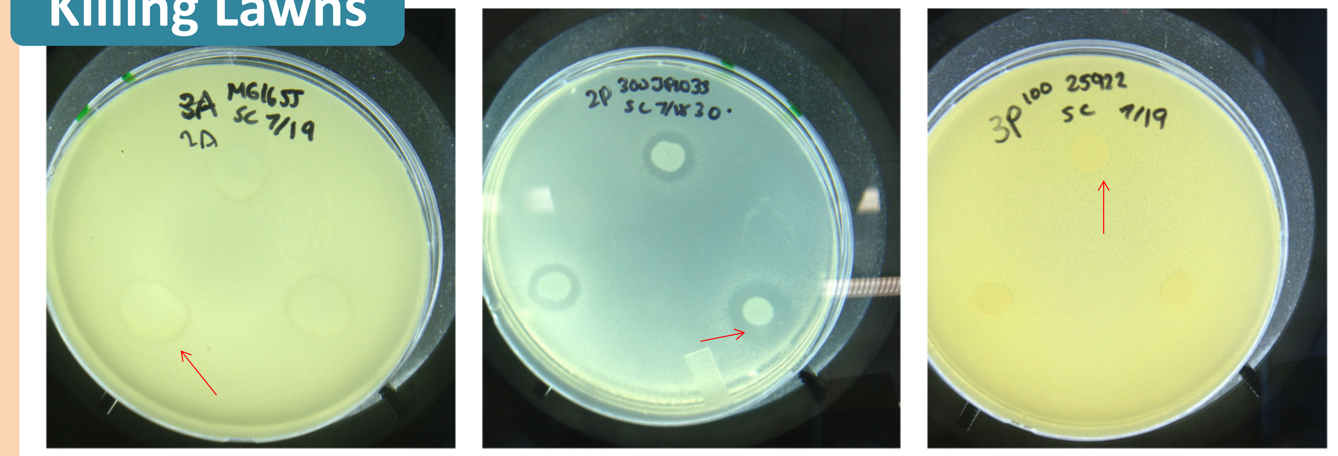


Strain 2A killed a weakened *E. coli* strain (*imp* mutant.)

Strain 2P killed a weakened *E. coli* strain (*imp* mutant.)

Strain 3P killed a weakened *E. coli* strain (*imp* mutant.)

Killing Lawns

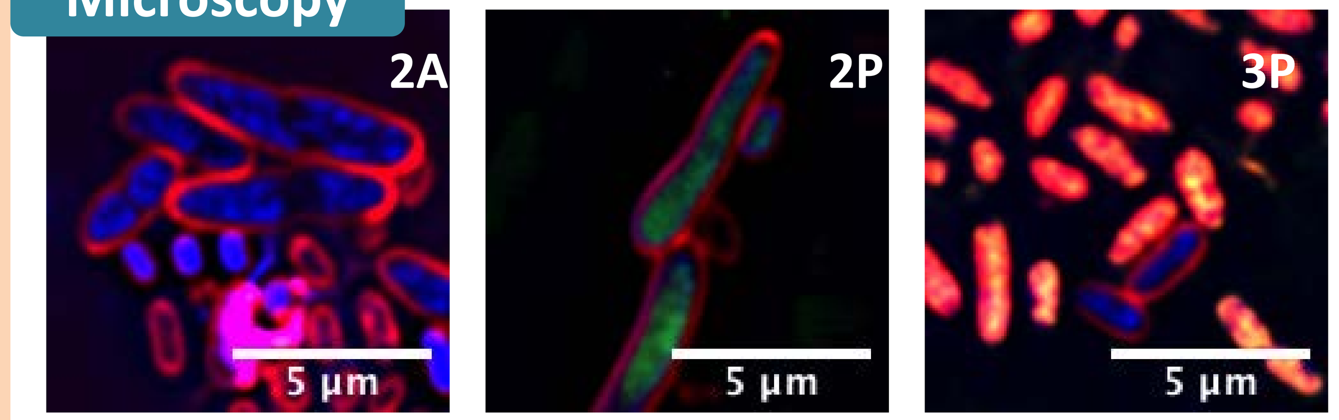


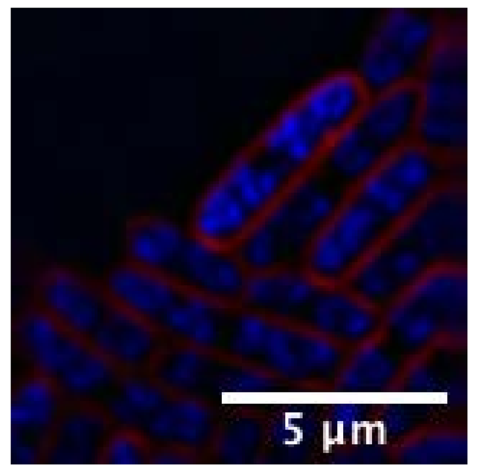
Strain 2A (from the centipede,) which originally grew on ISP2 at 30°C, grew as medium sized white dots. 2A killed the weakened *E. coli* strain (*imp* mutant) and MG1655 strains very well, and also killed strain 25922 adequately.

Strain 2P (from the centipede) which originally grew on MSGG at 30°C, grew as medium sized translucent dots that had a small white centers. 2P killed the weakened *E. coli* strain, (*imp* mutant.)

Strain 3P (from the pill bug) which originally grew on MSGG at room temperature grew as smaller translucent white color dots. 3P killed the weakened *E. coli* strain (*imp* mutant,) and 25922.

Microscopy





Slide JP1033 (bottom left) shows untreated *imp* mutant *E. coli* cells. The red dye, FM4-64, stains the cell membrane. In addition, the blue dye, DAPI, is a nuclear stain that stains DNA. A green dye, Sytox, also stains DNA but only enters the cell after their death. Since there is no green stain on the JP1033, we can tell that it is alive.

The pictures above show the effect of the three 'killer bacteria' on the *E. coli* cells. Some of these *E. coli* cells are sick and others are dead; this is shown by the circular shape of the cells as well as their green color.

Conclusion

Bacterial strains 2A and 2P (from the centipede) as well as strain 3P (from the pill bug) were all found to be able to kill at least one strain of *E. coli*. This proves that these three bacteria strains are able to produce antimicrobials that potentially could be used for antibiotic purposes. Insects are a good place to start looking for novel antibiotic drugs since certain bacteria live symbiotically with insects. These bacteria may produce antimicrobial compounds to protect their host from pathogens and infections. Extracting these bacteria from insects and testing them against human pathogens may be a preliminary step in the discovery of novel antibiotic drugs

Future Directions: First, to isolate the exact molecule that produces the killing effect by fractionating the extract and testing those fractions for killing. After the specific molecule is isolated, its structure may be determined and it may be developed as an antibiotic.

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