Exploring Microbial Dark Matter: Isolation of a Novel *Streptomyces* Strain Exhibiting Potent Antibacterial Activity Against Multidrug-Resistant *Staphylococcus* Species

Donnie Alverson[1], Aaron Koch[2], Ranga Rao Ravu[2], Kirk Maxey[2], and Lia Danelishvili[1]

[1]Department of Biomedical Sciences, Gary R. Carlson, MD, College of Veterinary Medicine, Oregon State University, Corvallis, OR, [2]Cayman Chemical Company, Ann Arbor, MI

KEY FINDING

An environmental *Streptomyces* isolate produces a series of potent antibiotics

INTRODUCTION

Widespread antibiotic resistance is outpacing the discovery of new antibiotics, creating a public health emergency.[1] Since the golden age of antibiotics discovery of the mid-20th century, the discovery and clinical development of new antibiotics have relied heavily upon the screening of bioactive secondary metabolites produced by soil microorganisms. However, these discovery efforts focused predominantly on screening of metabolites from culturable soil bacteria which represent a minor component of the total environmental microbial diversity. Overreliance on this limited pool of biological and chemical diversity has resulted in a sharp decrease in the number of new antimicrobial natural products being discovered and prompted new methods for investigating the underexplored natural product chemical space possessed by previously uncultured soil microorganisms.[2]

Herein, we report the use of the Isolation Chip (iChip) cultivation method for the discovery of a putatively novel environmental *Streptomyces* strain possessing potentially novel antimicrobial metabolites with strong inhibitory activity against multidrug-resistant strains of *Staphylococcus* spp.[3]

STRAIN ISOLATION

Soil culturing with iChip

Humus rich soil samples (Corvallis, OR) were incubated for 9 weeks in an iChip fashioned from a 96-well cell culture plate, enabling single bacterial cell isolation and growth under natural environmental conditions. A *Streptomyces* isolate was recovered and screened for antibacterial activity.

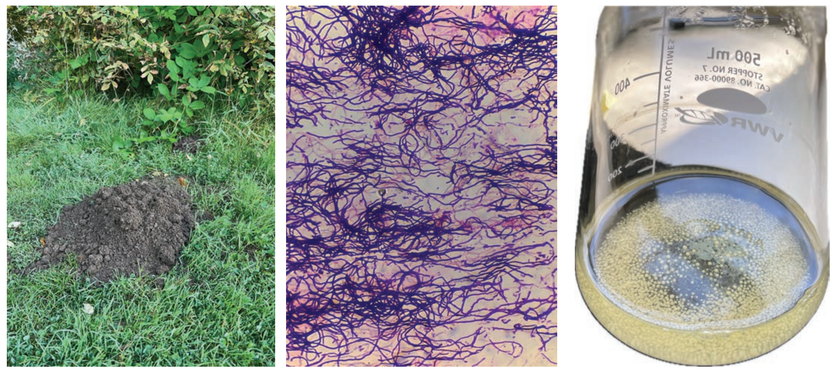

FIGURE 1 – Environmental *Streptomyces* isolate. An iChip culture device was inoculated with freshly dug soil and buried in the same location for 9 weeks (left) before disassembly and propagation of individual colonies. A subculture of a colony of interest exhibits gram-positive filamentous morphology (middle). In broth culture, the isolate forms white, spherical pellets in shaking condition (right). Both features are characteristic of bacteria belonging to the *Streptomyces* genus.

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NEW STRAIN DISPLAYS ANTIBIOTIC ACTIVITY

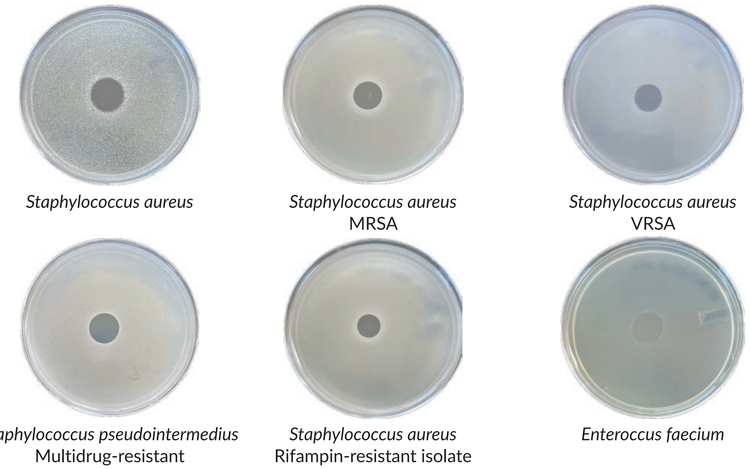

FIGURE 2 – Antibiotic testing of crude extract. Antibiotic activity was detected using agar diffusion assays of crude extract on lawns of clinically relevant gram-positive bacteria.

A PUTATIVELY NOVEL SPECIES OF *STREPTOMYCES*

Phylogenetic tree analysis

Partial 16S rRNA sequencing confirmed the isolate as belonging to the genus *Streptomyces* with a closest match with *Streptomyces niveus* (95.8% identity). Further multilocus sequence analysis (MLSA) using concatenated atpD, gyrB, rpoB, and trpB gene sequences was utilized to construct a phylogenetic tree. The novel isolate forms a distinct and well-supported lineage (bootstrap value, 98%), clearly separated from its closest relatives, including *Streptomyces niveus*, *S. albus*, and *S. violaceusniger*. This topology supports assignment of the isolate as a novel species within the genus *Streptomyces*.

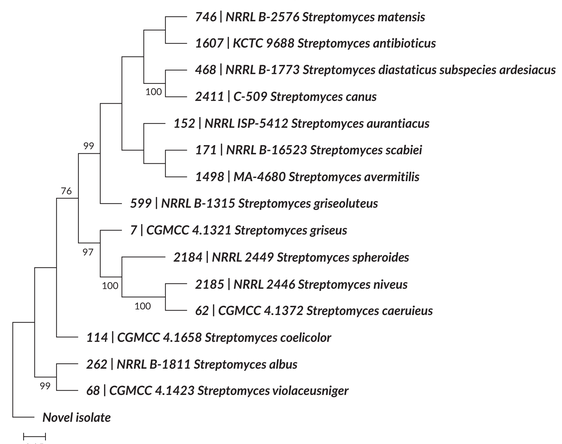

FIGURE 3 – Multilocus sequence analysis (MLSA)-based phylogenetic tree.

BIOACTIVITY GUIDED FRACTIONATION

Fermentation scaleup and extraction

The new *Streptomyces* spp. isolate was fermented for 7 days at 50 L scale. The clarified broth was subjected to workup with Amberlite XAD-16 resin to yield 12.8 g of bioactive crude extract. Bioactivity guided fractionation of the crude extract is in progress and has resulted in several enriched fractions displaying increased antibiotic activity. Initial dereplication of these fractions by analysis of their respective MS/MS spectra within the GNPS platform suggests the presence of previously unreported metabolites.[4]

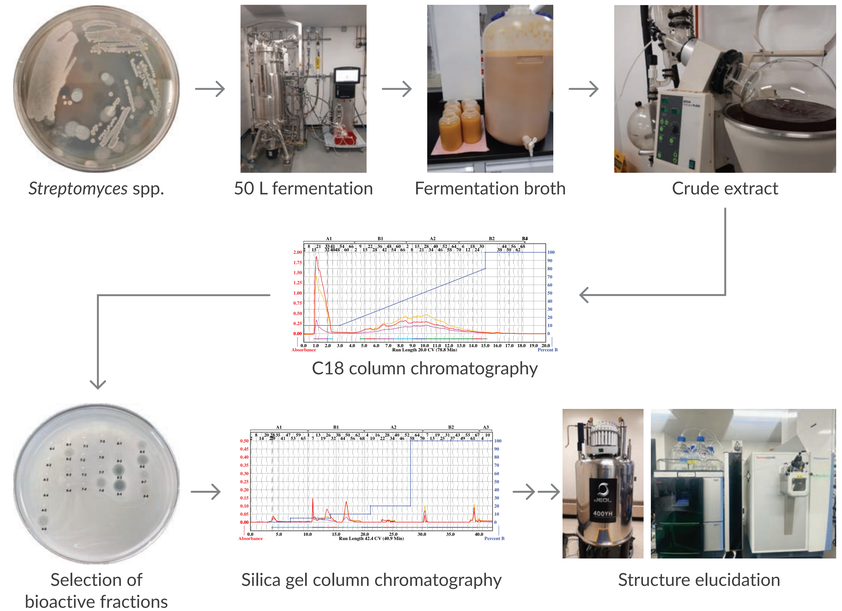

FIGURE 4 – Isolation Scheme. Bioactivity guided purification of the active metabolites is in progress. Once chemically pure, structure elucidation of the active metabolites will be pursued by NMR and LC-MS/MS analysis.

CONCLUSIONS

- Environmental soil sampling using the iChip culture method resulted in the isolation of a novel *Streptomyces* species with potent antibiotic activity.
- A large-scale 50 L fermentation and extraction yielded 12.8 g of active crude extract. Bioactivity guided fractionation of this crude extract yielded active fractions containing metabolites covering a wide range of polarities.
- Whole genome sequencing of the novel *Streptomyces* strain is in progress.
- Further purification for structure elucidation of the active metabolite(s) is in progress.

References

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