

## Isolation and Screening of Promising Novel Antibiotic-Producing Bacteria Cultivated from in-situ Diffusion Chambers

Olivia Farrar\*, Emily Morrison, Kevin Kiser

The idea of antibiotic discovery isn't a new concept, but in recent decades the misuse has led to an urgent need for new antibiotics which has increased their ineffectiveness against bacterial infections. Researchers must come up with new antibiotics and medications to fight these infections. One approach is to identify natural products of soil microbes that demonstrate antimicrobial activity. While there has been success with these methods, generally only 1% of soil microbes are culturable under standard laboratory conditions. To explore the remaining 99%, this project aims to mimic the natural settings in which these "unculturable" bacteria could grow. We use diffusion chambers from stainless-steel washers with two membranes glued to the top and bottom of it to sandwich agar suitable for the growth of slow-growing microbes, mixed with a soil inoculum. The diffusion chambers were incubated on soil so the membranes could allow for the free exchange of nutrients and growth factors from the soil into the agar, without introducing microbial contaminants. We compared bacterial colonies grown on chambers placed on soil versus nutrient agar and casein starch agar. Preliminary examination by Gram stain and microscopy was conducted followed by 16S rRNA sequence identification to determine whether novel species have been isolated. These isolates have already started being tested against ESKAPE pathogens by using cross streak testing methods. This project, overall, contributes a simple methodology that can be used and adapted by other researchers that are also looking for difficult-to-cultivate, antibiotic-producing bacteria in their local environments.

Commented [KK1]: I think these three statements are a little redundant. Try to consolidate into 1-2 sentences that state 1) misuse/overuse of antibiotics has led to increased resistance, 2) new antibiotics need to be discovered/developed.