ANTIBACTERIAL PROPERTIES OF ENDOPHYTIC FUNGI CULTURED IN NUTRIENT SUPPLEMENTED LUNAR AND MARS SIMULANTS

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Introduction

The evolution of antibiotic resistance is a critical concern for the future of human health. As humanity continues its exploration of space, the transport of microbial organisms is inevitable. Strains of antibiotic resistant bacteria have been found in the International Space Station. Conditions present in space (i.e. microgravity), can lead to increased virulence in pathogenic bacteria. As the number of resistant species of bacteria increases, the need for newer antibiotics also rises. Bioactive secondary metabolites produced by endophytic fungi are sources for novel antibiotics. Some of these bioactive compounds have shown antimicrobial properties against pathogenic bacteria. NASA's Artemis program has several planned missions that will return humans to the surface of the Moon and is a critical stepping stone that will place the first humans on Mars. In recent years, research into the growth of plants, fungi, and other microbial organisms in low-Earth orbit and in Lunar and Mars regolith simulants has increased. With the high likelihood that antibiotic resistant bacteria will be transported as humans explore space, the Moon, and Mars, the need for antibiotics capable on inhibiting their growth will be needed. Making use of materials that are present on the Moon and Mars as part of culture media present a novel approach to the production of bioactive secondary metabolites that inhibit bacteria while also minimizing the need to fund the transport of materials during space missions.

Methods

Collection of plants on campus and plant identification. Isolated endophytic fungi from each plant.

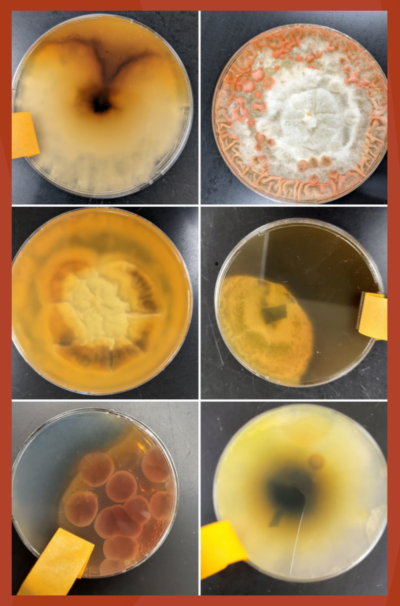
Endophytic fungi isolates cultured on PDA, SDA, and YMA plates.

Lunar and Mars regolith simulants (16.5g) supplemented with 10 ml of agar (PDA or SDA).

Isolated endophytic fungi grown in semi-solid fermentation cultures of supplemented Mars and Lunar simulants.

Organic extraction using ethyl acetate. Followed by well diffusion bioassay against four species of bacteria.

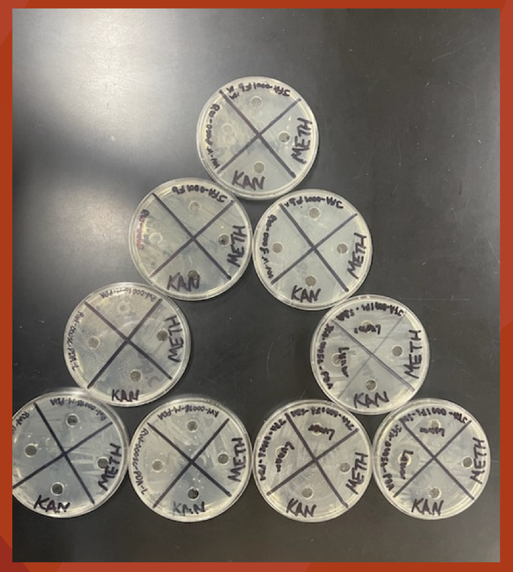
Musa sp. sampled



Endophytic fungi growth on agar plates

Endophytic fungi growth on regoliths

Endophytic fungi extraction



Agar Well Diffusion Bioassays

Results

A total of 12 plants were sampled on the DDCC campus resulting in 32 total cultures of plant tissues on PDA, YMA, and SDA. Thirty-one isolates were cultured from plant tissues. A total of 6 isolates were chosen to culture in simulants supplement with nutrient agar (PDA and SDA) for 6 weeks followed by organic extraction. Of the six organic extractions tested against four species of bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella (Enterobacter) aerogenes*, and *Staphylococcus epidermidis*) in agar well diffusion bioassasys, five had inhibitory effects against *B. subtilis* and/or *S. epidermidis*. No isolates exhibited inhibitory properties against *E. coli* or *K. aerogenes*.

Inhibitory Properties of Organic Extracts of Endophytic Fungi Cultured on Nutrient Supplemented Regolith Simulants

Sample ID	Plant Species Sampled	Regolith Simulant	Agar Supplement	*E. coli*	*S. epidermidis*	*B. subtilis*	*K. (E.) aerogenes*
SP-6193	*Acer rubrum*	Lunar	Sabouraud Dextrose Agar	-	+	+	-
SP-7291	*Acer rubrum*	Mars	Sabouraud Dextrose Agar	-	-	+	-
SP-1720	Unknown	Lunar	Potato Dextrose Agar	-	+	+	-
SP-4293	*Musa spp.*	Mars	Potato Dextrose Agar	-	+	+	-
SP-1038	*Dichanthelium boscii*	Lunar	Potato Dextrose Agar	-	+	?	-
SP-9909	*Bryoandersonia illecebra*	Mars	Potato Dextrose Agar	-	-	?	-

Inhibition of four species of bacteria by organic extracts from endophytic fungi grown on lunar or mars regolith simulants supplemented by nutrient containing agar. Species inhibitied (+) had zones of inhibition significantly larger than a negative control (methanol). Those that did not differ significantly (-) from negative control or were inconclusive (?) are also indicated.

Conclusion

The rise of antibiotic reisistance in bacteria pose problems not only for human populations in the biosphere, but also in regards to human exploration of space. Sources of novel antibiotics are crucial for continued treatment of infections caused by pathogenic bacteria whether on earth or space. Here we presented the results of organic extractions of endophytic fungi isolated and cultured on Lunar and Mars regolith simulants supplemented with nutrient containing agar and tested them against four species of bacteria in bioassays. Our results indicated that five of the six isolate organic extractions had inhibitory properties against *B. subtilis* and *S. epidermidis*. Whether the presence of regolith simulants resulted in the inhibitory properties must be researched further by comparing growth of isolated endophytic fungi in the absence of simulants and comparing those outcomes to those we observed. The potential for novel secondary metabolite production by endophytic fungi exposed to new culture media is not a new concept. However, when culture media may contain materials from the Lunar or Mars' surface, those would present novel situations. More research using simulants that more accurately reflect the true materials and conditions found in Lunar or Mars regolith should be conducted. For example, our Mars simulant does not contain perchlorate, which is a known hazard to human health. A simulant containing perchlorate would be a more accurate media for further culturing of endophytic fungi and testing organic extractions for antimicrobial properties. Whether needed by future astronauts or humanity in general, sourcing new antibiotics should be a top priority.

Works Consulted

SCAN ME

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