Introduction

- Lichens are complex symbiotic structures that emerged from a symbiotic relationship between an alga (photobiont) and fungus (mycobiont)
- Can grow on a variety of substrates: plastic, rubber, metals, and glass.
- Environmentally important bioindicators for pollution (Ardelean, Keller, Scheidegger 2015)
- Low metabolic activity -> inhabit majority of habitats and substrates & act as pioneer organisms
- 3,600 species known in North America -> 18,000 species worldwide
- Studies have shown that lichens have shown to be a new supply of antibiotics and they contain a wide range of biologically active chemicals, most of which have antimicrobial properties. (Sierra, M. A., et al. 2020)
- Because of the high demands of antibiotics, medical fields are running out of antibiotic resources.

Rationale

- The goal that is to be achieved for this study is to evaluate the extent of antimicrobial properties of varying lichen extracts have on various strains of bacteria.
- Applying what is done in this study to medical sciences to hopefully benefit and aid in the discovery of new antibiotic technology.
- Using the following methods, obtaining an understanding in the potential antimicrobial effects that lichens posses and how they can help cease or prevent the reproduction of harmful bacteria and it's resistance to antibiotics.

Problem Statement

The rising antimicrobial resistance of pathogenic organisms towards medical antibiotics.

Materials

DNA Barcoding
- Lichen extracts
- Microcentrifuge tubes
- Centrifuge
- Micropipettes
- Chelex, Silica, and Rapid protocol
- PCR machine and protocol
- Gel electrophoresis gel and chamber
- TBE buffer

Kirby Bauer Disk Diffusion
- Lichen extracts
- Microcentrifuge tubes
- Centrifuge
- Micropipettes
- Beakers
- Methanol
- Bacterial strains
- Agar and plates
- Paper disks
- Filter paper

Hypothesis

H0 (null): Lichen extract and bacterial growth of *E. coli K-12* and *S. epidermidis* occurs independently of each other.

H1 (alternative): *E. coli K-12* bacterial growth is dependent on the presence of lichen extract.

H2 (alternative): *S. epidermidis* bacterial growth is dependent on the presence of lichen extract.

Statistical Analysis

- Used R-value to measures statistical strength between variables.
- 1 = very strong positive correlation
- -1 = very strong negative correlation
- *E.coli K-12* having an r value of 0.59 -> positive moderate correlation
- *S.epidermidis* having an r value of 0.30 -> positive weak correlation

Discussion

Kirby Bauer Disk Diffusion:
- Extract was moderately effective at inhibiting *S. epidermidis* and mildly effective at inhibiting *E.coli K-12*.
- 50% concentration was the most effective.
- The high standard deviation of the zone of inhibition of *S.epidermidis*.
  Limitations/Difficulties:
  - Unable to test on other pathogenic strains due to safety restrictions.
  - The pure lichen extract was viscid and was difficult to apply to paper disks for Kirby Bauer test.

DNA Barcoding:
- Samples A1 and A2 had successful barcodes.
- Sample A3 had inconclusive results -> species: unknown.
- ITS primer is compatible with lichens DNA.
  Limitations/Difficulties:
  - The Silica DNA isolation method was extensive and could easily leave room for human error.
  - Rapid DNA isolation was not thorough.

Conclusion

Disk Diffusion Analysis:
- Weak correlation between antimicrobial activities in *S. epidermidis* with an r value of 0.30.
- Weak correlation between antimicrobial activities in *E. coli K-12* with an r value of 0.59.
- P value for *S.epidermidis* = .04 -> Reject null hypothesis.
- P value for *E.coli K-12* = .23 -> Accept null hypothesis.

DNA Barcoding Analysis:
- ITS fungal primer was successful in PCR amplification.
- The rbcL primer was not compatible with the lichens genetic code.

  Sample A1 lichen species: *Physcia biziana*

  Sample A2 lichen species: *Physcia stellaris*

  Sample A3 lichen species: Unknown

Future Research

- Using the conclusions of this study, medical field specialists can possibly create new antibiotics using lichens or other natural organisms.
- Analyzing the effectiveness of varying lichen species on preventing bacterial growth.
- How would different methods of suspension of lichen extracts affect growth of inhibition.
- Varying pathogenic strains on lichen species.
- Testing lichens from varying environmental conditions to evaluate atmospheric pollution.

Exploring Antimicrobial Properties of Lichen Extracts

Methodology

Lichen Sample Collection

Sample A1: *Physcia biziana*
GPS coordinates:
40.59459 N, 73.63543 W

Sample A2: *Physcia stellaris*
GPS coordinates:
40.59459 N, 73.63466 W

Sample A3: Unknown
GPS coordinates:
40.59093 N, 73.65985 W

Figure 1
Photographs of samples A1, A2, and A3 used in Kirby-Bauer disk diffusion test.
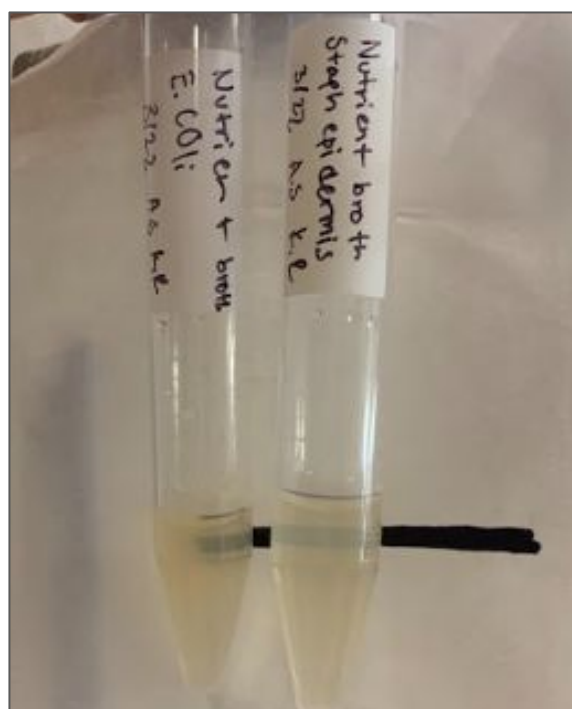
Antimicrobial Lichen Extract

Extract	A1	A2	A3
Concentration	100%*	100%*	100%*
	50%	50%	50%
	25%	25%	25%
	0*	0*	0*

Figure 2
Bacterial species E.coli K-12 and S.epidermidis.

Figure 3
*100% *undiluted extract was scraped after evaporation*
*0 * pure methanol (control)*

Kirby Bauer Disk Diffusion Set-Up
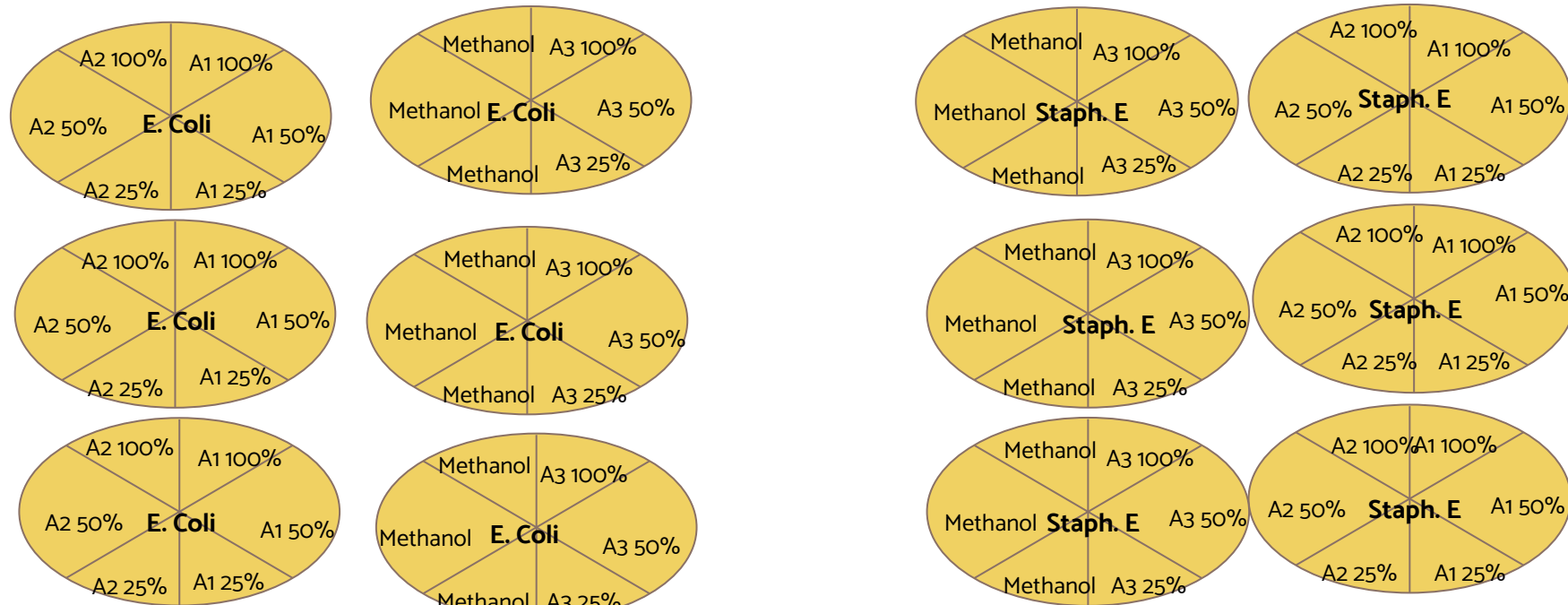
Figure 4
Mueller Hinton Agar plate setup for samples A1, A2, A3 and their designated concentrations.

Gel Electrophoresis
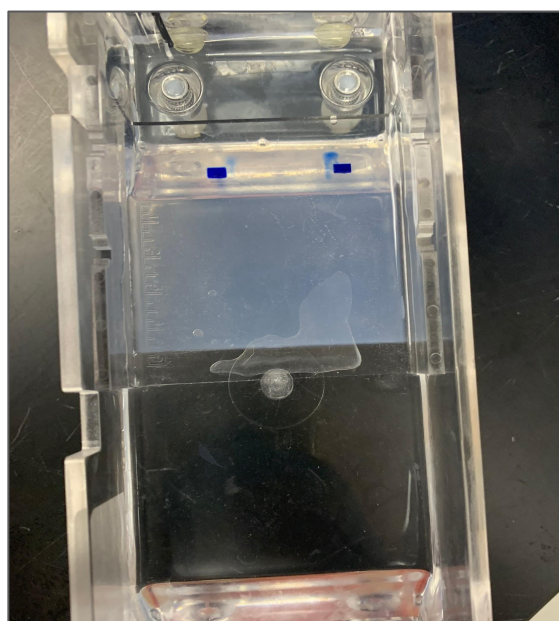
Figure 5
Sample A1 DNA isolated using Chelex loaded in agarose gel wells.
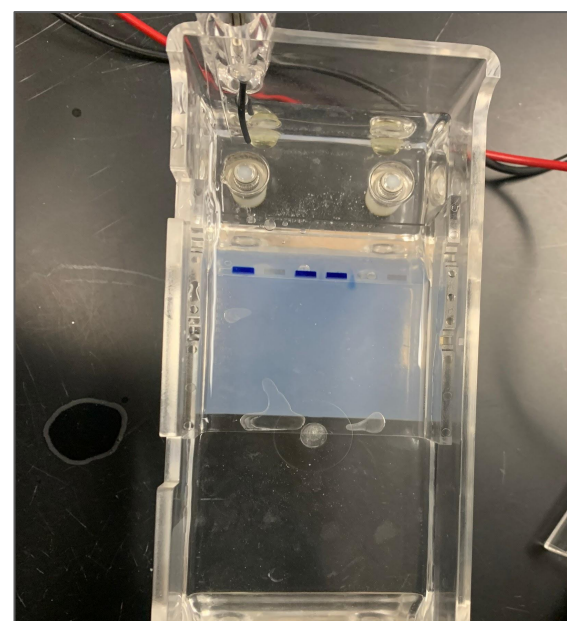
Figure 6
Sample A1 DNA isolated using Silica and Rapid loaded in agarose gel wells.

Data

Zone of Bacterial Growth Inhibition

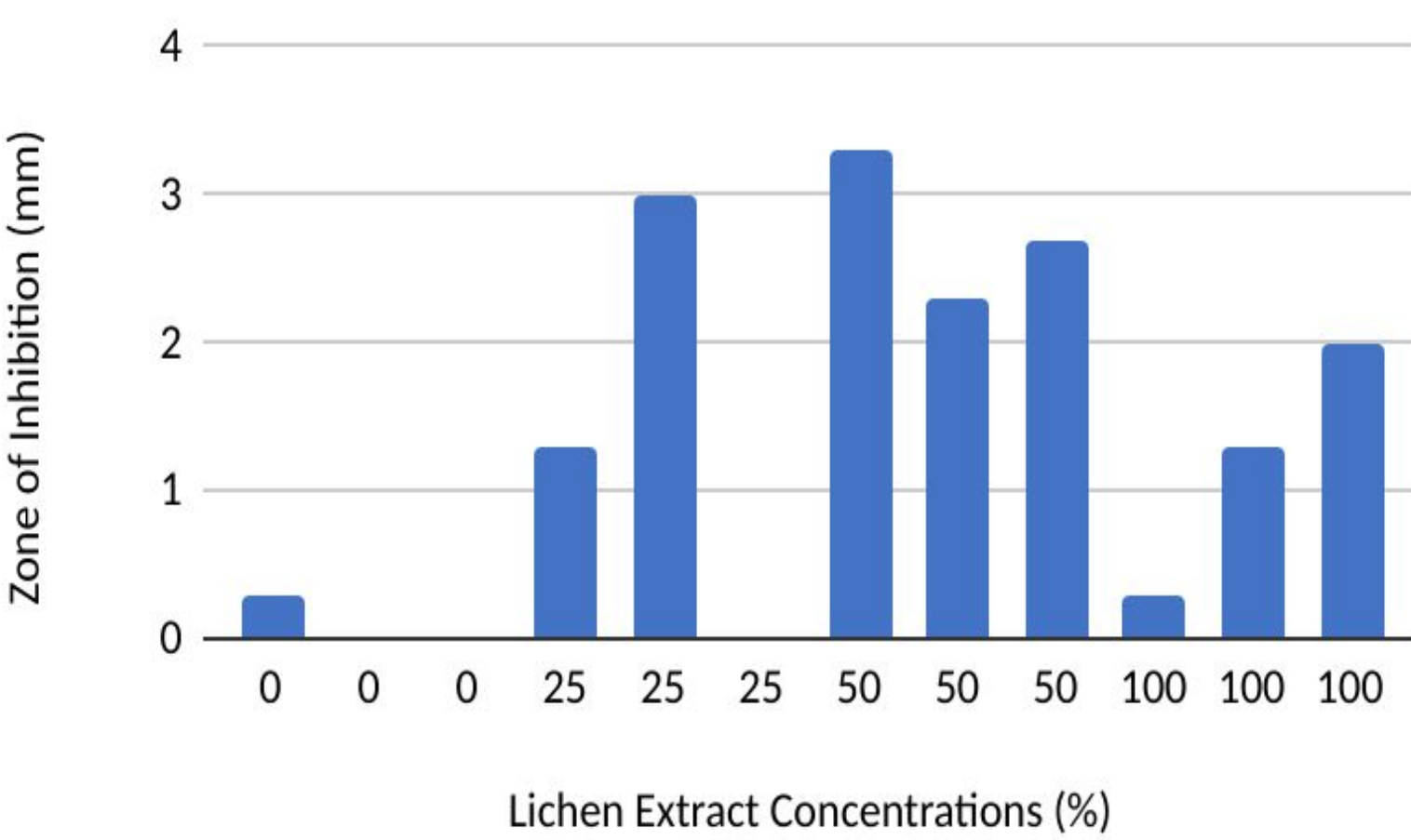Figure 7 *Zone of inhibition for E.coli K-12.*
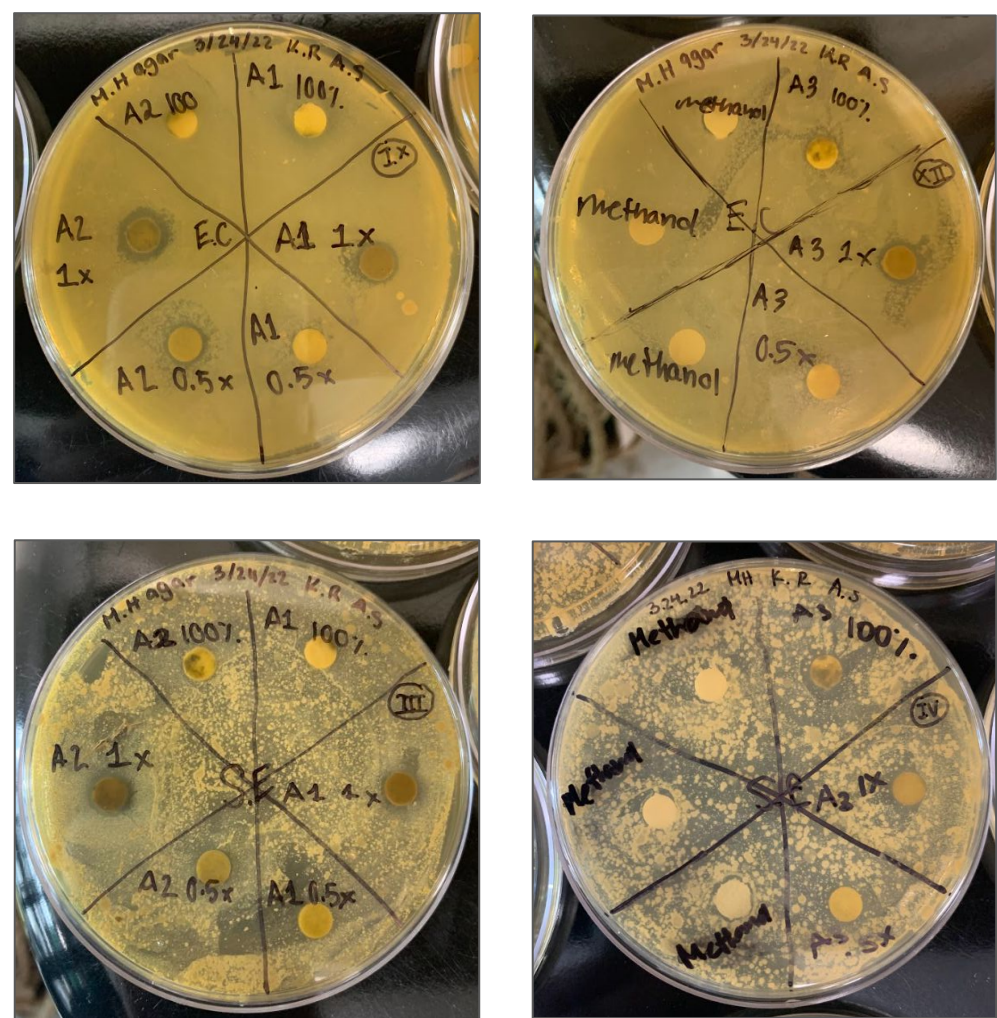

Figure 8 *Zone of inhibition for S.epidermidis.*

Figure 9
Photographs of Mueller Hinton agar plates for each concentration and bacterial strain showing zone of bacterial growth inhibition.

Pearson's Correlation Coefficient

r=0.30
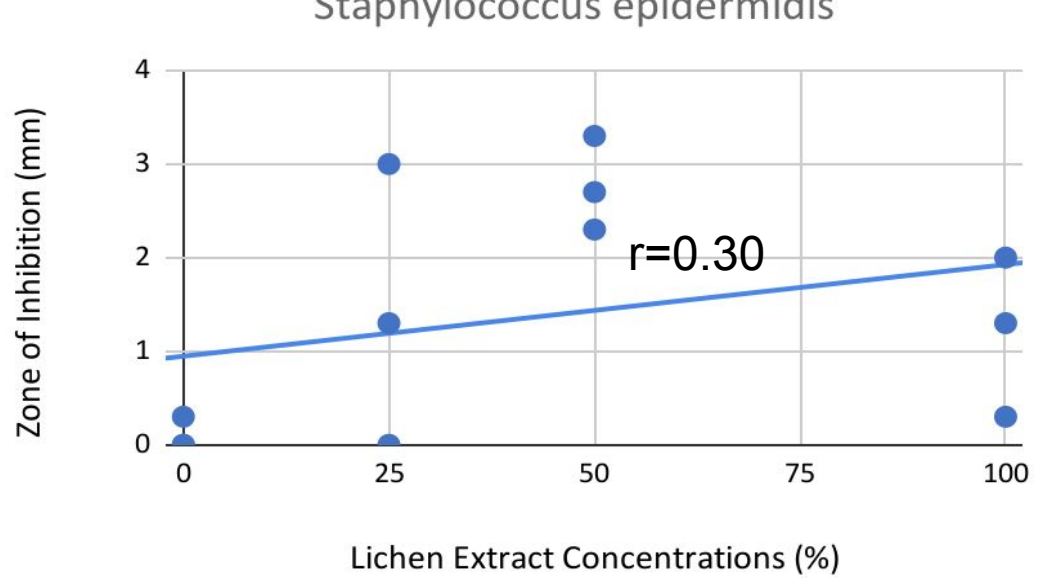
Figure 11
This indicates the weak correlation between the lichen extract concentration and zone of growth inhibition of S.epidermidis.
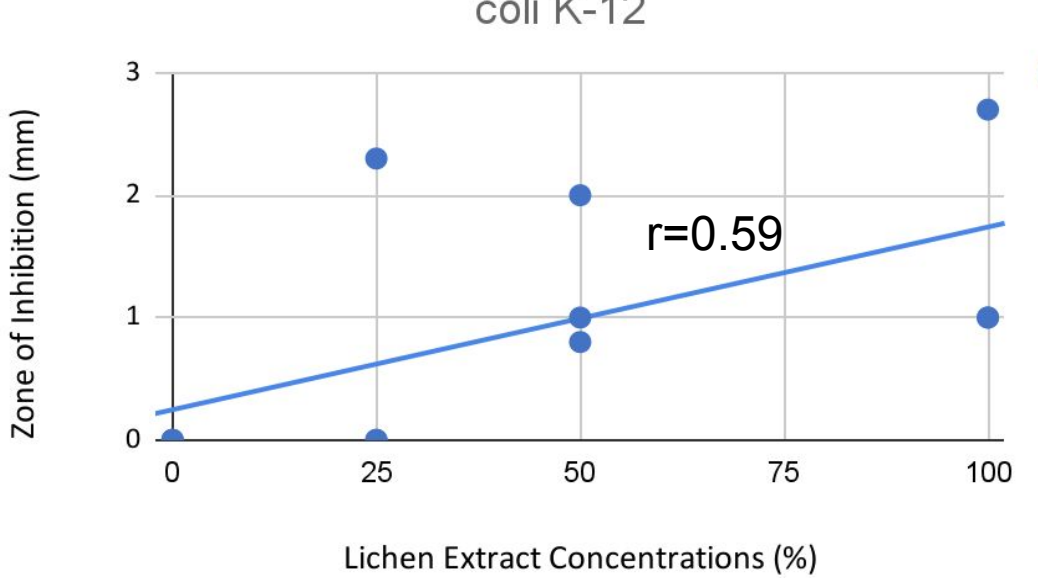

r=0.59

Figure 10
This shows a moderate correlation between the lichen extract concentration and the zone of growth inhibition of E.coli K-12.
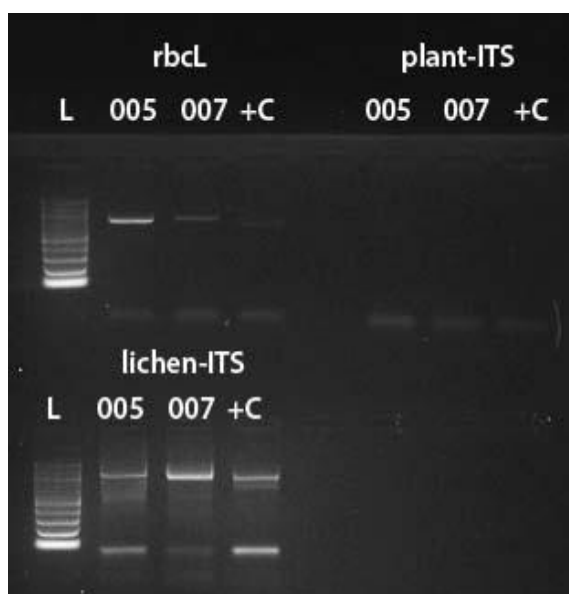
DNA Barcoding Gel Bands

Figure 12
The gel electrophoresis results for sample A1 (007) and sample A2 (005) for the rbcL and ITS primers.
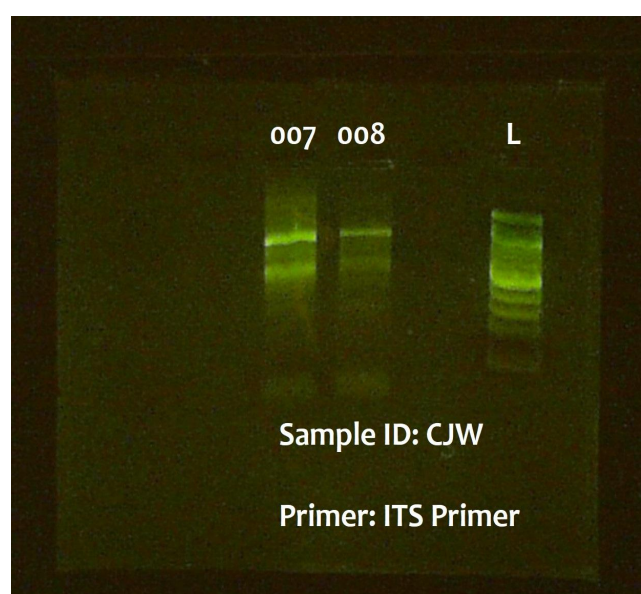
Figure 13
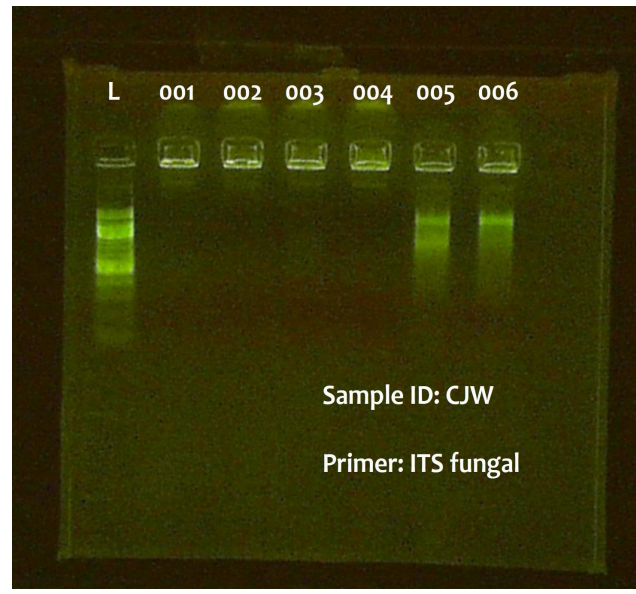*The gel electrophoresis results for sample A1 (007 and 008) using the ITS primer.*

Figure 14
The gel electrophoresis results for samples A2 (005) and sample A3 (006) using the ITS primer.