Purpose of research 3 sept 7

Identifying Research topic

- → Bacterial necrosis in saguaro population
- → Application of plant extracts to compare inhibition to antibiotic streptomycin
- → Environmental resistance
  - starA

Research questions

→ Will the extracts of different ayurvedic products exhibit antimicrobial potential against cactus necrosis?

→ Which of the ayurvedic products will exhibit the highest antimicrobial potential?

Sept 12

Background Research

Saguaro Cacti

- largest in Sonoran Desert
- life span 125 – 300 yrs
- When deformed / diseased it is no longer an asset and may present danger to collapse
- Not troubled by many pests / disease - However rot disease (bacterial necrosis) is common cause of death.

Bacterial Problems

- Rot disease prevelant throughout its population
- Use of antibiotics has lead to drug antibiotic resistance.

Bacterial Necrosis (Rot disease)

- The catastrophic freeze in 1937 was determined as the real culprit.
- Took yrs. for impact of the freeze to be evident as rotting and collapsing cacti.
- caused by bacterium Erwinia Carotuda and other species/ Erwinia cainaeanna (rejected)

Causes

1. Bacterial colonization (surface)
2. Inner tissue becomes water-soaked (brown/black in color) as it progresses inside.
3. Area enlarges and takes purple coloration.
4. External tissue dry and begins to break up into pieces as necrosis progresses internally.

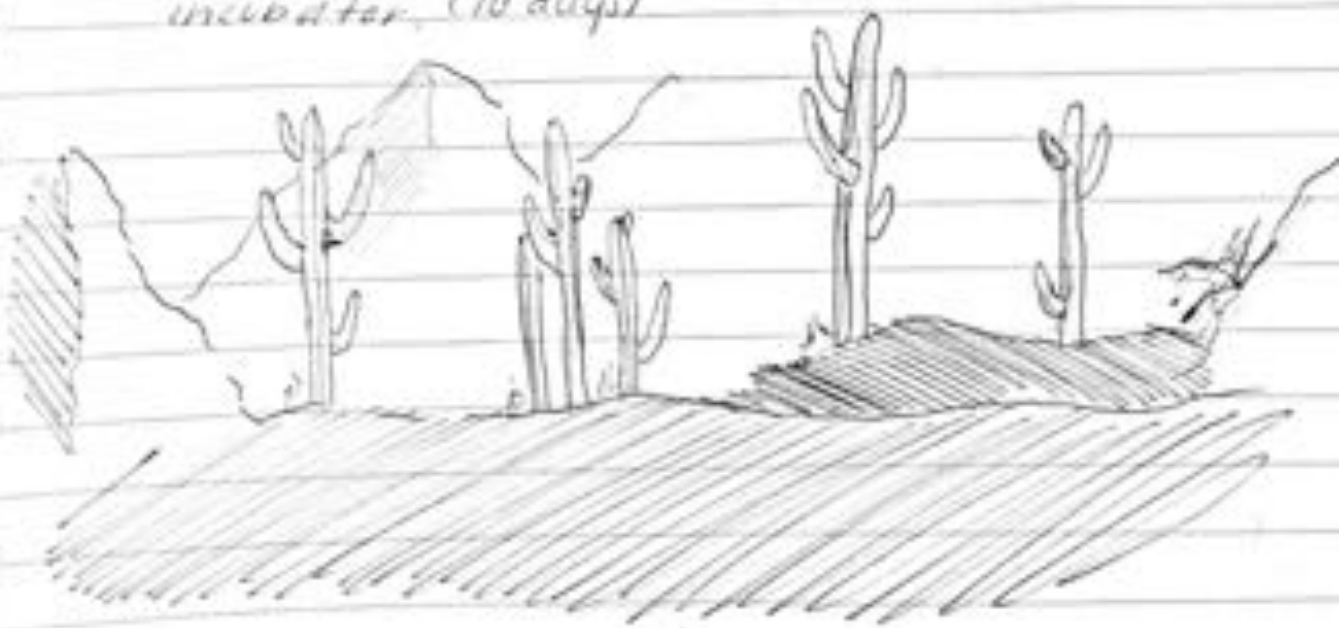
Final stage of sagauro cactus disintegration as tissue begins to decay.

Pre-Experimental Procedures.

Field Work. (November 6, 21)
Reserva de la Biosfera del Pinacate
Gran Desierto de Altar

→ Area of 62,500 m^2 (approx) was explored and analyzed prior to ~~data~~ sample collection.
- approx 80% of cacti were infected / sick

→ Prior to sample collection materials were sterilized w/ hypochlorite solution (1:9)
→ 4 cactuses were selected.
→ Incision was made, with a cotton swab (sterile) of tissue (water-like/moist) sample was streaked in the Miller-Hinton agar plate.
→ Parafilm to seal ~~dishes~~ plates.
→ transported to lab and stored in an incubator. (10 days)



Experimental Procedures

Bacterial Dilution Procedures

→ 200ml distilled water w/ saline packet solution (magnetic stirrer to create isotonic solution (bacteria grew better in isotonic solution))

→ 2ml of previously prepared solution was poured into test tube.

→ Using an inoculating loop (sterile (bunsen burner)) bacterial colonies were collected and integrated using a mixer to dissolve and create a homogeneous concentration. (test tube)

→ Using sterile cotton swab solution was streaked into new MH agar plates.
→ Plates were incubated at 37 °C.

Experimental Procedures

Preparation of antibiotic paper disks.
(Dec 10, 21)

→ Obtaining plant extracts
- Oregano, garlic, clove, and cinnamon were separately soaked in 50ml of ethanol.

→ (Dec 14, 21) Solutions were poured into the rotary evaporator flask (conditions: 60°C (boiling point of ethanol) at 100 rpm (standard))

(Dec 14, 21)
(Procedures for bacterial ~~count~~ Dilution preparation were repeated)
- saline solution in 200ml distilled water
- bacterial colonies ~~traded~~ incubated
- test tube were placed in vortex to dissolve bacteria into saline solution (Following McFarland Standard in preparing final bacterial dilution - 1.5×10^8 CFU/mL (checked using Wickerham card))

Paper disks were soaked in the plant extracts and streptomycin:
1) oregano extract ONLY
2) garlic extract ONLY
3) clove extract ONLY
4) Cinnamon extract only
5) (control) Streptomycin ONLY

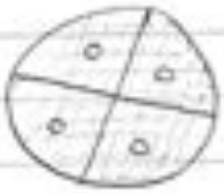
Experimental Procedures

Kirby- Bauer Sensitivity Assay

(Dec 14, 21)

→ Preparation of paper discs. Soaked 10mg of extracts and streptomycin.

→ Inoculate Mueller- Hinton plates w/ prepared bacterial cultures using sterile cotton swabs.

→ 

- paper discs were placed onto the inoculated MH plates.
- Placed in the incubator overnight

→ (Dec 15, 21) zones of inhibition were measured and recorded using a digital caliper.

Data Collection

Data collected was compared and analyzed

Data Collection Dec 15, 21

Oregano	B1	B2	B3
T1	24.9	21	13.7
T2	19.9	21.4	16.7
T3	27.4	22.4	23
T4	22.15	21.9	19.1
~~Garlic~~ Control (Streptomycin)			
T1	13.9	14.7	24.6
T2	9.4	19.1	26.5
T3	0	15.3	25.2
T4	12.6	16	20.6
Clove			
T1	16.4	19	19.5
T2	17.3	15.1	15.7
T3	16.1	13.8	11.5
T4	23.2	12.2	20.4
Cinnamon			
T1	~~19.4~~ 15.8	14.3	15
T2	~~9.4~~ 16.7	14.9	22
T3	~~0~~ 12.8	9	18
T4	19.5	15.3	19.3
Garlic			
T1	24.9	11.1	16.9
T2	25.2	11.2	17.6
T3	20.9	13.3	18
T4	20	11.7	15.9

Result Stats

Sample 1

	Avg zone of inhibition	t-test
Oregano *	24.15	0.033
Garlic *	22.6	0.016
Clove *	18.25	0.042
Cinnamon	16.2	0.408
Control (streptomycin)	8.85	

Sample 2

Oregano *	21.67	0.0147
Garlic *	11.95	0.0495
Clove	15.025	0.564
Cinnamon	13.375	0.1395
Control	16.275	

Sample 3

Oregano	18.13	0.0509
Garlic *	17.1	0.0039
Clove	16.78	0.8924
Cinnamon *	17.95	0.0054189
Control	24.25	

* denotes significance (significant difference between treatment and control ($p < 0.05$)(paired t-test))

DNA TEST
(Week of 7/Feb 22)

→ Selected 3 clones from each plate (predominant)

→ Ran a Polymerase chain reaction test (PCR). Copy of genom - 16 ribosomal DNA. Short sequence (600 base pairs)

→ Take results and placed in machine that reads nucleotide

→ sequences into a data base

- 2 different sequencesets for / reverse

Analysis of Results
(DNA subway)

→ Determine sequence Relationship

→ Selected 16S

→ Import Dr. Anderson 2nd file

→ Select all samples

→ Trim the sequences (remove N's)

→ Use Blast → NCBI stores sequences that have been published

→ Results of bacteria w/ similar / exact same sequence.

→ Blast each sequence and look at mismatches (analyze sequence viewer to choose most liable sequences)

Matches (DNA subway) 9/Feb/21

name

1a F - no sequence

1a R - no sequence

1b F - Enterobacter sp / Bacillus sp.

* Bacteria that apparently benefits plants found in soil (mineralized and protects) involved in Plant growth.

1b R (Viable) (quality sequence)

2a F - Micrococcus sp. (Viable) (quality sequence)

2a R - Micrococcus sp

2b F

2b R - R Planococcus sp.

3a F - Bacillus sp (in Bacteria) / Chryseomicrobium imtechese

3a R - Chryseomicrobium (more viable ?)

3b F - no sequence

3b R - Protein fusion vector

2c F - Isoptericola variabilis strain (Viable)

2c R - Isoptericola variabilis strain

* Observation

1b R → Pseudochrobactin vulneris
→ klebsiella grimontii
→ Unimicrobial resistance
E. Coli → Enterobacter
→ Escherichia