Mining the Aerobiome for Novel Antimicrobial Compounds

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Introduction

The rapid emergence of multi-drug resistant (MDR) bacteria, including select agents and "ESKAPE" pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*), pose a serious and growing threat to human health. Despite spectacular success with the discovery and application of antibiotics during the "golden era," the discovery of new antibiotics steeply declined after the 1960s. Furthermore, the use of traditional antibiotic screening technologies frequently results only in the rediscovery of previously known compounds. Nevertheless, nature provides a massive reservoir of microbes that produce potentially beneficial compounds to be discovered and explored.

Outdoor bioaerosols potentially offer a rich source of novel prokaryotes, such as actinomycetes, whose spores are able to survive atmospheric stresses such as dessication and UV light. Filamentous actinomycetes are already a major source of microbial natural products, producing 90% of known antibiotics (1). Recently available genome sequences from actinomycetes species have revealed many biosynthetic gene clusters for the production of unknown secondary metabolites. We believe that the discovery of novel antimicrobials is dependent on the activation of secondary metabolites. Factors that trigger expression of many biosynthetic pathways for cryptic secondary metabolites are currently poorly understood, but it is believed that secretion of the resulting bioactive compounds, naturally produced by microorganisms, confer a significant selective advantage in nature.

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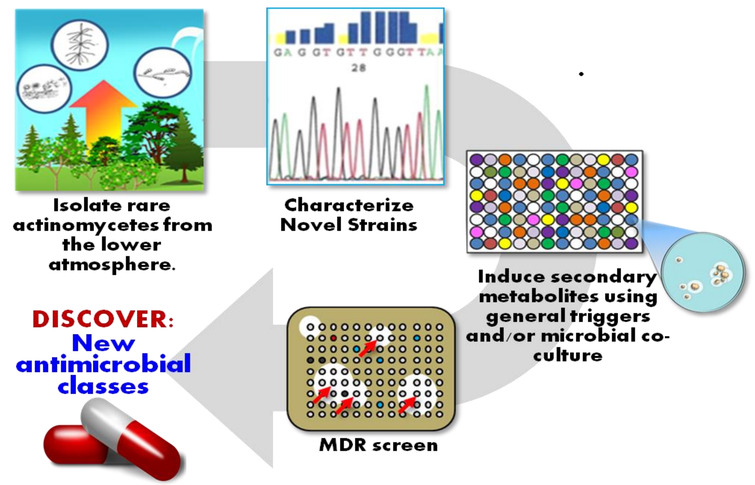
Project Objective and Overview

Objective: Develop an antimicrobial discovery pipeline that queries bacteria found in atmospheric aerosols for potential antibiotics effective against MDR threats and correlating these findings with geographic location, altitude, and underlying terrestrial biome.



Experimental Approach

Isolation of novel, rare actinomycete strains from aerosol microbiome – A stringent selective isolation strategy will be used to isolate bacterial strains from aerosol samples above diverse habitats to enrich for rare actinomycete species, particularly outside the genus *Streptomyces*. To optimize the aerosol sampling strategy, the genomic DNA from culturable microorganisms and culture-independent samples will be extracted and the 16SrRNA gene sequenced.

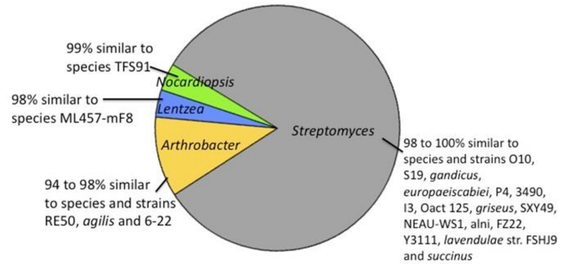


Figure 2 : 16S-rRNA gene sequence analysis of Idaho State University collection of antibiotic-producing actinomycete strains.

Experimental Approach – continued

Induce cryptic biosynthetic pathways –
Using ECBC's robotic liquid handling systems, we will employ a high-throughput strategy aimed at eliciting secondary metabolite production by:

1) Co-cultivation of two or more different bacteria to mimic natural ecological situations and exploit interspecies signaling

2) Chemical stimulation to elicit metabolite production (e.g. histone deacetylase (HDAC) inhibitors, N-acetyl-glucosamine, subinhibitory concentrations of antibiotics)

Figure 3. A liquid handling robot will be used as a screening platform for the discovery of novel biological activities from airborne microbes (2).

Antimicrobial compounds will be isolated and characterized from strains determined to have novel, potent antimicrobial activities.

Potential Risks

A major risk to this approach is the well-documented tendency to re-discover known compounds in a poorly designed screening effort. Screening against MDR bacteria will reduce the likelihood of this pitfall.

References

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