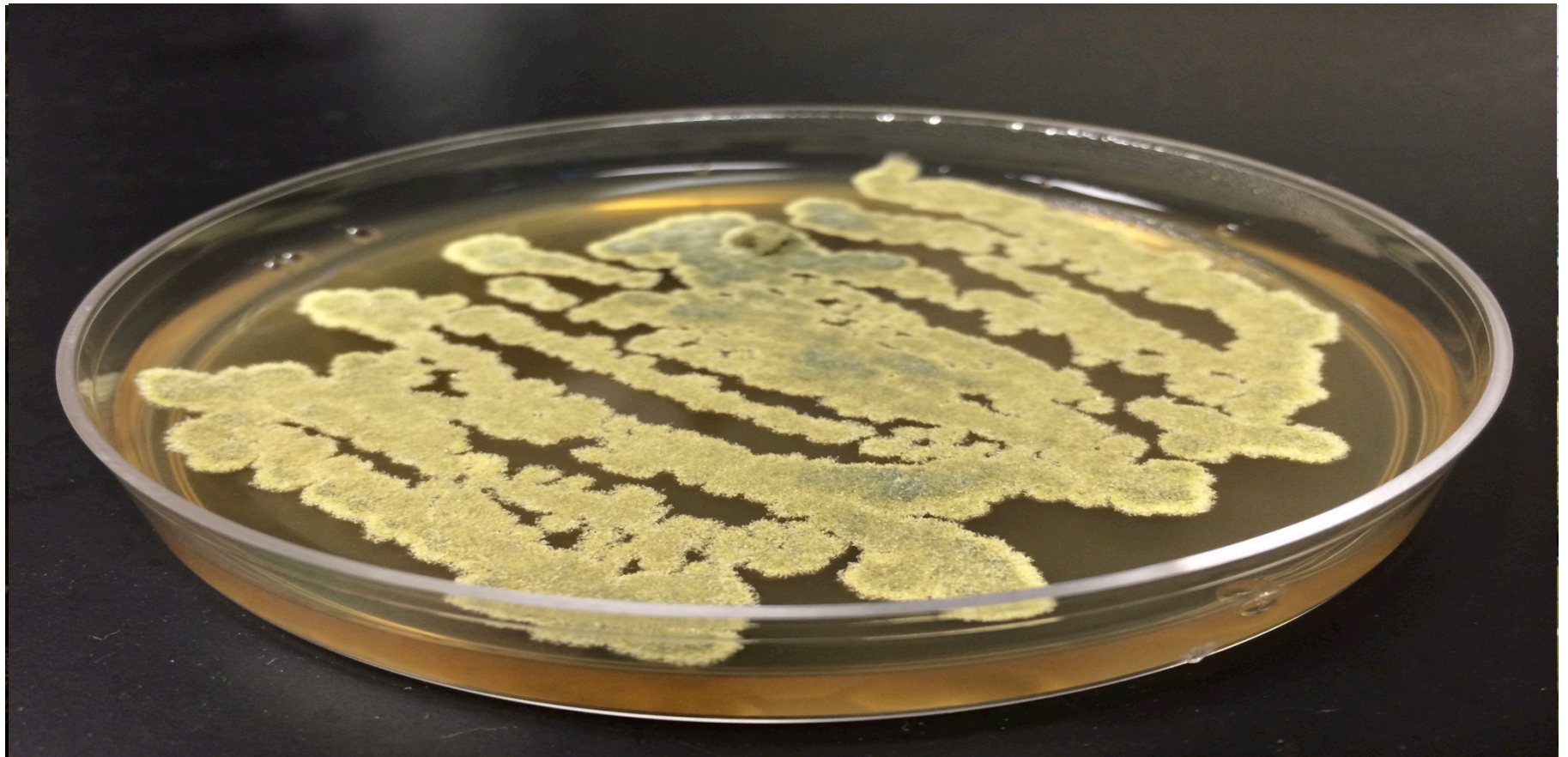




Chemical Screen and Antibacterial Testing of Fungal Endophytes

Andrew Pham | Dr. Sandra Loesgen | Department of Chemistry



What is an Endophyte?

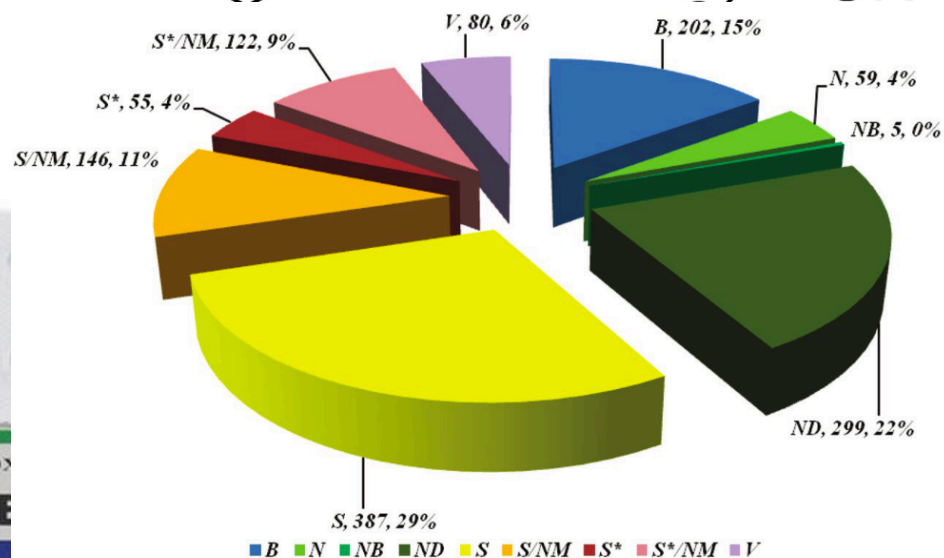
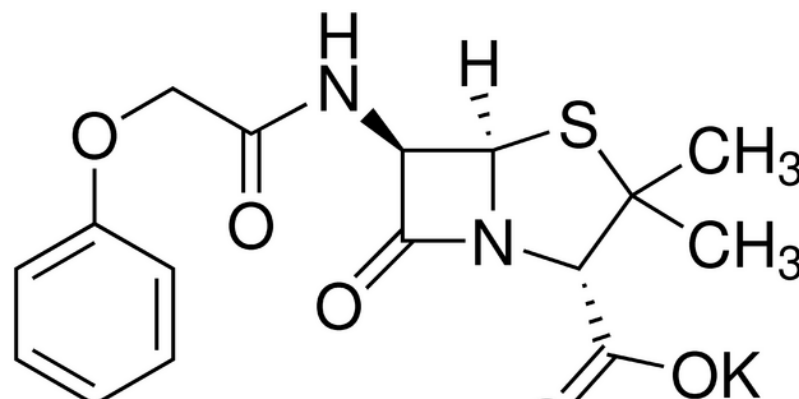
- Bacteria or Fungi
- Literally means “in the plant”
- Greek
 - Endon – Within
 - Phytan – Plant
- Endophytes are involved in mutualistic symbiosis with their plant host providing **bioactive metabolites** in exchange for nutrients

Algae collected from Strawberry Hill, Oregon



Natural Product

“Natural products are organic compounds that are formed by living systems”



2 Hanson JR (2003) RoyalSocietyChem.
D. Newman; G. Gragg; *J. Nat. Prod.* 2012 75: 311–335

Project

- Strains
 - Gozo 1
 - Gozo 4-Y
 - Kreta 2/23
 - Kreta 2/28



Fungal Media

M2

Malt extract
Yeast extract
D-glucose
pH = 7.0

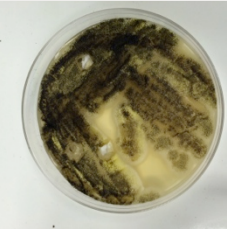
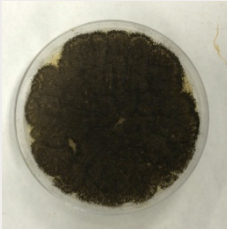
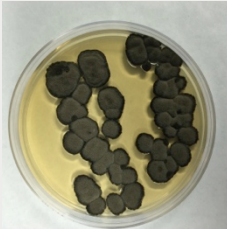
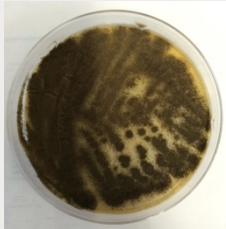
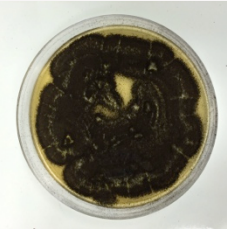
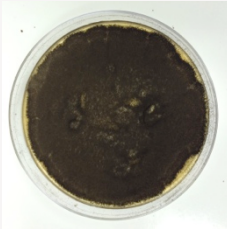
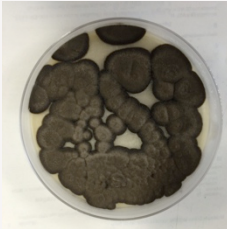

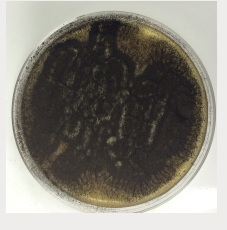
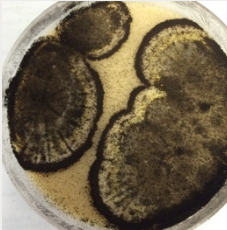
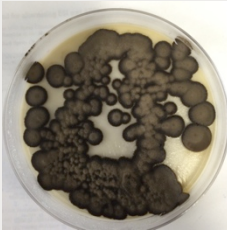

1158

NH_4HSO_4
Malt Extract
D-glucose
Yeast Extract
pH = 6.0

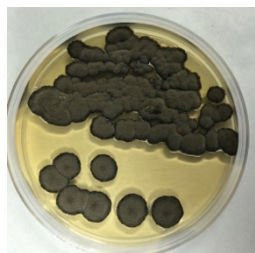
G20

Glycerine
Malt extract
Yeast extract

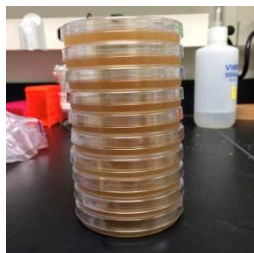
Project

	Gozo 1	Gozo 4-Y	Kreta 2/23	Kreta 2/28
M2				
1158				
G20				

Method - Work Flow



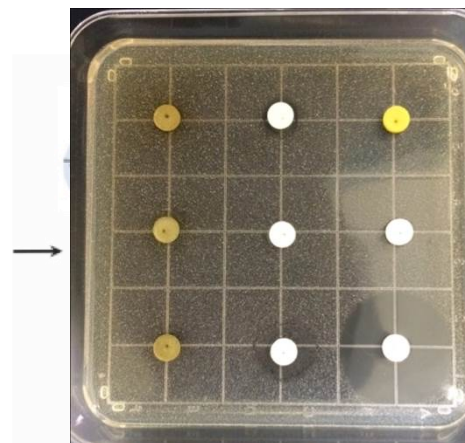
Isolation



10 Plate Work Up

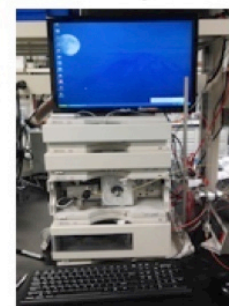


Extraction/Work up



Bioactivity

↓ → TLC



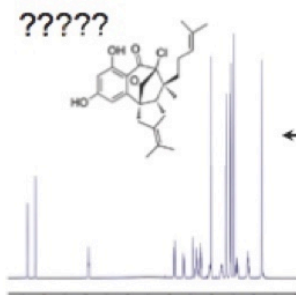
Purification HPLC



Analysis by LC-MS



Analysis by NMR



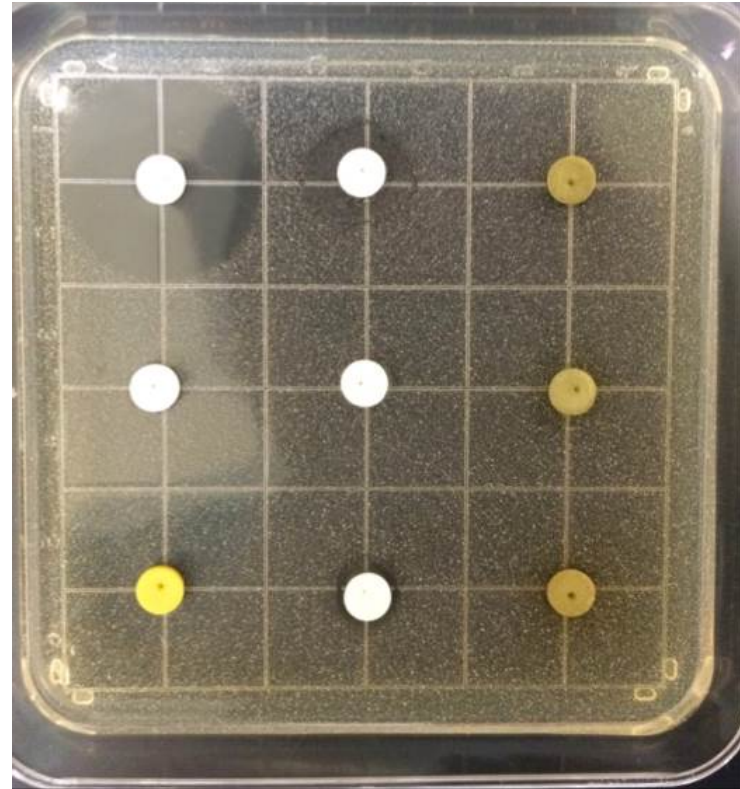
Structure determination

Bioassay

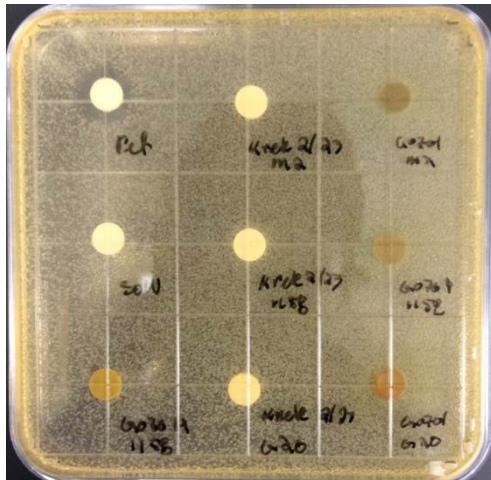
Diffusion Disk Assay

- Test for
 - Activity
- Looking for
 - Zone of inhibition

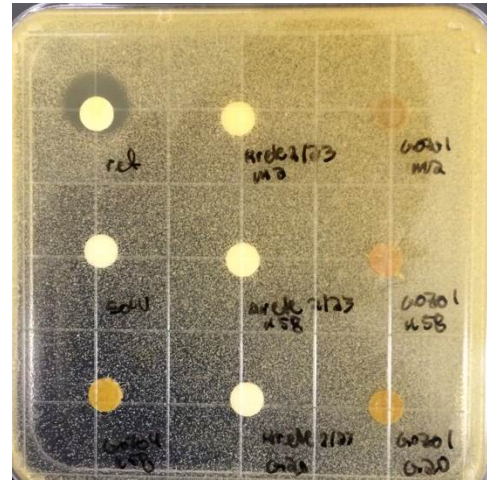
Target Strain
<i>Escherichia coli</i>
<i>Pseudomonas aeruginosa</i>
<i>Staphylococcus aureus</i>
<i>Enterococcus faecium</i>
<i>Bacillus subtilis</i>
<i>Candida albicans</i>



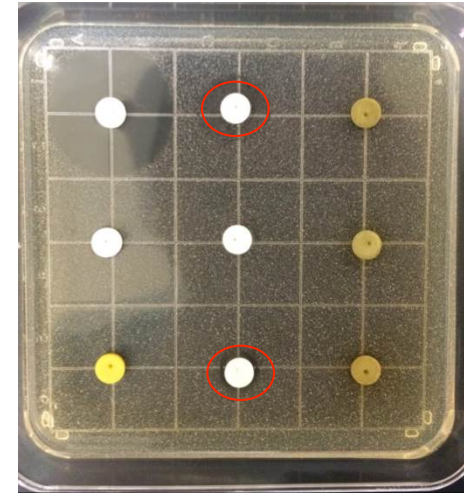
Antibacterial testing (Diffusion Disk Assay)



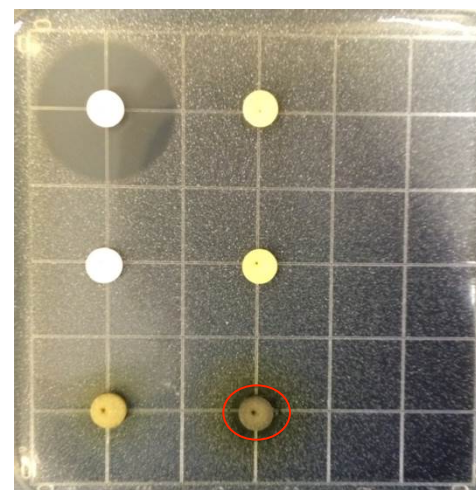
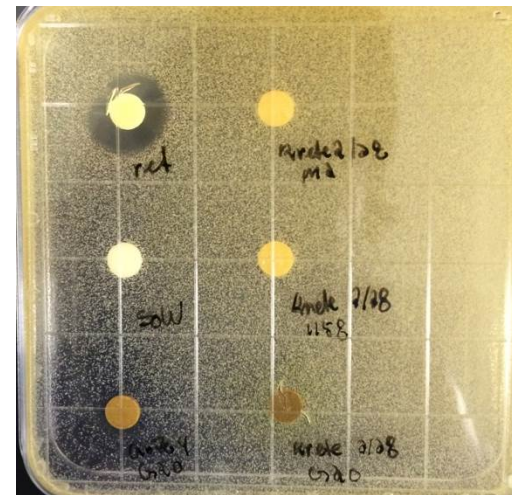
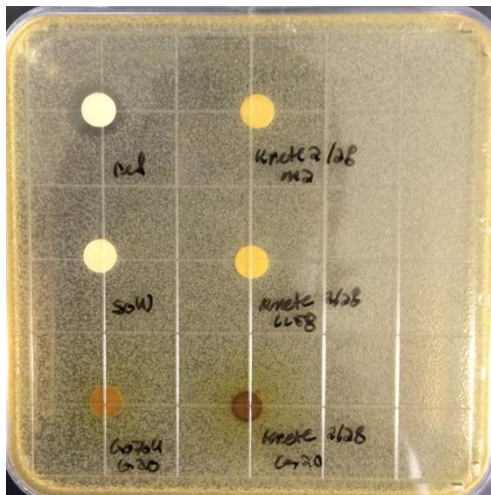
P. aeruginosa



C. albicans



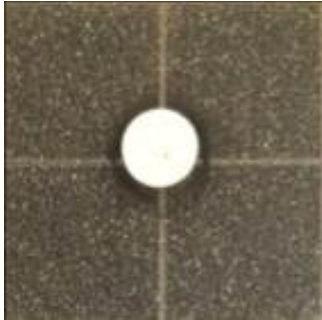
S. aureus



Diffusion Disk Assay - Results



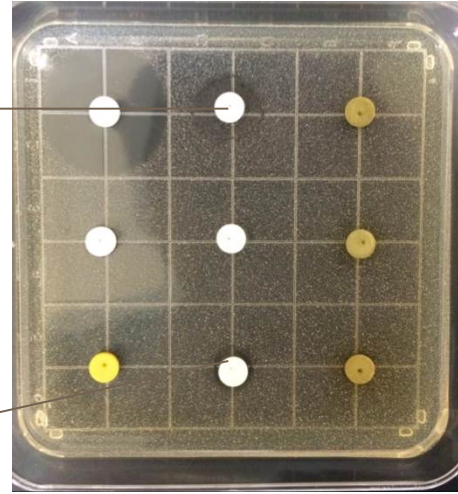
Krete 2/23 M2



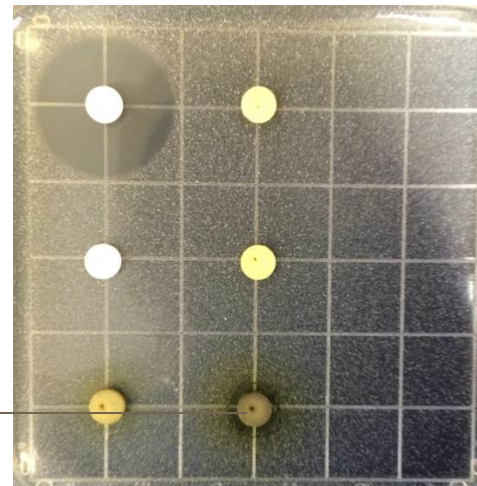
Krete 2/23 G20



Krete 2/28 G20

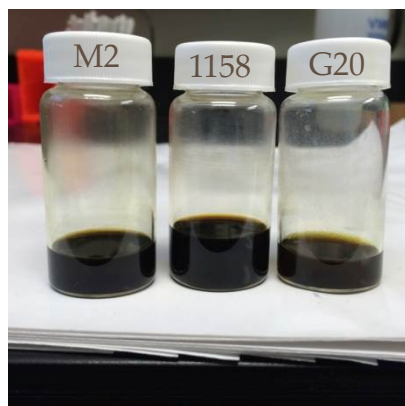


Staphylococcus aureus

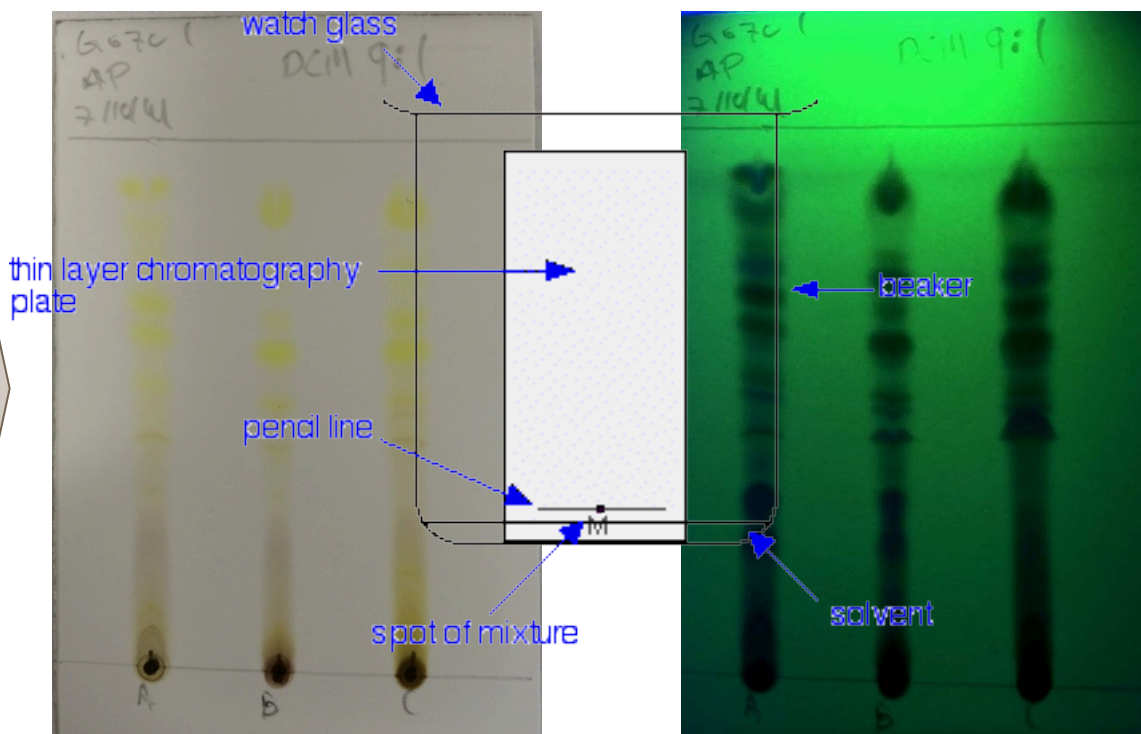
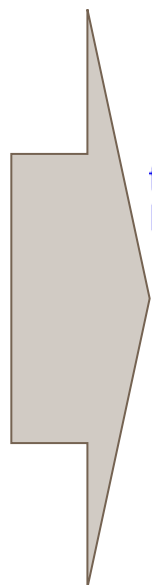


Chemical Screening

Chemical Screening - Thin-Layer Chromatography (TLC)



Gozo 1



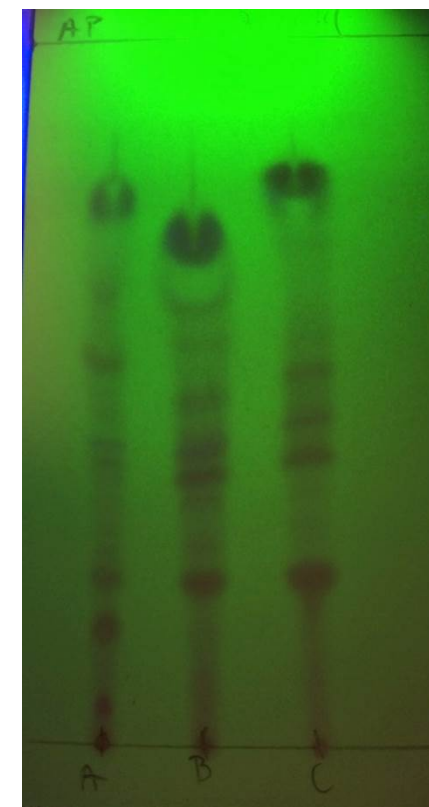
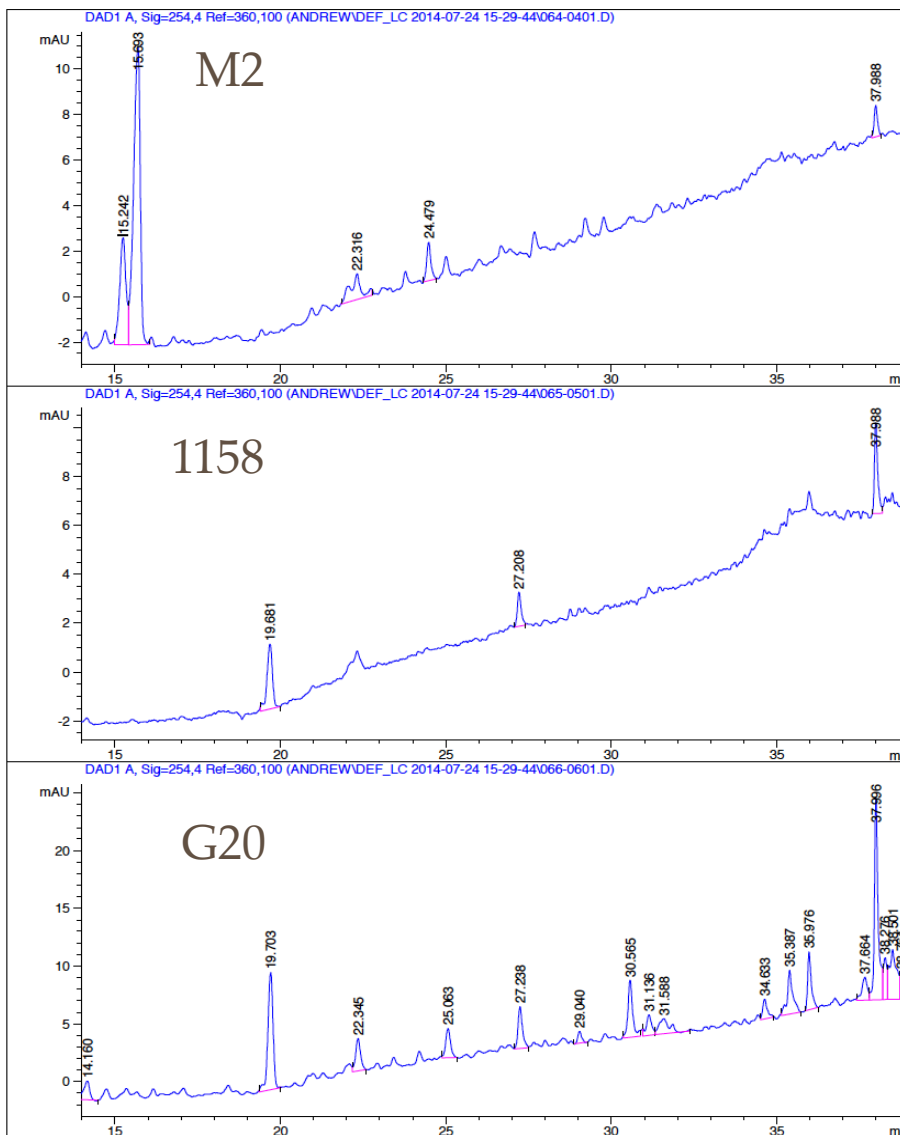
M2 1158 G20

254 nm

Solvent System

Dichloromethane 9 : 1 Methanol

Chemical Screening - Kreta 2/23

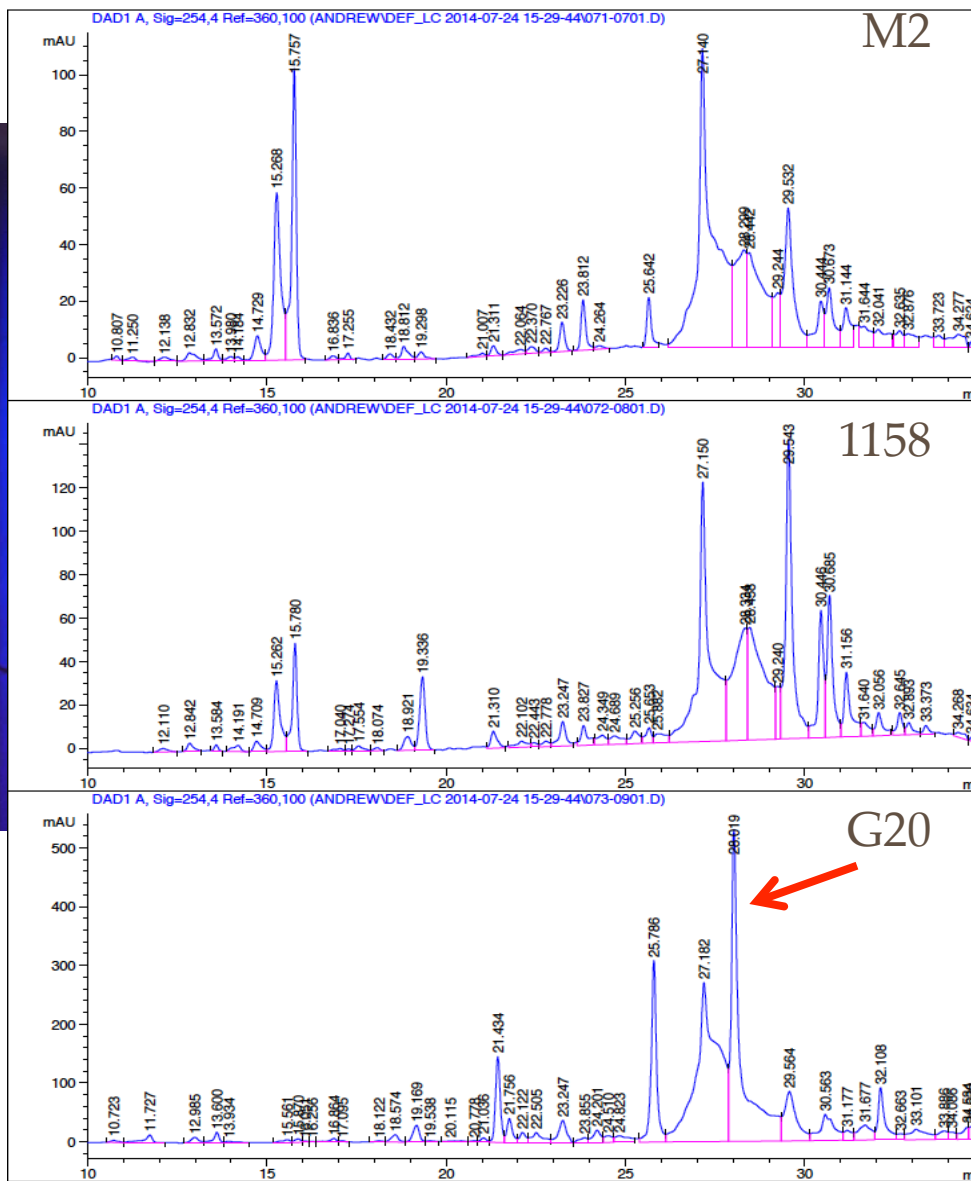


M2 1158 G20

Chemical Screening - Kreta 2/28

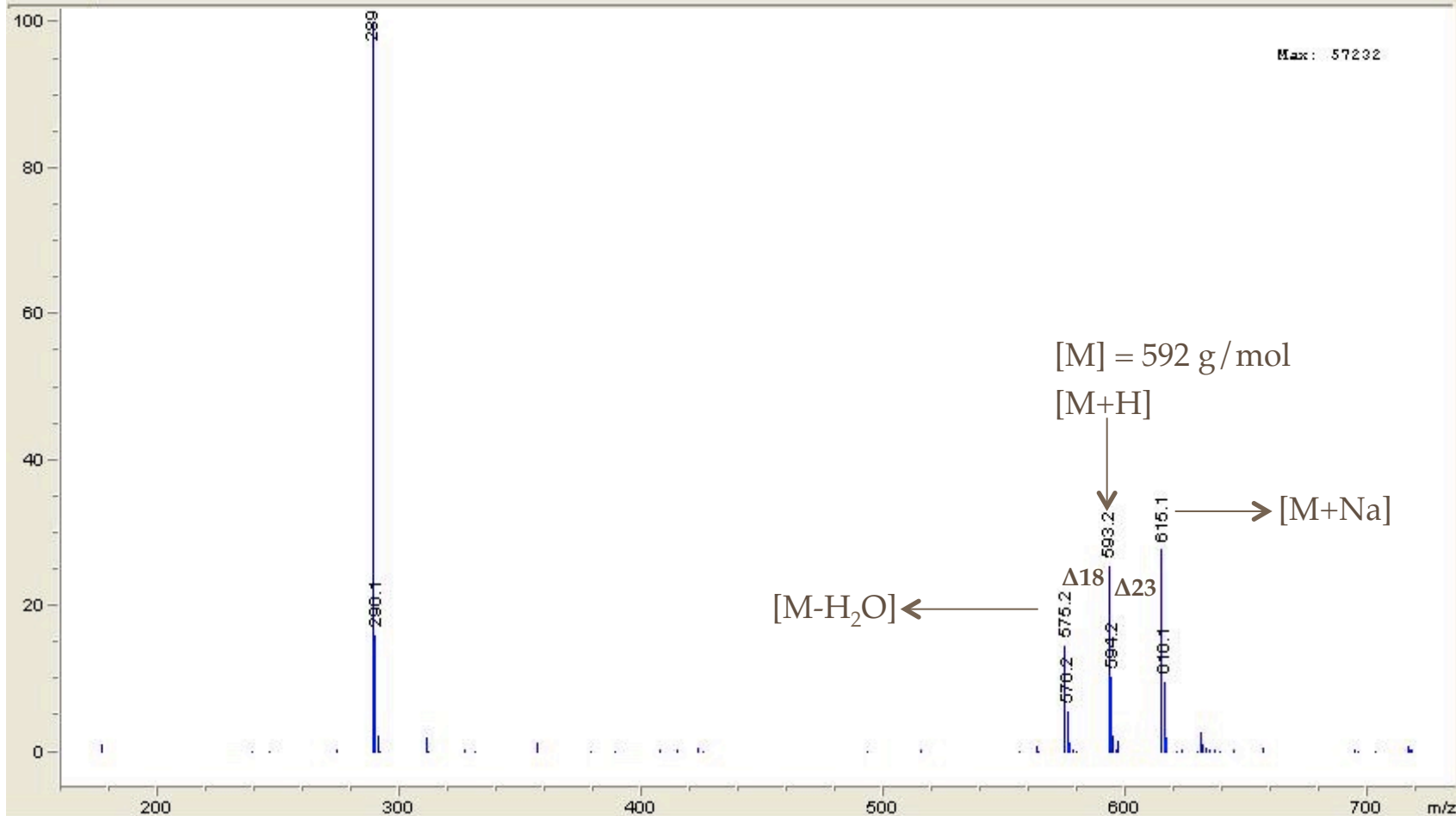


M2 1158 G20



Chemical Screening - MS (Kreta 2/28 G20) - 28 min

*MSD1 SPC, time=28.079 of C:\CHEM32\1\DATA\ANDREW\DEF_LC 2014-07-24 15-29-44\073-0901.D ES-API, Pos, Scan, Frag: 70

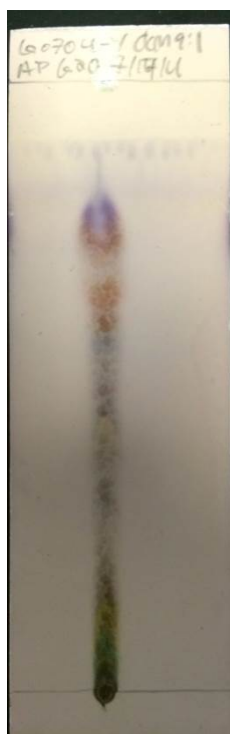
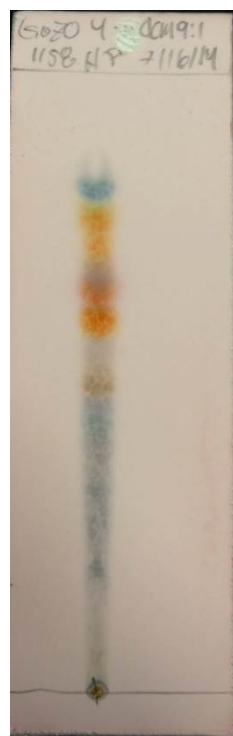


Chemical Screening - TLC

Gozo 1



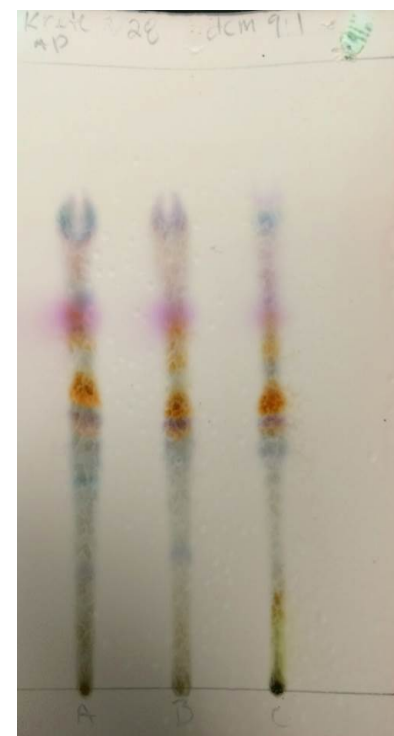
Gozo 4-Y



Krete 2/23



Krete 2/28



Stain: p-Anisaldehyde

Conclusion

- Endophytes produced different natural products under different medium

Future Work

- Continue onto new strains
- Kreta 2/23 & Kreta 2/28
 - Large scale
 - Further purification
 - Bioassay & Structure Elucidation
- Isolate new entophyte from Oregon Algae sources

Acknowledgement

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Chenxi (Zoe) Zhu

Department of Microbiology

Oregon State University

Questions?

