Microbiology, Immunology and Molecular Genetics 109AL— Research Immersion Laboratory in Microbiology

Research Project Presentation

Cultivation-independent and cultivation-dependent analysis of microbial soil near burned Laurel Sumac bushes in the Skirball hills

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INTRODUCTION & BACKGROUND

Relevance to Research

WHY ARE WE CONDUCTING THIS RESEARCH?

Skirball Hills 2017

- 2017, **second deadliest, most destructive hit from wildfires in California history**.
- 9,133 fires burned over 1.3 million acres of land (California Department of Forestry and Fire Protection)
- December of 2017, Skirball Fire consumed more than 422 acres of Southern California
- Impacted species: Laurel Sumac

Skirball Hills 2019

Malosoma Laurina or LAUREL SUMAC

- Rhizosphere sampled from the base of a burned Laurel Sumac
- Soils supporting Laurel Sumac are acidic to neutral, well drained, dry and often rocky or gravelly
    - Favored on soils with **high exchangeable potassium levels,** and peak abundance occurs on coastal sites with heavy litter layers USDA—**Fire Effects Information System (FEIS)**

LAUREL SUMAC SOIL

- Laurel sumacs typically have their outer layers burned, although more severe fire may result in some shrub mortality
- Sampled plant from had burned roots and visible damage to the branches. USDA—**Fire Effects Information System (FEIS)**

Goal of our research:

1) What are the current soil characteristics/how do they differ from a normal Laurel Sumac?
2) What type of bacteria are present in the soil? Who is there?
   a) What are the different functions of the bacteria? What are they doing?

Healthy Laurel Sumac

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PROJECT GOALS & HYPOTHESES

OVERALL PROJECT GOALS

- Connection between soil microbial community ***structure and function***
- Overall purpose of our project is to use a cultivation independent (CI) and a cultivation dependent (CD) approach in order to *evaluate the effect a wildfire has on a soil microbial community.*
- **CI approach**—Analyze the difference in microbial community diversity of recovering and healthy Laurel Sumac through 16S rRNA eDNA
- **CD approach**—Identify how bacteria within the rhizosphere are interacting with each other and their environment.

OVERALL PROJECT HYPOTHESES:

Since plant and microbial communities in soil are so heavily impacted by the physiochemical changes caused by ***wildfires*** (Mahmood et al. 2003, Insam et al. 2009), ***if*** soil taken from around Laurel Sumacs have been affected by the fire and community structure altered, ***then*** culture-dependent analysis of the soil will show the presence of bacteria capable of nitrogen-fixing, cellulolytic ability, plant growth, and oxidation.

OVERALL PROJECT HYPOTHESES:

Also, ***since*** plants can determine the composition of rhizosphere bacteria (Bowen et al. 2017), ***if*** recovering laurel sumac shrubs exhibits an effect on microbial community structures, ***then*** culture-independent analysis of soil eDNA will show a greater number of bacterial lineages capable of nitrogen-fixing, cellulolytic ability, and oxidation than in healthy shrubs.

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EXPERIMENTAL APPROACH

Methodology

HOW ARE WE CONDUCTING THIS RESEARCH?

- Experimental approach divided into 2 main portions

Cultivation Dependent (AL)

- Focus on isolating microbes + functional assays
- Soil Characterization
- 4 main functional assays. (3 trials per assay)
  - Nitrogen fixing activity
  - Cellulase activity
  - Oxidative fermentation assay
  - Siderophore assay

Cultivation Independent (to be done in BL)

- Focus on genomic sequence analysis
- 16s rRNA PCR and gene sequencing
  - Sequence comparison between healthy and burned Laurel Sumac

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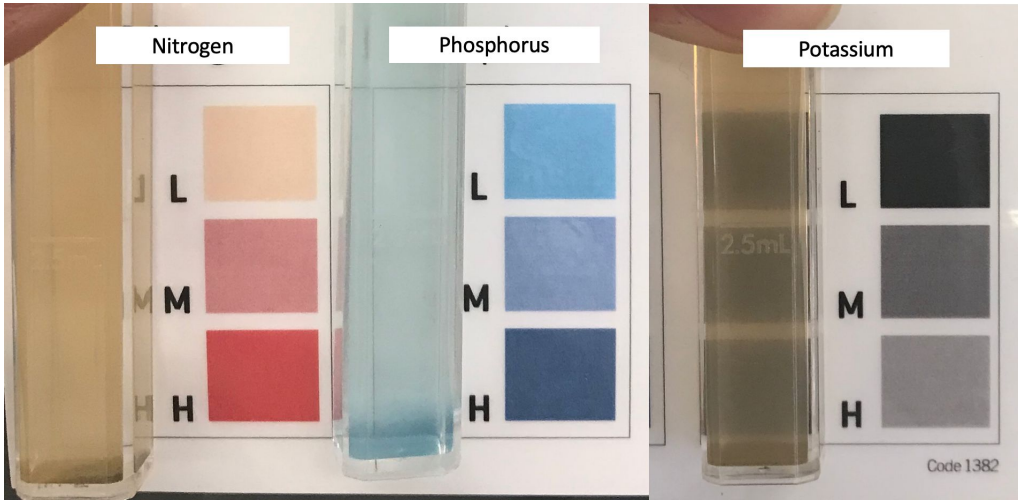
RESULTS

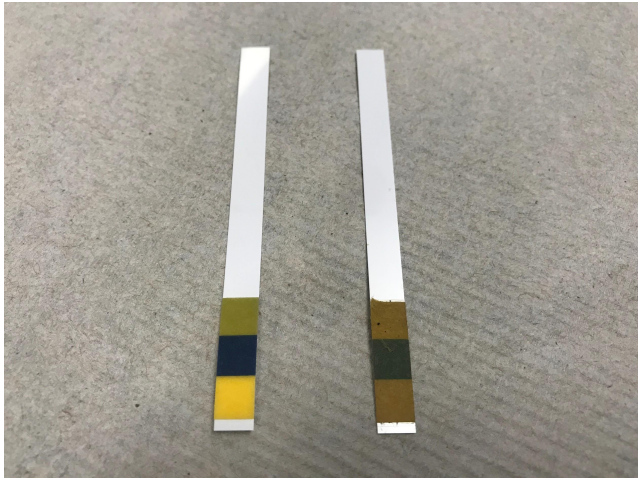
Soil Characterization

- Soil characterization—Muddy, Thick, Lots of Debris
- Low nutrients—Low Nitrogen, Phosphorus, Potassium
- pH results indicated 6.0

NPK Testing



pH Strip Testing



Isolations + Purifications - Gram Stains

Gram Stains - R2A Isolates

Isolate	Gram Status
W19UCLA1091BLCS30R01	Negative
W19UCLA1091BLCS30R02	Negative
W19UCLA1091BLCS30R03	Positive
W19UCLA1091BLCS30R04	Negative
W19UCLA1091BLCS30R05	Positive
W19UCLA1091BLCS30R06	Negative
W19UCLA1091BLCS30R07	Negative
W19UCLA1091BLCS30R08	Positive
W19UCLA1091BLCS30R09	Positive
W19UCLA1091BLCS30R10	Negative
W19UCLA1091BLCS30R11	Negative
W19UCLA1091BLCS30R12	Positive
W19UCLA1091BLCS30R13	Positive
W19UCLA1091BLCS30R14	Positive
W19UCLA1091BLCS30R15	Negative
W19UCLA1091BLCS30R16	Positive
W19UCLA1091BLCS30R17	Positive
W19UCLA1091BLCS30R18	Positive
W19UCLA1091BLCS30R19	Positive
W19UCLA1091BLCS30R20	Negative

Gram Stains - N_2-BAP Isolates

Isolate	Gram Status
W19UCLA1091BLCS30N01	Positive
W19UCLA1091BLCS30N02	Mixed
W19UCLA1091BLCS30N05	Negative
W19UCLA1091BLCS30N09	Positive
W19UCLA1091BLCS30N10	Inc.
W19UCLA1091BLCS30N11	Negative
W19UCLA1091BLCS30N12	Positive
W19UCLA1091BLCS30N13	Negative
W19UCLA1091BLCS30N14	Mixed
W19UCLA1091BLCS30N17	Positive
W19UCLA1091BLCS30N18	Negative

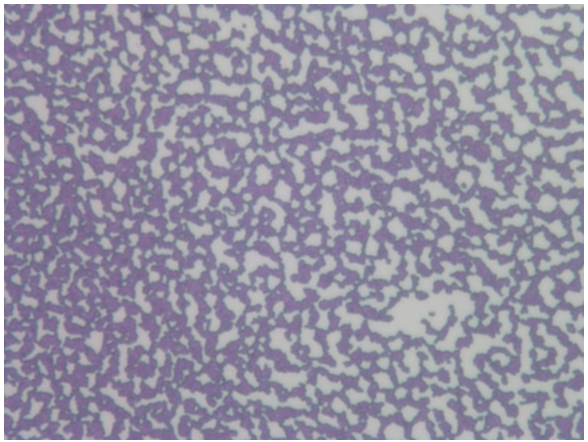
CUMULATIVE RESULTS FOR ISOLATE GRAM STAINS

	R2A	N2-BAP
Positive	11	3
Negative	9	4
Mixed	0	2

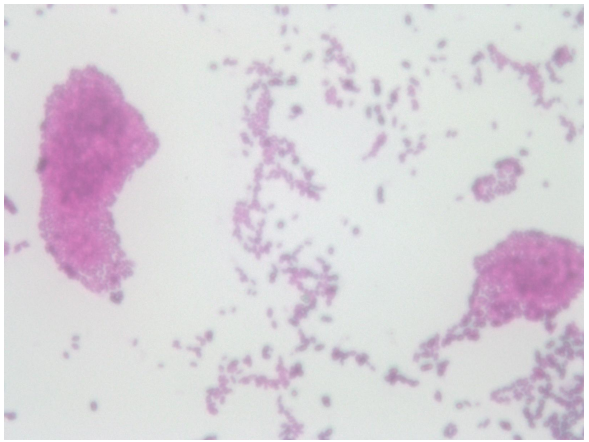
* N2 Isolate #10 not included as gram stain inconclusive

Isolations + Purifications - Gram Stains

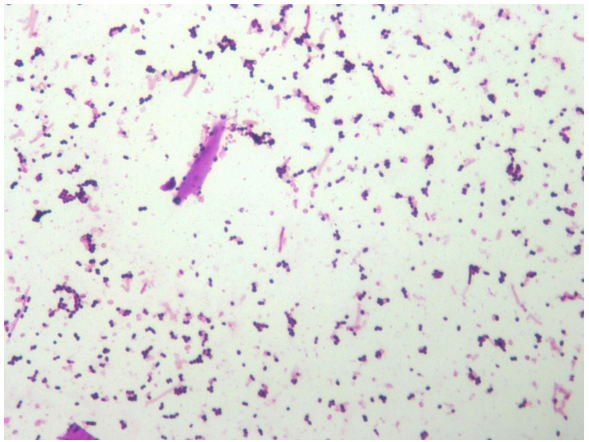
R2A Isolate #19
(Gram Positive)



R2A Isolate #15
(Gram Negative)



N2-BAP Isolate #2
(Gram Variable)



Siderophore Assay

R2A isolate number	trial 1	trial 2	trial 3
1	no	no	yes
2	no	no	no
3	yes	no	no
4	yes	yes	yes
5	yes	yes	yes
6	yes	yes	yes
7	no	yes	yes
8	no	no	yes
9	no	yes	yes
10	no	yes	yes
11	no	no	yes
12	no	no	yes
13	no	no	yes
14	yes	yes	yes
15	yes	yes	yes
16	yes	no	yes
17	no	no	yes
18	no	no	yes
19	no	yes	no

Siderophore Assays were done on 19 R2A ioslates (#1-19) for 3 trials

Table shows isolates per trial

Siderophore production is indicated by color change:

- Yes -> color change
- no -> no color change

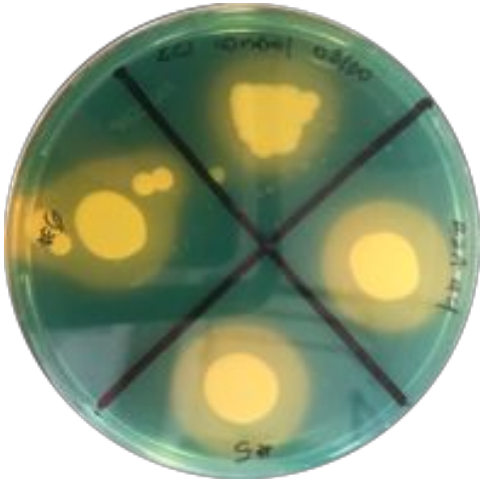
Only 6 R2A isolates were consistent throughout the 3 trials

Siderophore Assay

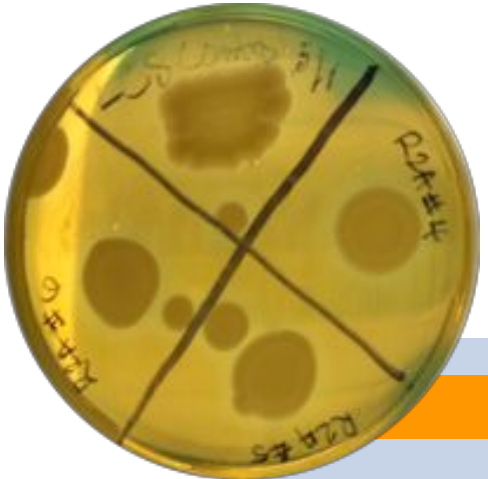
The amount of Siderophore produced varied between isolates and trials (indicated by size of color change region)
CAS plates for R2A isolates 4, 5, and 6

Trial 1: R2A Isolate 4-6

Trial 2: R2A Isolate 4-6



Trial 3: R2A Isolate 4-6



Cellulose Assay - Xylan (R2A Isolates)

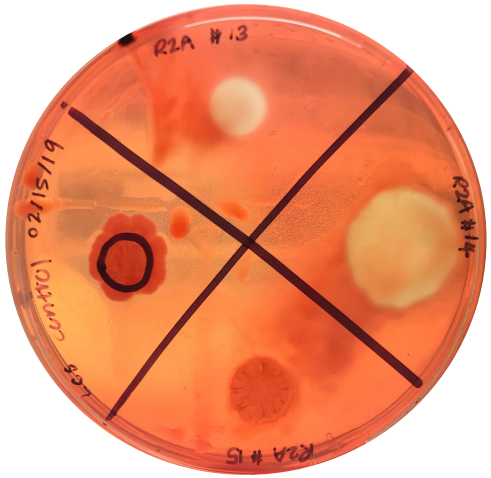
Xylan - R2A Isolates			
Isolate	**Trial 1**	**Trial 2**	**Trial 3**
W19UCLA1091BLCS30R01	Positive	Negative	Positive
W19UCLA1091BLCS30R02	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R03	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R04	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R05	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R06	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R07	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R08	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R09	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R10	Negative	Positive	Negative
W19UCLA1091BLCS30R11	**Negative**	**Negative**	**Negative**
W19UCLA1091BLCS30R12	Negative	Positive	Positive
W19UCLA1091BLCS30R13	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R14	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R15	Negative	Positive	Positive
W19UCLA1091BLCS30R16	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R17	Negative	Positive	Positive
W19UCLA1091BLCS30R18	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R19	**Positive**	**Positive**	**Positive**
W19UCLA1091BLCS30R20	Inc.	Negative	Positive

XYLAN CUMULATIVE RESULTS FOR R2A ISOLATES

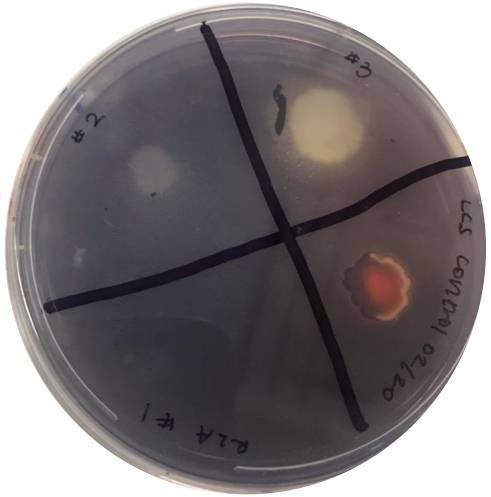
Positive	13
Negative	1
Mixed/Inc.	6

Cellulose Assay - Xylan (R2A Isolates)

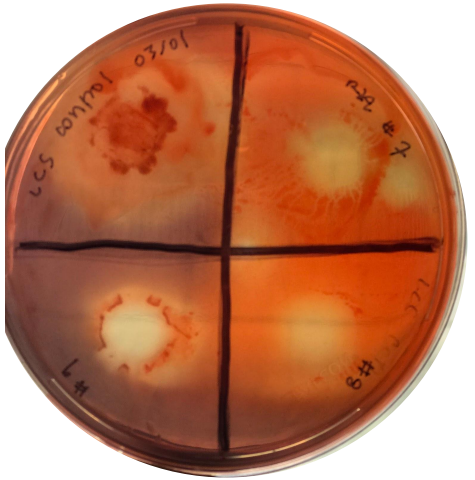
The consumption of xylan varied between isolates and trials (indicated by size of color change region)



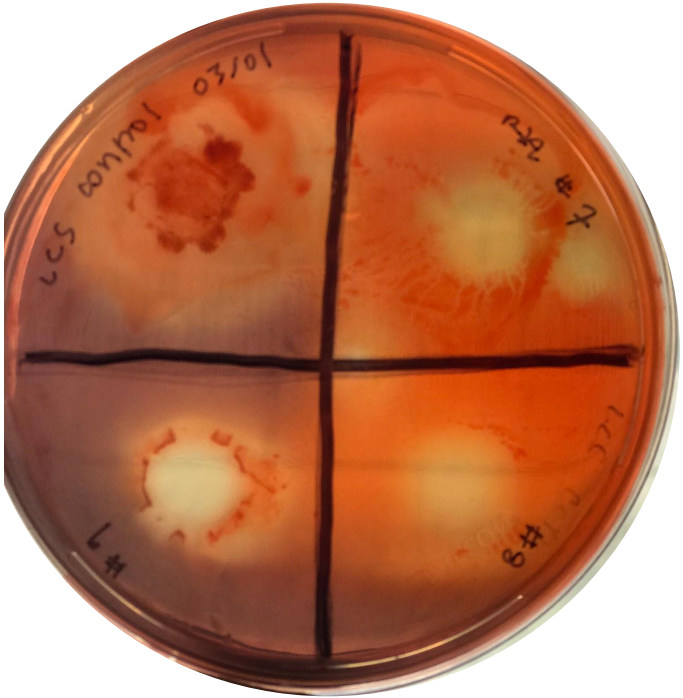
Trial 1: Isolates 13-15



Trial 2: Isolates 1-3



Trial 3: Isolates 7-9


#7
#9

Cellulose Assay-Cellulose degradation (TY+CMC)

R2A isolate number	trial 1	trial 2	trial 3
1	negative	negative	negative
2	positive	positive	positive
3	positive	positive	positive
4	negative	positive	positive
5	negative	positive	positive
6	positive	positive	positive
7	positive	positive	negative
8	positive	negative	negative
9	negative	positive	positive
10	negative	positive	positive
11	positive	negative	positive
12	negative	positive	positive
13	negative	positive	positive
14	negative	positive	positive
15	negative	positive	negative
16	negative	positive	positive
17	negative	positive	negative
18	negative	positive	positive
19	positive	positive	positive

Cellulose assay done on all R2A isolates (1-19)

Expected Results:

If isolates degrade cellulase, then we will see a zone cl
Congo Red dye is added.

Table shows R2A isolates per trial

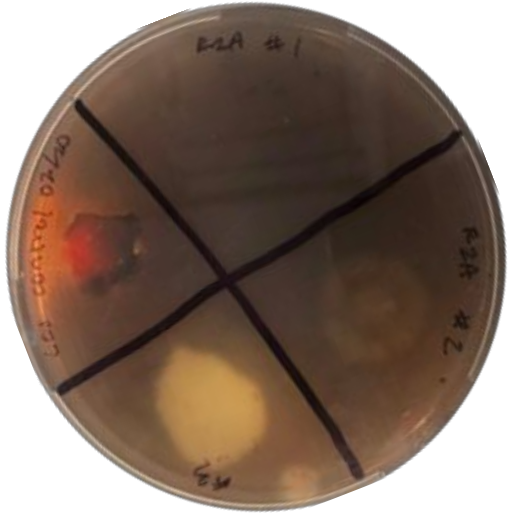
- Positive = there were zone clearings around isol
- Negative = no zone clearing

Cellulose Assay-Cellulose degradation (TY+CMC)

R2A isolates 1, 2, and 3 spot inoculated on TY+CMC plates for trials 1, 2 and 3

Trial 1

Trial 2



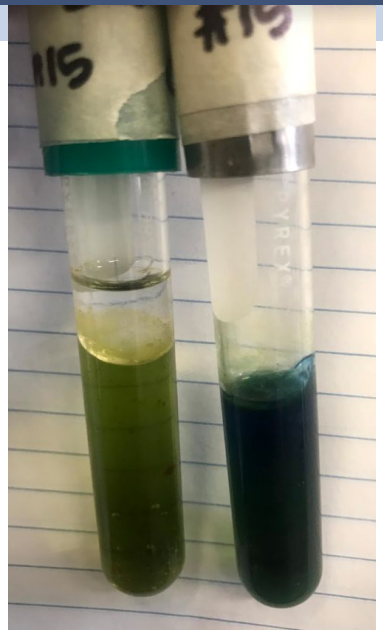
Trial 3

OF Assay of R2A Isolates

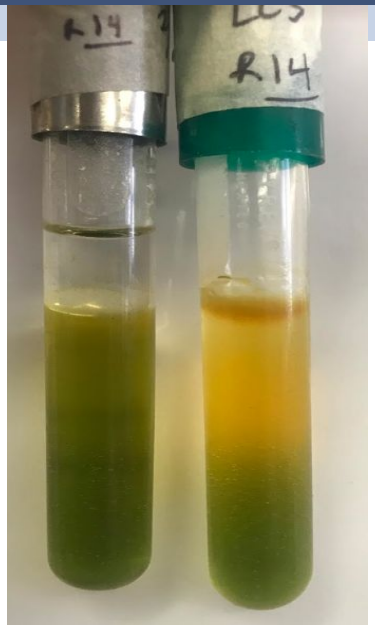
- The OF test is designed to differentiate bacteria on the basis of fermentative or oxidative metabolism of carbohydrates.

- In this medium, aerobic organisms oxidize the carbohydrate to CO_2, H_2O, and energy.

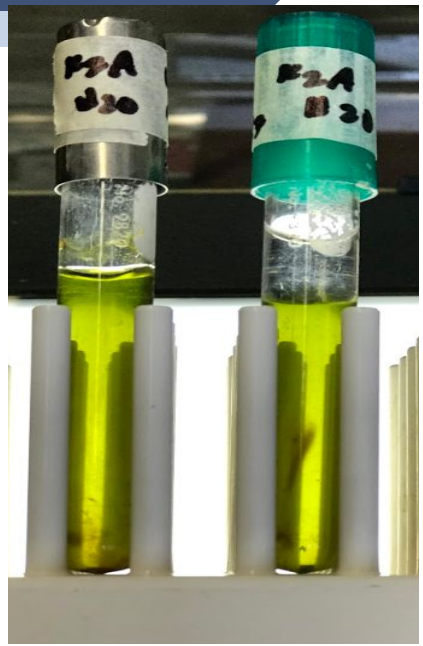
OF Assay



Trial 1 of R2A Isolate #15.
Indicates Peptone Production

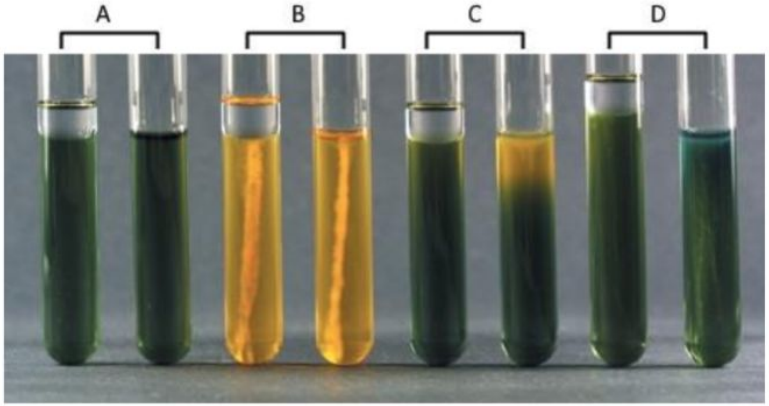


Trial 2 of R2A Isolate #14.
Indicates Catabolism of Carbohydrates in the Presence of Oxygen



Trial 3 of R2A Isolate #20.
No Fermentation took place

OF Assay



A: No Fermentation Took Place
B: Fermentation Took Place
C: Catabolism of Carbohydrates in the Presence of Oxygen
D: Peptone Production Took Place

OF ASSAY TABLE: Shows the status of each isolate of the OF assay over three trials

R2A ISOLATE NUMBER	TRIAL 1	TRIAL 2	TRIAL 3
1	A	A	A
2	A	A	A
3	A	A	A
4	C	C	C
5	C	C	C
6	A	A	A
7	A	A	A
8	A	A	C
9	A	A	A
10	A	A	A
11	C	A	C
12	A	A	A
13	A	A	A
14	C	C	C
15	D	D	A
16	A	A	A
17	A	A	A
18	A	A	A
19	A	A	A
20	A	A	A

TABLE KEY:

A: No Fermentation Took Place

B: Fermentation Took Place

C: Catabolism of Carbohydrates in the Presence of Oxygen

D: Peptone Production Took Place

Letters highlighted in **RED** indicate they do not match the other trials

OF Assay

PIE CHART KEY:

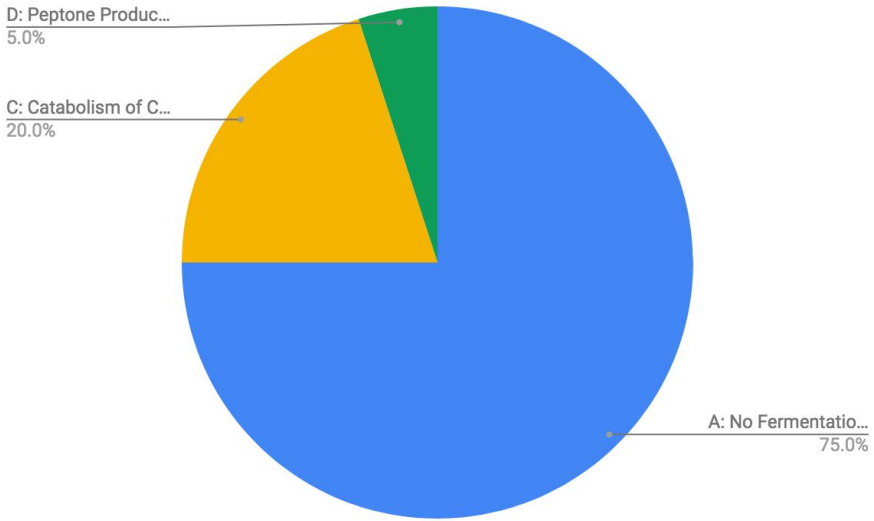
A: No Fermentation Took Place

B: Fermentation Took Place

C: Catabolism of Carbohydrates in the Presence of Oxygen

D: Peptone Production Took Place

PIE CHART OF OF ASSAY RESULTS



Nitrogen-Fixation Assays

Assays done on isolates from N2-BAP

Each table shows the nitrogen fixing capabilities of the N2-BAP isolates per trial. Tables show the presence of nitrogen fixing indicated by by growth (cloudiness) (YES) or no nitrogen fixing present (NO) or VARIABLE meaning nitrogen fixing status could not be determined

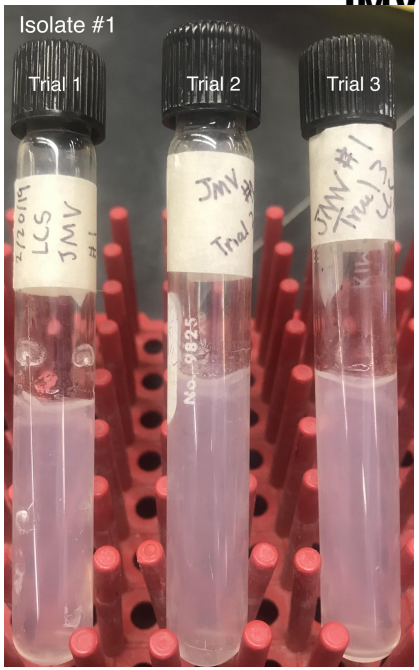
JMV			
Isolate	Trial 1	Trial 2	Trial 3
1	yes	yes	yes
2	yes	yes	yes
5	yes	yes	yes
9	yes	yes	yes
10	yes	yes	yes
11	yes	yes	yes
12	yes	yes	yes
13	yes	yes	yes
14	yes	yes	yes
17	yes	yes	yes
18	yes	yes	yes

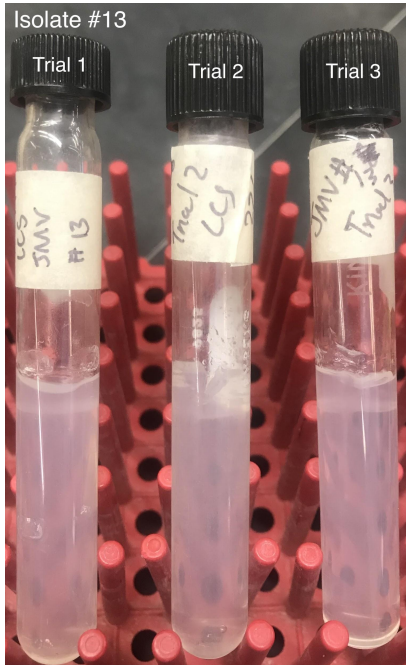
DF			
Isolate	Trial 1	Trial 2	Trial 3
1	yes	yes	yes
2	yes	yes	yes
5	yes	yes	yes
9	yes	yes	yes
10	yes	yes	yes
11	yes	variable	yes
12	yes	yes	yes
13	yes	yes	variable
14	yes	variable	yes
17	yes	yes	yes
18	yes	yes	yes

Nitrogen-Fixation Assays

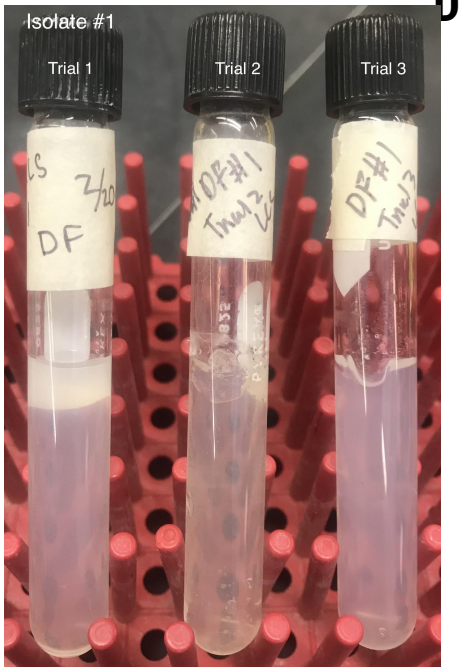
Assays done on isolates from N2-BAP

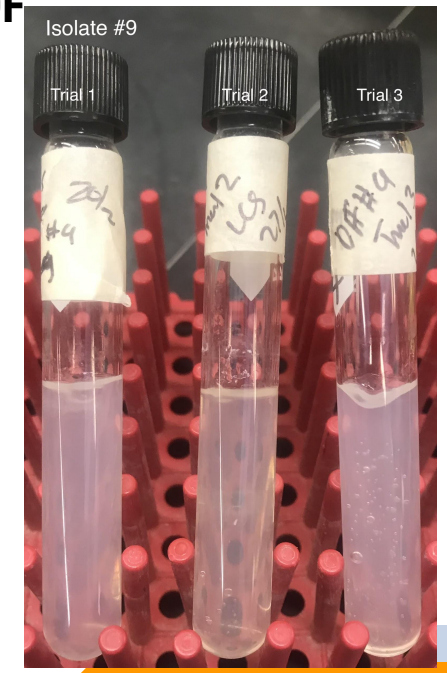
JMV





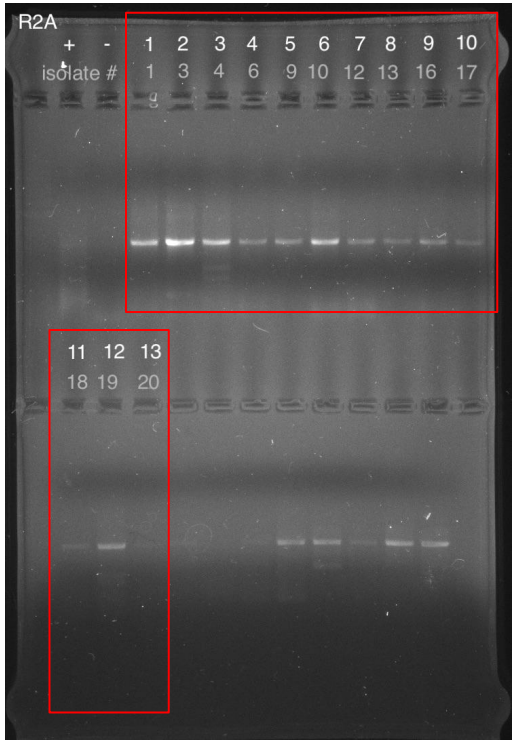
DF





PCR

- **R2A:** 18 isolates sent out for sequencing
- **N2-BAP:** 11 isolates sent out for sequencing



DISCUSSION OF RESULTS

- Most data supports our hypothesis
  - Nitrogen Fixing Bacteria?—**YES**
  - Oxidative Bacteria?—**YES**
  - Cellulolytic Activity in Bacteria?—**YES**
- Big Picture:
  - We do not know who is there...YET⟶ Know more in BL
  - BUT we mostly know what they are doing

Bias and Significance of Results

- Bias
  - Soil
  - Cultivation
  - Isolates
  - Assays
  - PCR
  - Databases
- Significance
  - Bioremediation
  - Climate Change
  - New Species

5

FUTURE DIRECTION

Continuation of Research

Next Quarter

109BL-S19

- Comparison of two environments
  - Healthy Sumac
  - Recovering Sumac
- 16S PCR Data
- Phylogenetic Tree

Analysis

- Comparison of
  - Composition
  - Function
  - Biomass (Bonk et al. 2018)

https://doi.org/10.1016/j.mimet.2018.a09.015

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https://doi.org/10.1016/j.mimet.2018.09.015