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The Role of Antibiotics in Modulating Secondary Metabolite Production of Streptomycesgrown in Co-Culture

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The role of antibiotics in modulating secondary metabolite production of *Streptomyces* grown in co-culture.

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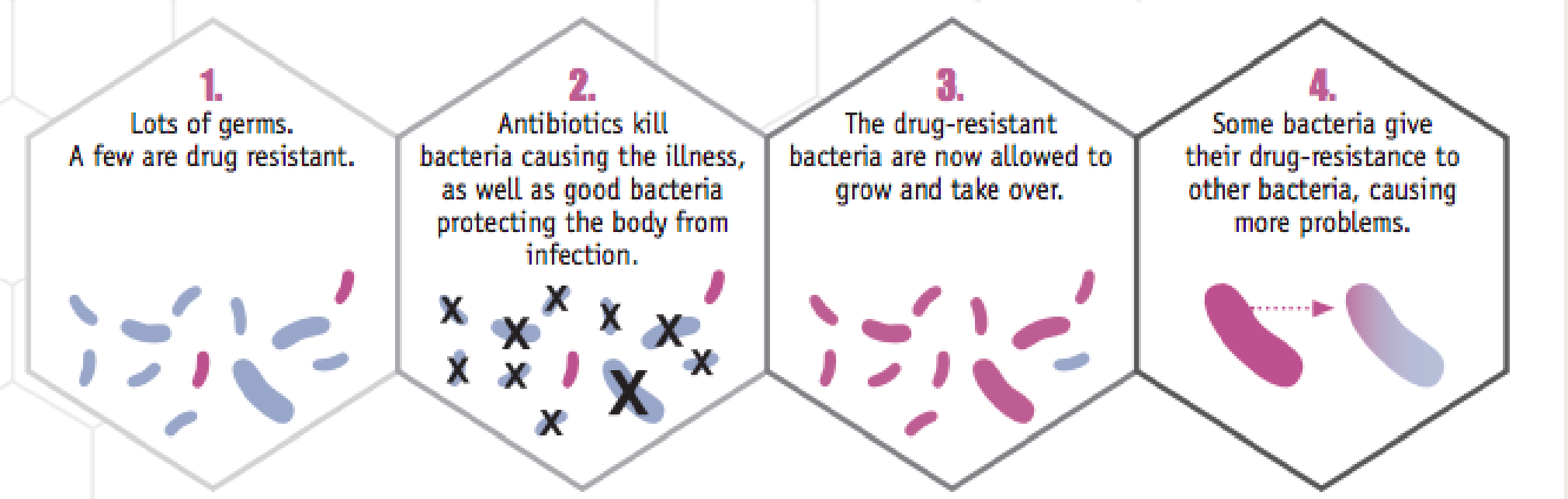
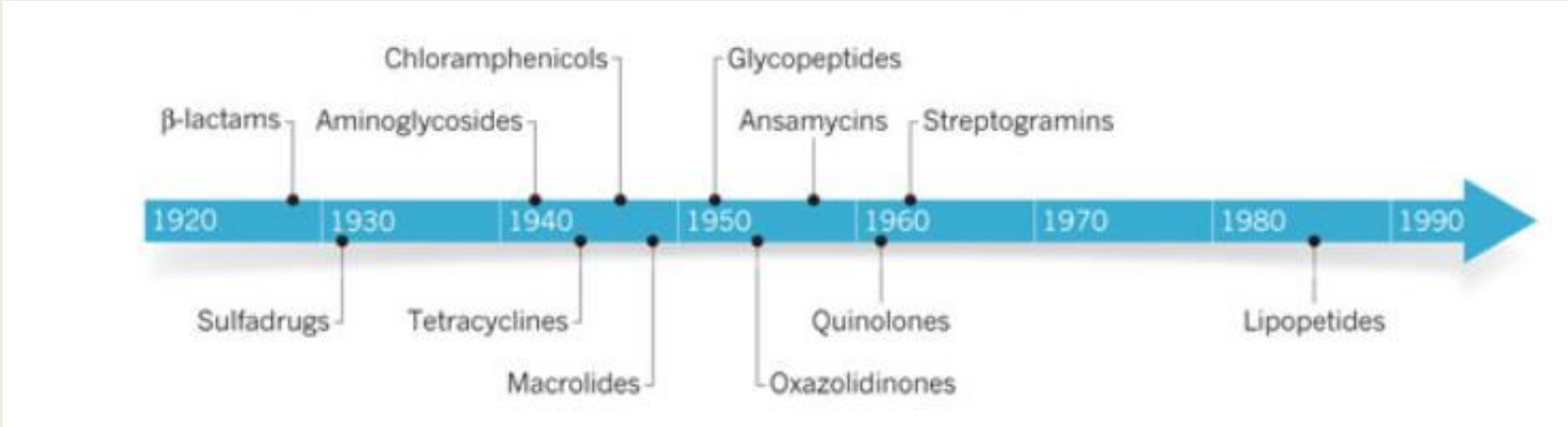
College of Natural Sciences

How Antibiotic Resistance Happens



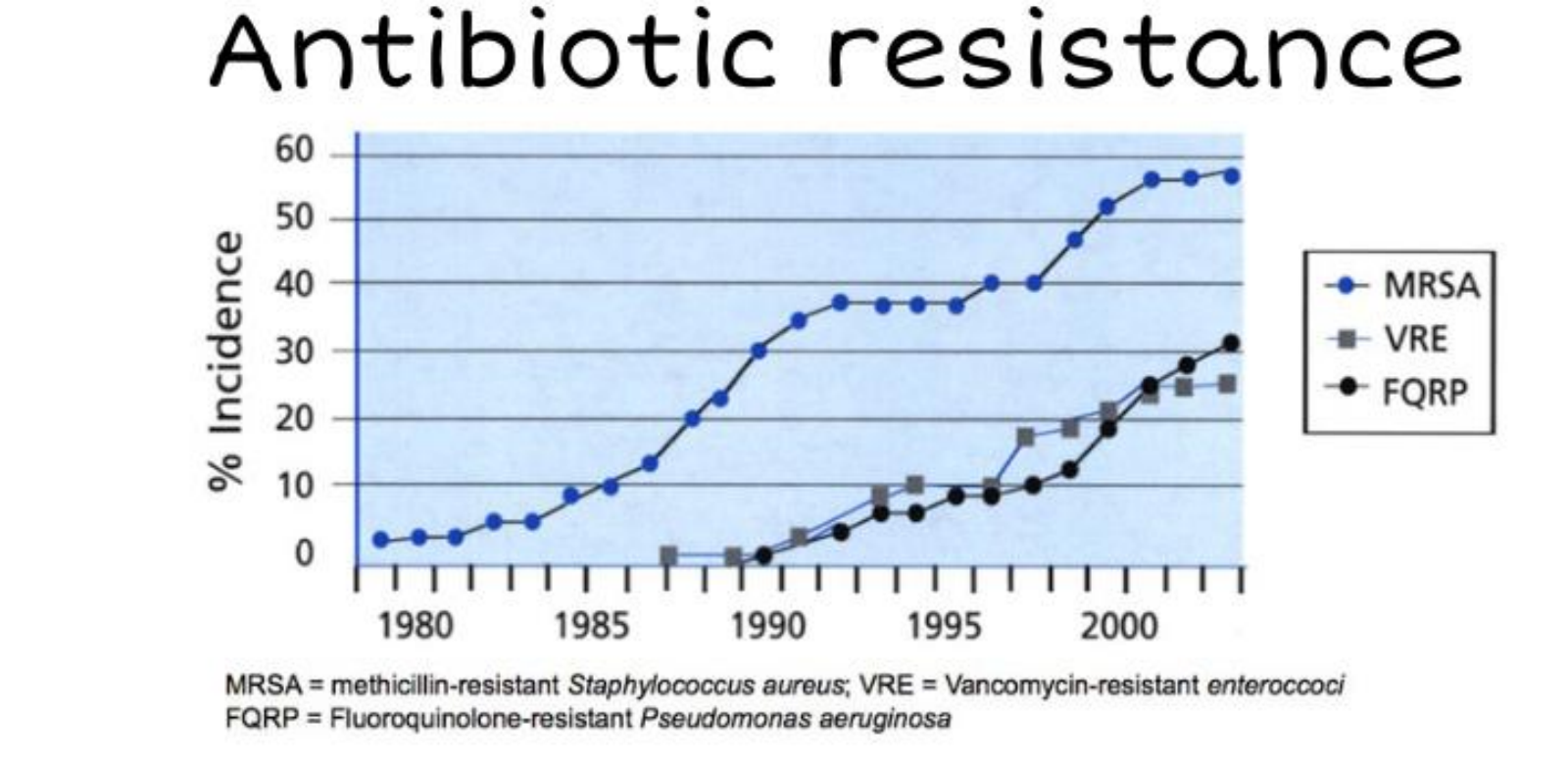
Examples of how it spreads:

1. Livestock
2. Prescriptions

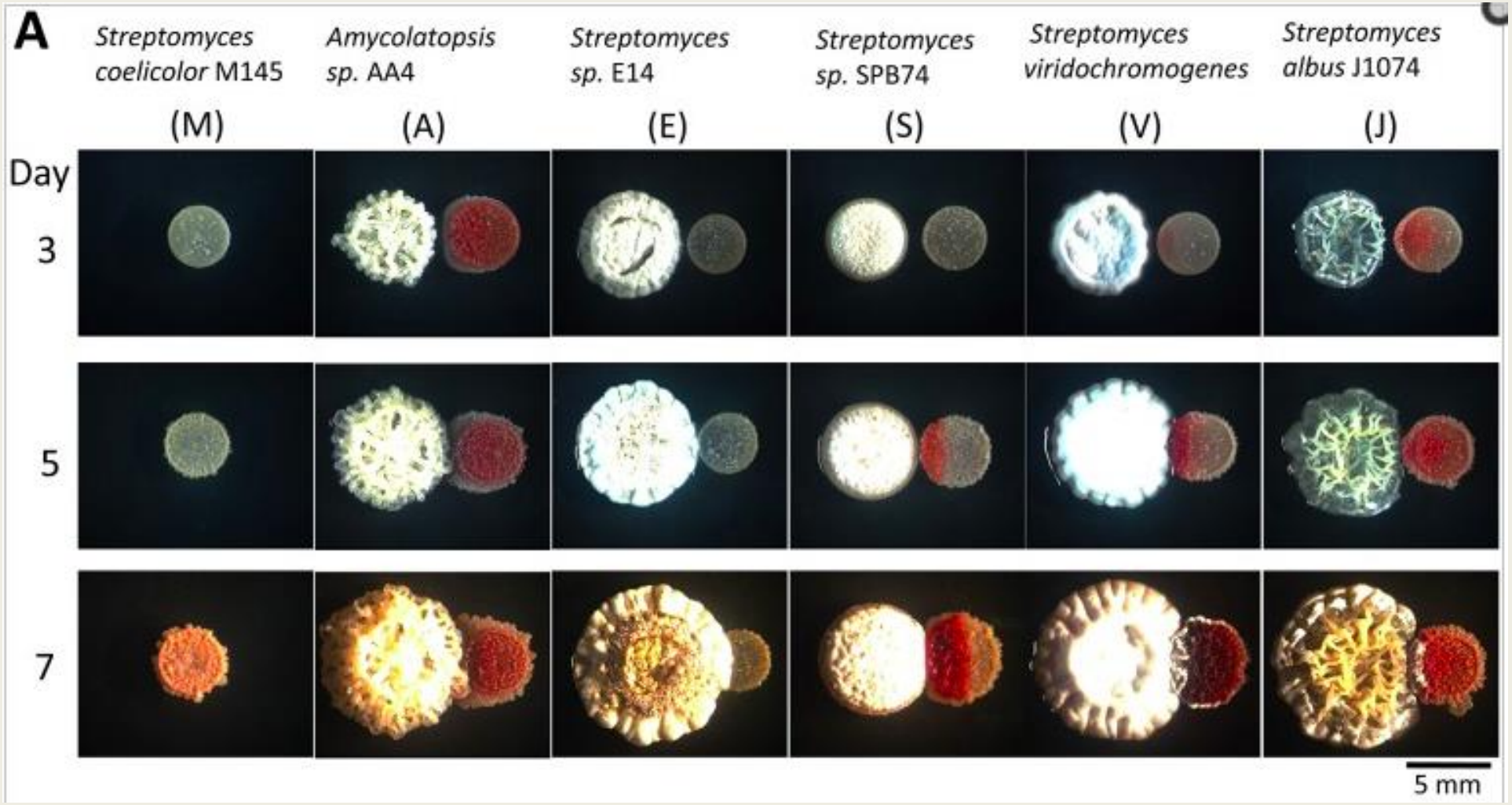


The Golden Era of Antibiotics

- Considered to be between the 20th century; specifically the 50's – 60's



Interspecies Interactions in co-cultures has led to new secondary metabolites.

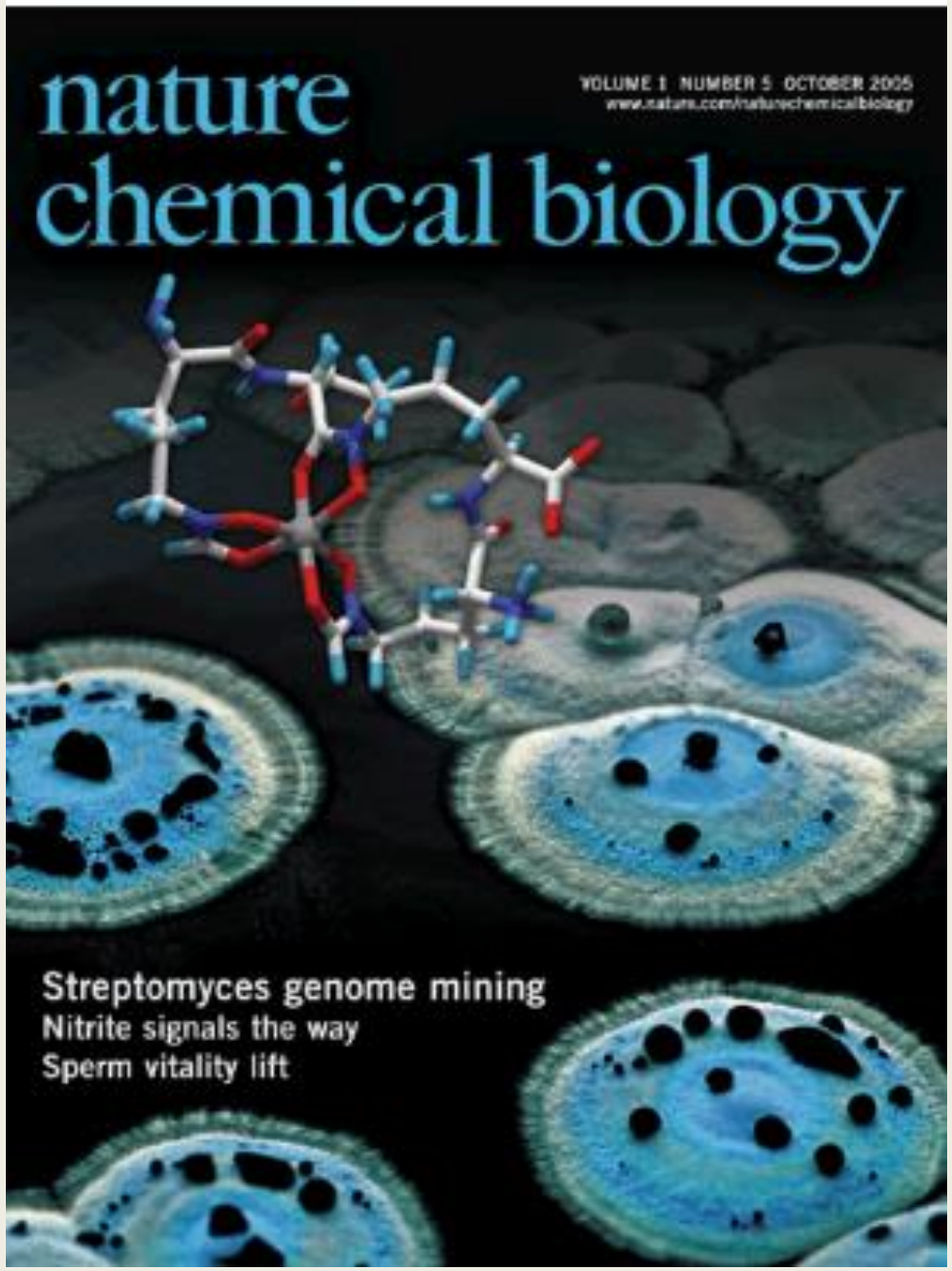


Traxler *et al.* 2013

PURPOSE:
CAN ANTIBIOTICS BE USED IN CO-CULTURE EXPERIMENTS TO PRODUCE NEW SECONDARY METABOLITES

An Introduction to *Streptomyces*

- *Streptomyces* is a genus of bacteria that contains many antibiotic producers
- *Streptomyces coelicolor* (wild type)
- *Streptomyces coelicolor* (M145)
- *Streptomyces osmaniensis*



Nature Chemical Biology cover from 2005

Isolating potential *Streptomyces*

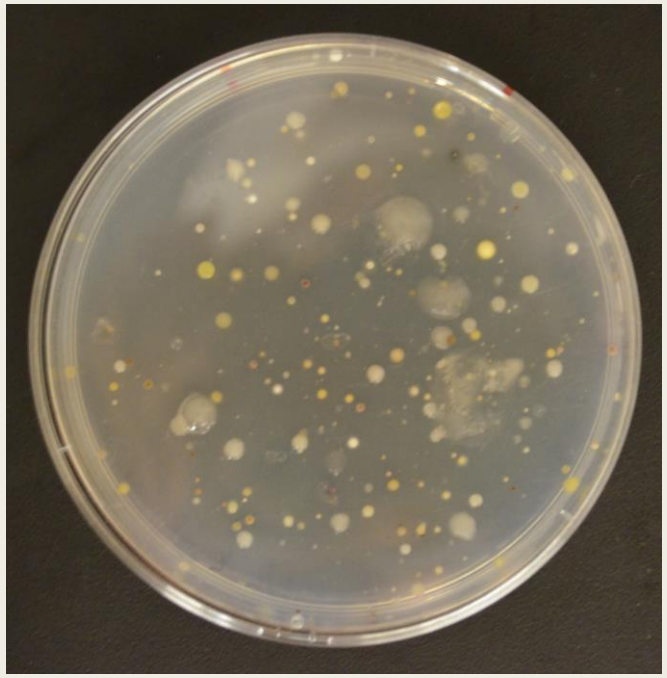


Plate by Matthew Co

Streptomyces osmaniensis gene for 16S ribosomal RNA, partial sequence, isolate: CDF2L1D13

Sequence ID: LC136922.1 Length: 1434 Number of Matches: 1

Range 1: 163 to 1163 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1805 bits(977)	0.0	989/1001(99%)	0/1001(0%)	Plus/Plus

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Sbjct  163   CTGGACGGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTGTTG  222
Query  61    GTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCAC  120
Sbjct  223   GTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCAC  282
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Sbjct  283   ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA  342
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Streptomyces osmaniensis was identified through sequencing.

Liquid cultures

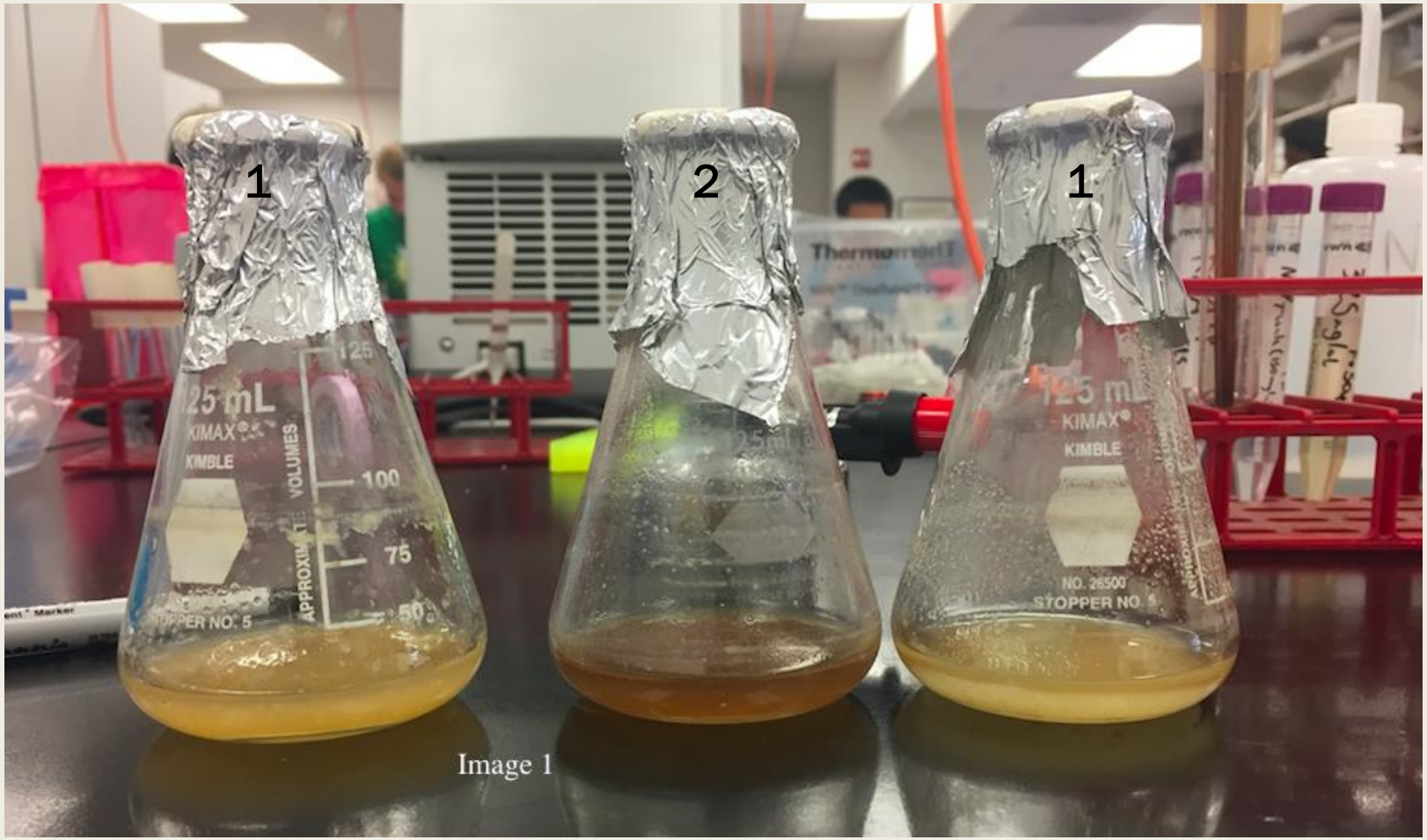
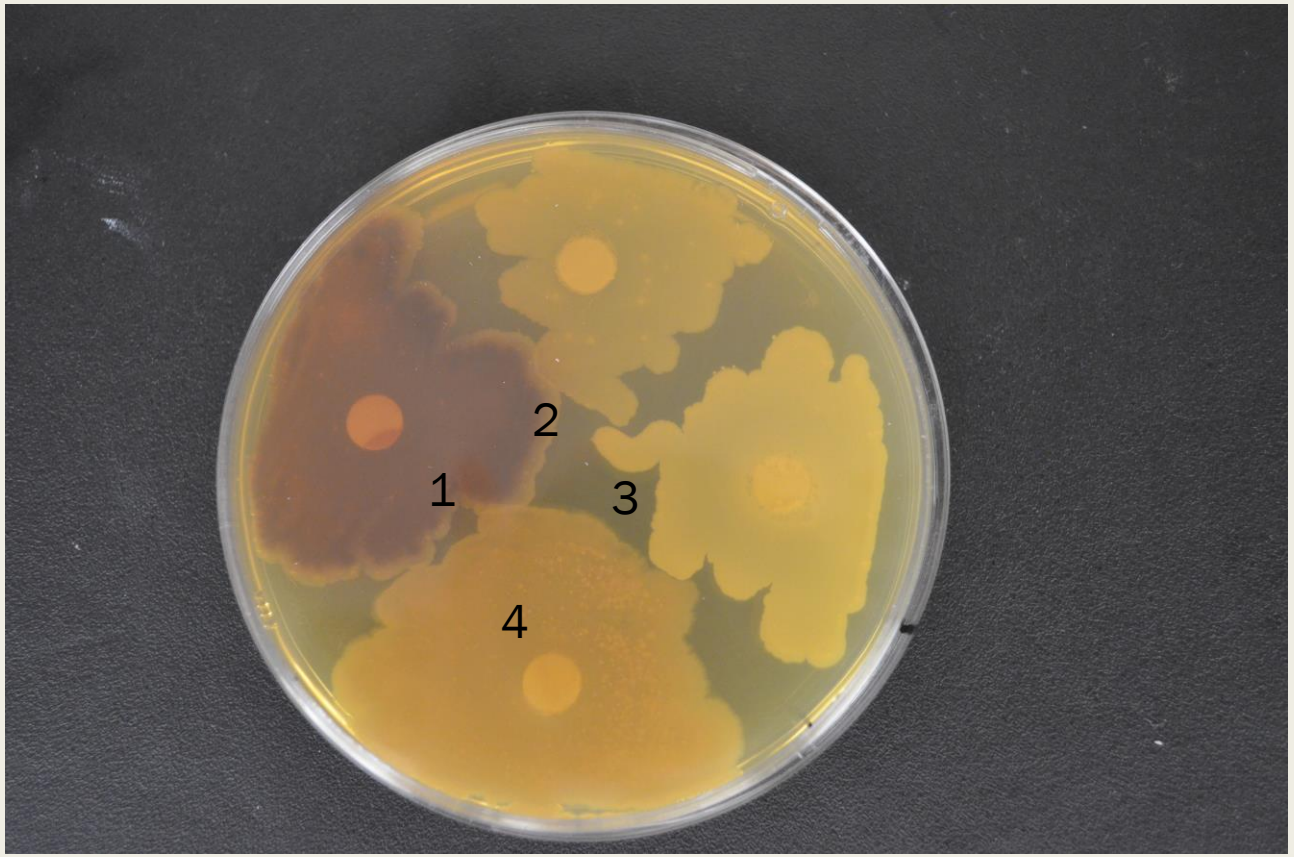


Image 1

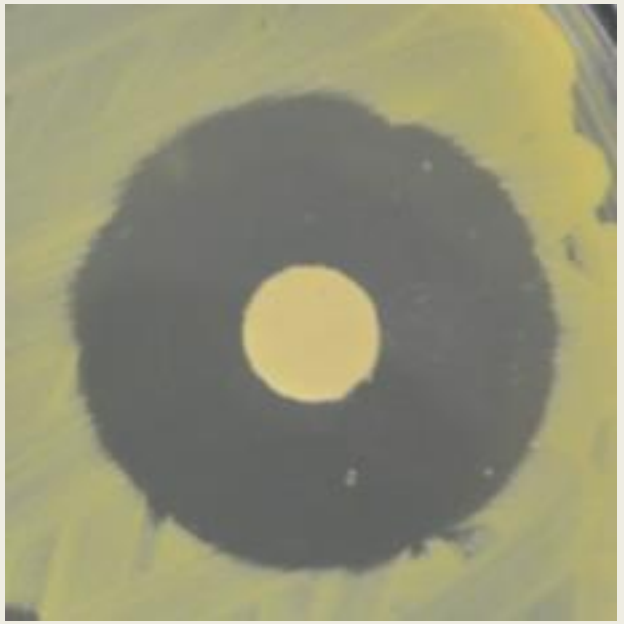
- TSB-YE [1]
- ISP2 [2]

Identifying what media is ideal for secondary metabolite production.

Cell Disk Assay

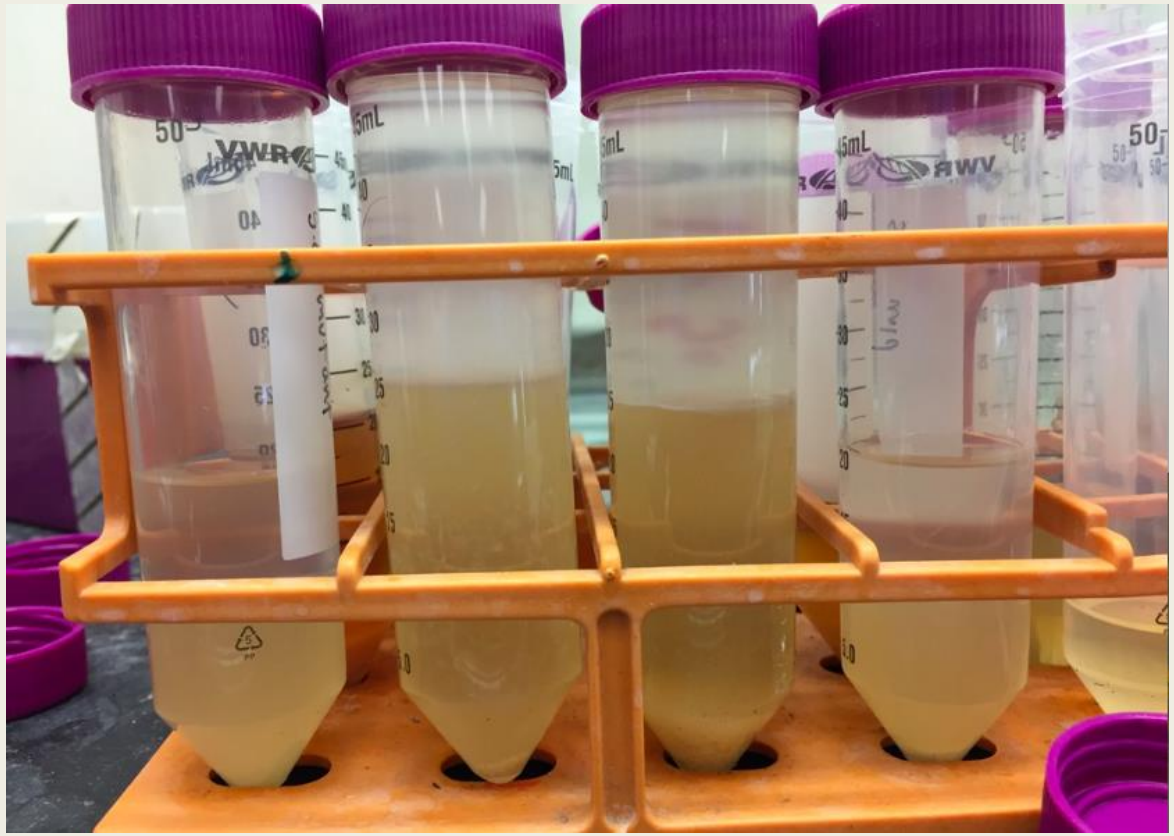


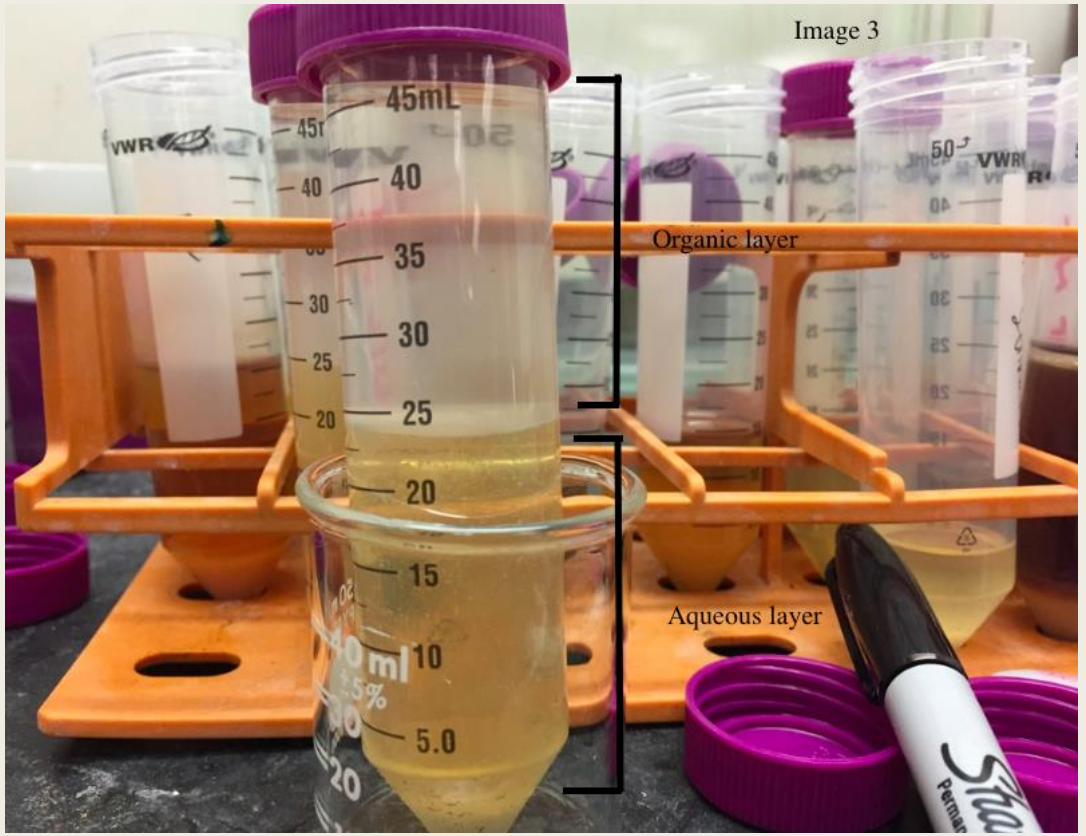
1. *Serratia marcescens*
2. *Pseudomonas sp.*
3. *Staphylococcus aureus*
4. *Bacillus sp.*



No inhibition when conducting ”live” cell disk assays.

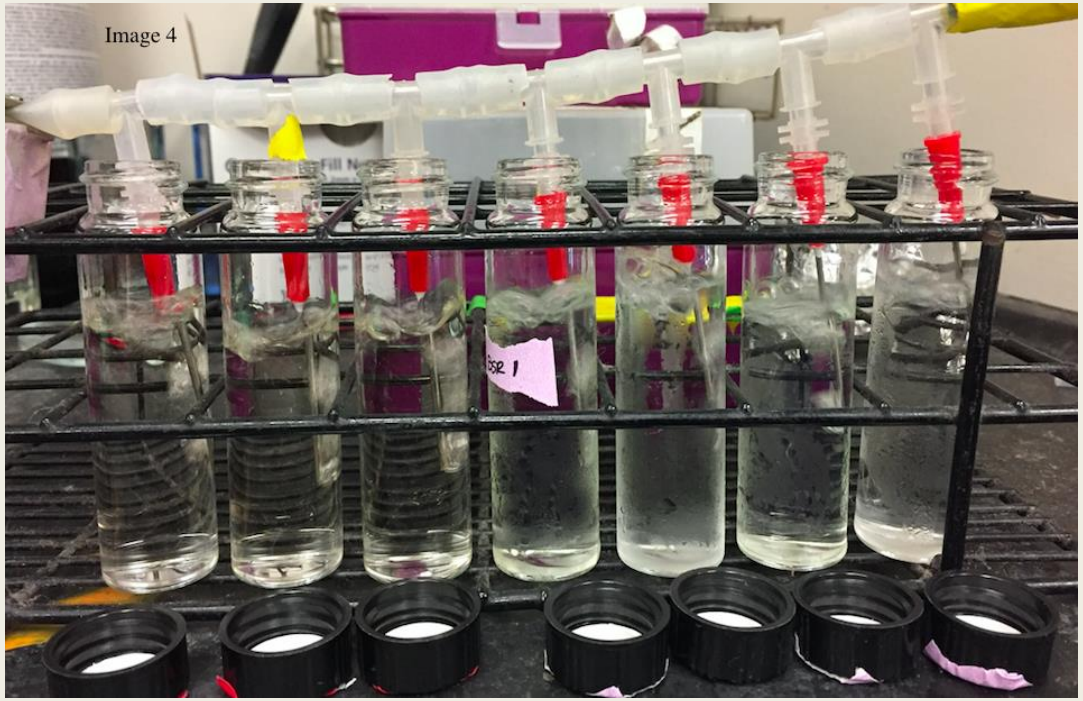
Ethyl Acetate extractions

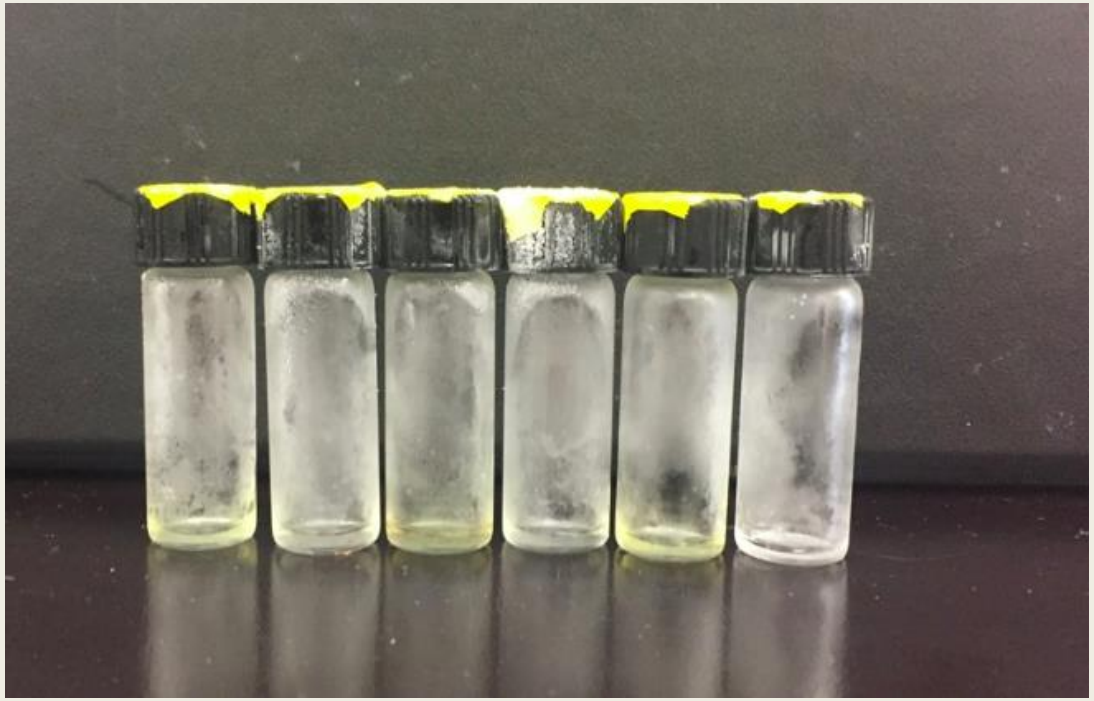




Ethyl Acetate extractions help isolate and test for antibiotics more efficiently.

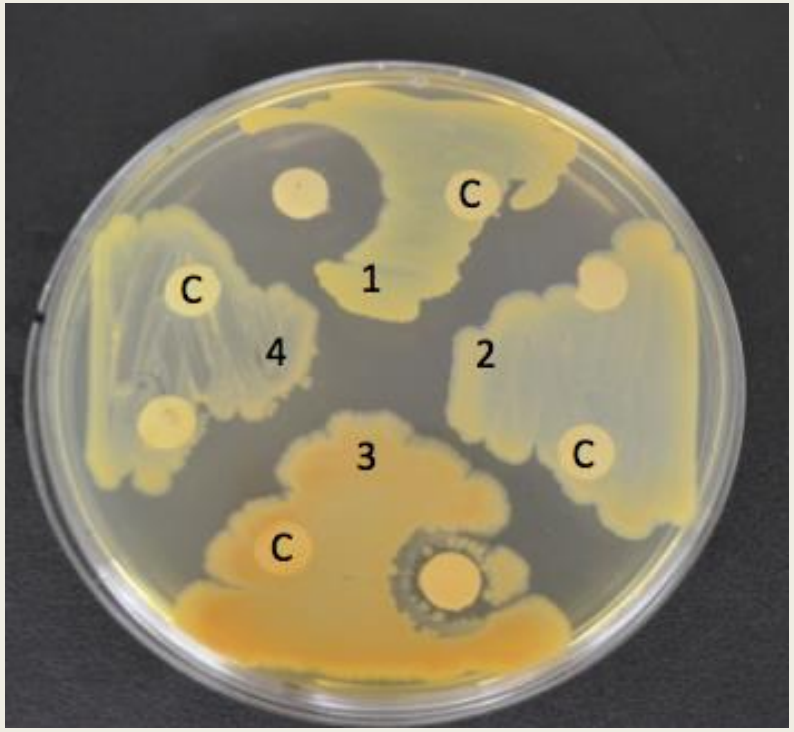
Drying down





Organic layer dried down to collect potential antibiotics.

Extract disk assay

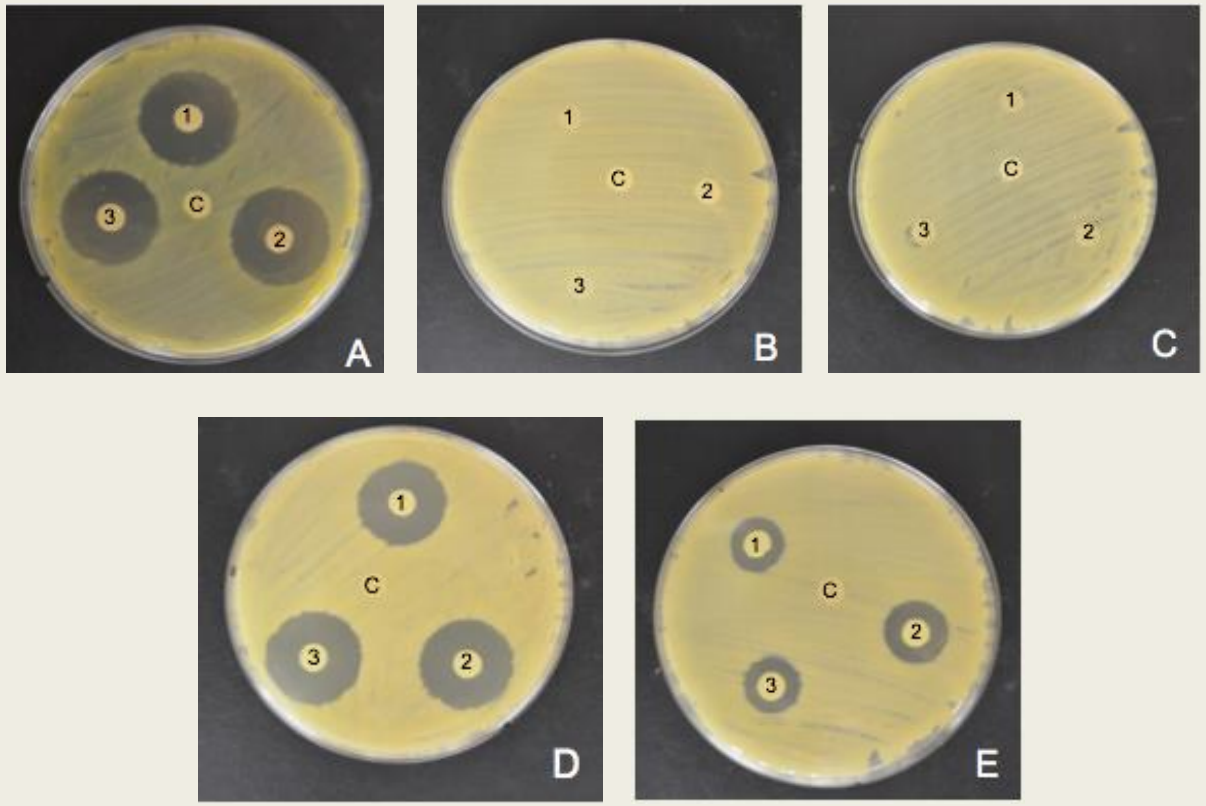


1. Pseudomonas sp.
2. Rhodococcus sp.
3. Bacillus sp.
4. E. coli

C : Control

Tests using Ethyl Acetate extractions led to inhibition on *Pseudomonas.*

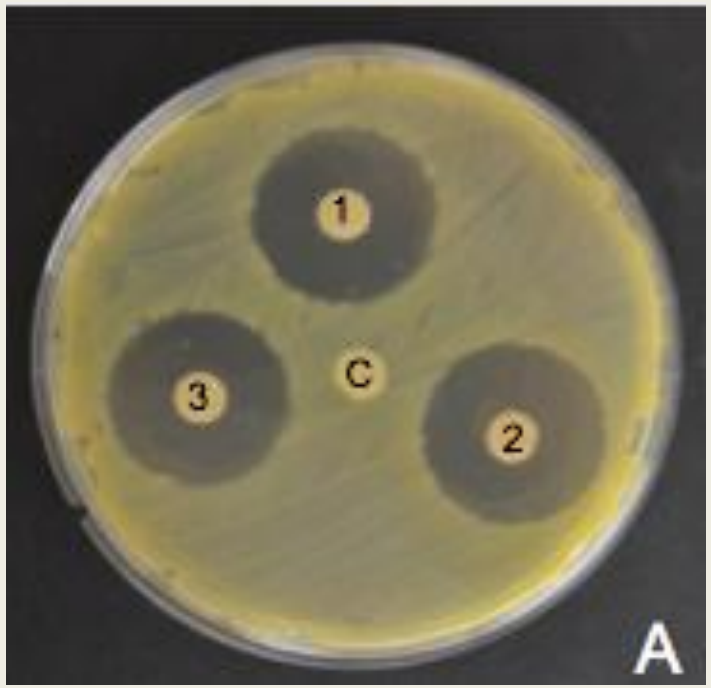
Disk Assays



A. S. osmaniensis
B. S. coelicolor
C. S. coelicolor (M145)
D. S. osmaniensis + *S. coelicolor* (M145)
E. S. osmaniensis + *S. coelicolor*

Compared inhibition of mono-cultures vs. co-cultures to determine benefits of interspecies interactions.

Results



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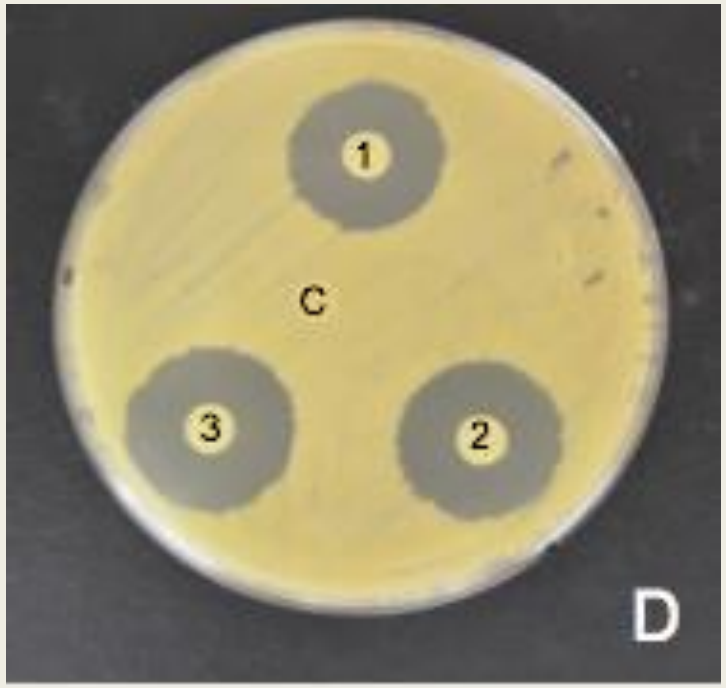

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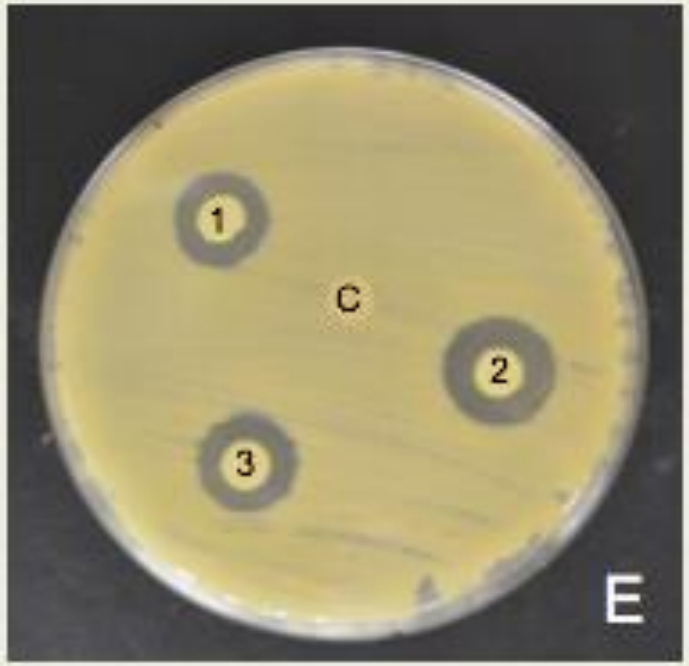

S. osmaniensis

24 mm

S. osmaniensis + *S. coelicolor (M145)*

22 mm

S. osmaniensis + *S. coelicolor*

15 mm

Average diameter (mm) of mono-cultures of *S. osmaniensis* led to larger zones of inhibition compared to that of co-cultures with both wild and mutant type.

Future Directions

- Co-culturing of different antibiotic producing *Streptomyces spp.*
- Different nutrient media
- Antibiotics produced by the isolate should be characterized by using Liquid Chromatography Mass Spectrometry.

Antibiotic secretion of *Streptomyces spp.* colony

Acknowledgements

- I thank Dr. Gregory C. Palmer and the mentors in the Antibiotics Discovery and Function laboratory at the University of Texas at Austin for their guidance and support and Matthew Co for his soil sample plate image. I thank the Summer Undergraduate Program for Experiential Research (SUPER) 2017 program at the University of Texas at Austin and the Undergraduate Opportunities Center (UROC) at California State University, Monterey Bay.

Reference

- Traxler M., Watrous J., Alexandrov T., Dorrestein P., Kolter R. Interspecies Interactions Stimulate Diversification of the *Streptomyces coelicolor* Secreted Metabolome. *PubMed*. 2013. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3747584/
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- Davies J. Where have All the Antibiotics Gone? The Canadian Journal of Infectious Diseases & Medical Microbiology. 2006;17(5):287-290.

Thank you for your time.
Any Questions?