

# Isolation of soil *Bacillus* spp with inhibitory effects on methicillin-resistant *Staphylococcus aureus* (MRSA)

Kaitlin Moore & Eric T. Gillock

Department of Biological Sciences, Fort Hays State University

## Introduction

The overuse of antibiotics in both human medicine and agriculture has contributed greatly to the crisis we experience today. In the United States alone, **at least 2 million people acquire resistant infections with approximately 23,000 of these cases resulting in death each year** (CDC, 2013). MRSA infection, in otherwise healthy individuals, affects the superficial skin and soft tissues. More serious complications can arise though, affecting the lungs (Figure 1), bone, and deep soft tissues. MRSA is able to avoid the body's immune system through the production of biofilm and certain toxins. These virulence factors, in combination with multidrug resistance, result in high morbidity and mortality rates (AAM, 2015).

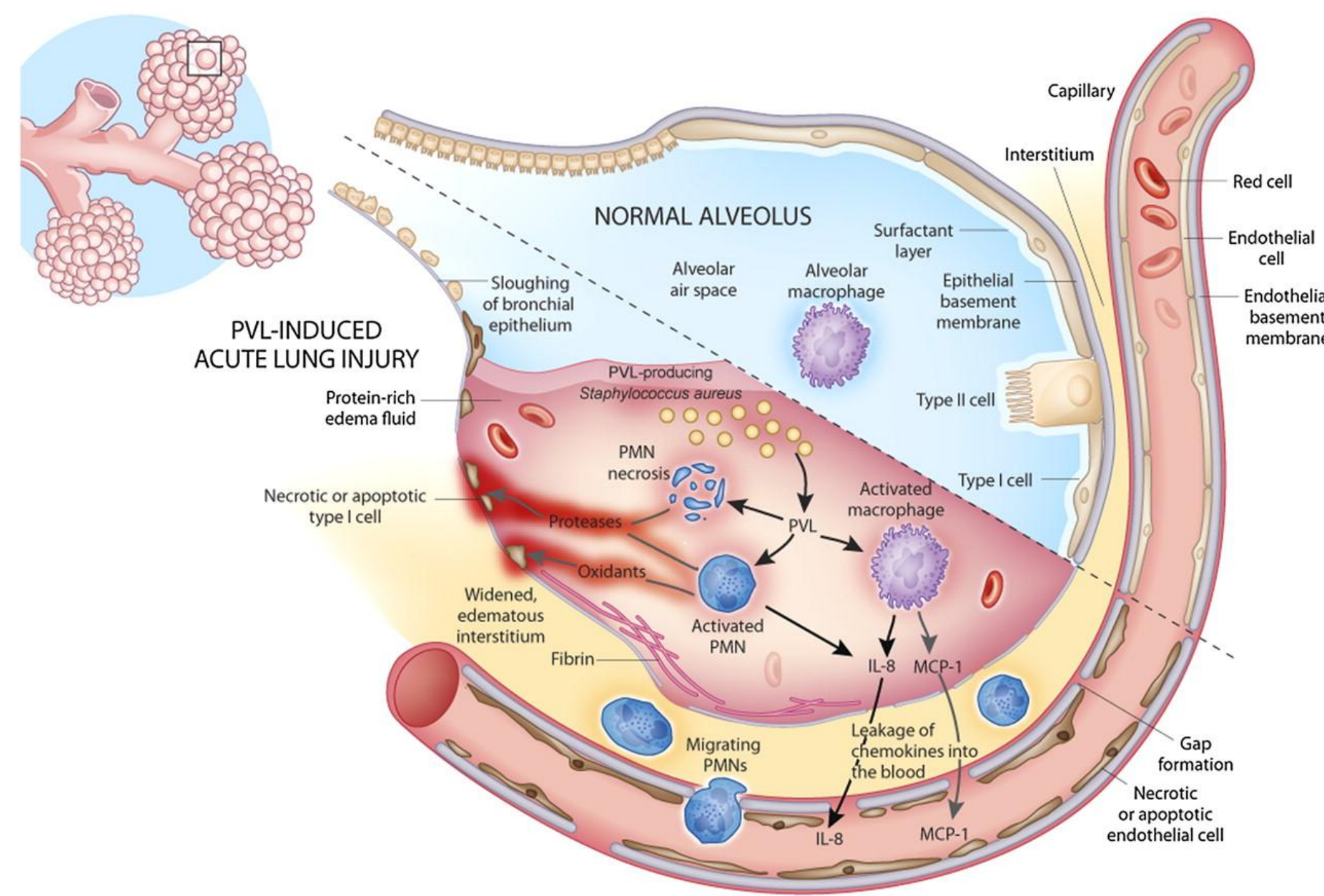


Figure 1: Some strains of *S. aureus* are capable of producing the PVL toxin which can induce necrosis of lung tissue (Diep, 2010).

## Why *Bacillus* spp?

After a fruitless search for pathogens in soil, Selman Waksman suggested their destruction may be a result of the soil inhabiting microbes. The most commonly antagonistic soil organisms can be divided into four major genera, one of which, the spore forming bacteria *Bacillus*, is effective against Gram-positive pathogens (Waksman & Woodruff, 1940).

**Ubiquitous microorganisms employ strategies to survive in diverse environments, thus controlling the growth of their neighbors.** One such organism, *Bacillus subtilis*, was found to inhibit the growth of *S. aureus* as a result of these strategies. Gonzalez, et al., (2011) hypothesized that this may explain the lack of infection in the 30% of the population colonized with *S. aureus*.

## Approach

### Isolation of soil *Bacillus* spp (Figure 2)

- 0.25g soil, 4.5mL TSB, & 500μL sodium acetate trihydrate
- Incubate at 30°C for 4 hours on shaker at 250 rpm
  - While *B. anthracis* spores will germinate, other *Bacillus* spores will not
- Heat 200μL at 80°C for 10 minutes
  - Vegetative *B. anthracis* cells are killed
- Swab a lawn on TSA & incubate at 30°C for 48 hours

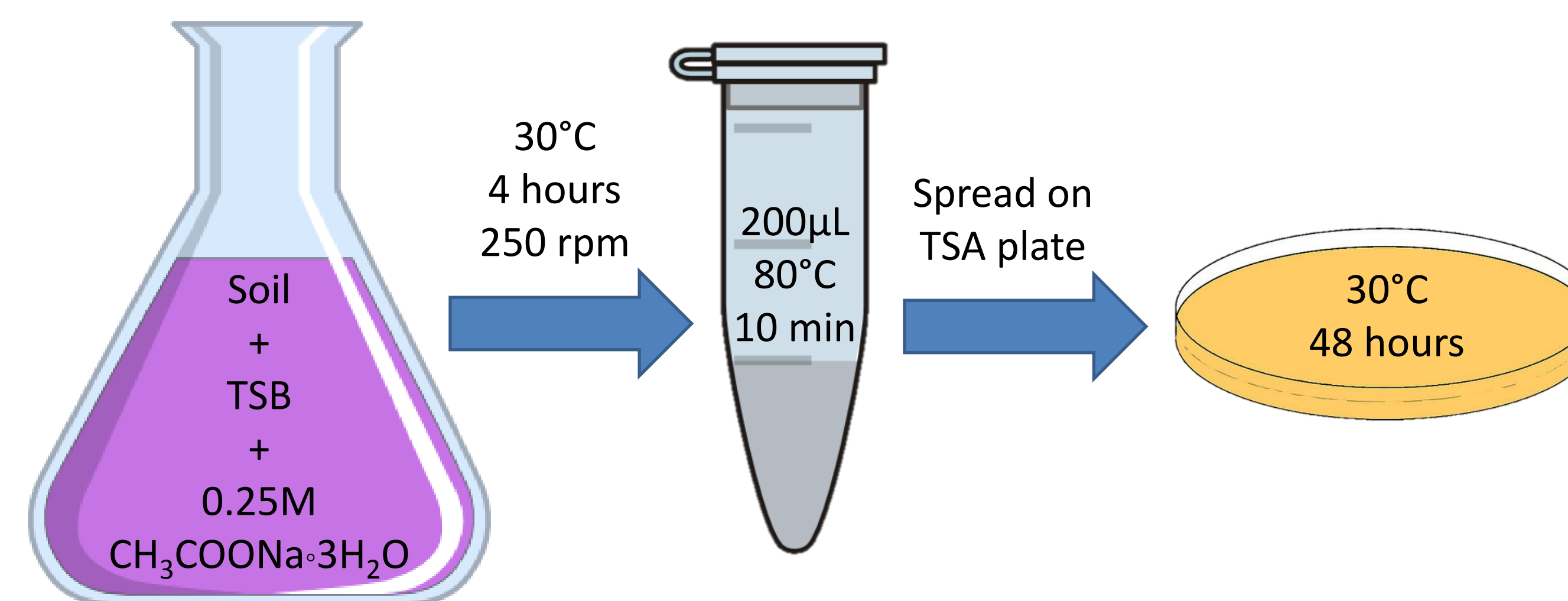


Figure 2: Isolation of soil *Bacillus* spp using protocol devised by Travers et al., (1987).

### Screening (Figure 3)

- 4mL molten STSA & methicillin-sensitive *S. aureus* (MSSA)
- Overlay on previously cultured *Bacillus* colonies
- Incubate at 37°C for 12 hours
- Select colonies producing zones of inhibition (ZOIs)
  - Those colonies purified via 3 subsequent isolation streak plates

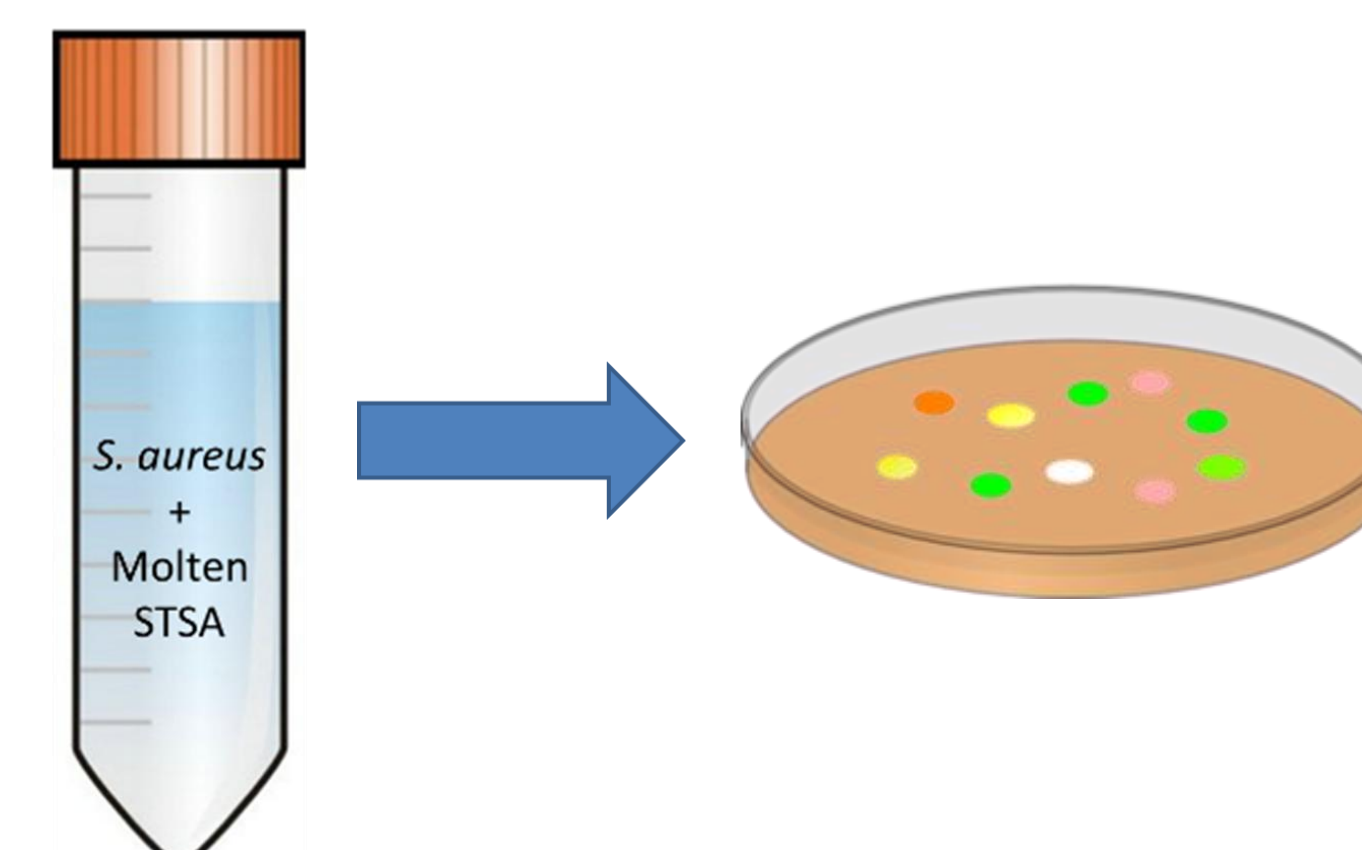


Figure 3: Initial screening of soil samples against MSSA.

### Perpendicular streak tests (Figure 4)

- Test purified environmental isolates against MRSA strains

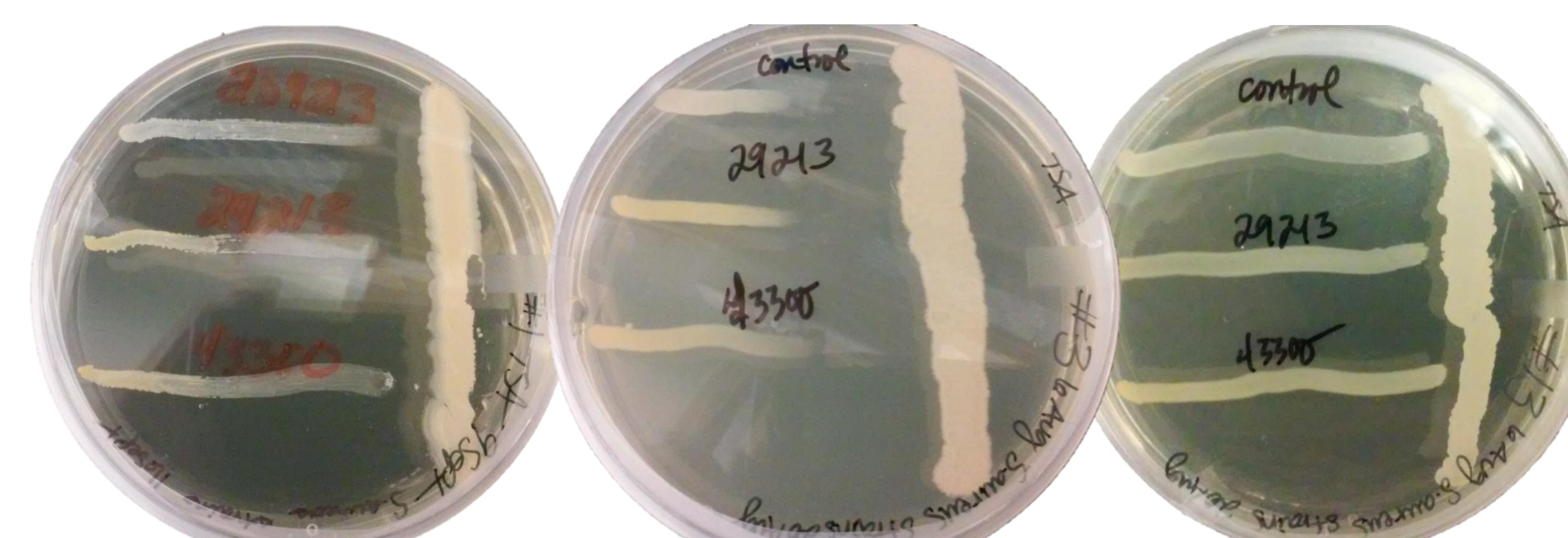


Figure 4: Purified environmental isolates tested against both MSSA & MRSA strains.

## Results

Currently, three isolates of approx. 30 soil samples have demonstrated significant activity against MSSA and MRSA strains in the perpendicular streak tests (Figure 4). These isolates were sent to MIDI labs for ID (Table 1). A spent media analysis has been performed on environmental isolate “#3.” Though no zones of inhibition have been observed to date, there has been an obvious impact on the density of the surrounding lawn of *S. aureus*.

Isolate	MIDI Labs ID
#1	<i>Bacillus amyloliquefaciens</i>
#3	<i>Bacillus subtilis</i>
#13	<i>Bacillus mojavensis</i>

Table 1: Classification of environmental isolates based on 16S ribosomal subunit sequence.

## What Next?

*Bacillus* is still a promising genus as a source for antimicrobials effective against Gram-positive organisms. In addition to continuing spent media analyses, high pressure liquid chromatography (HPLC) will soon be employed to determine the components of the promising environmental isolates.

## Acknowledgments

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