Biology Fall Quarter Lab Report

Authors

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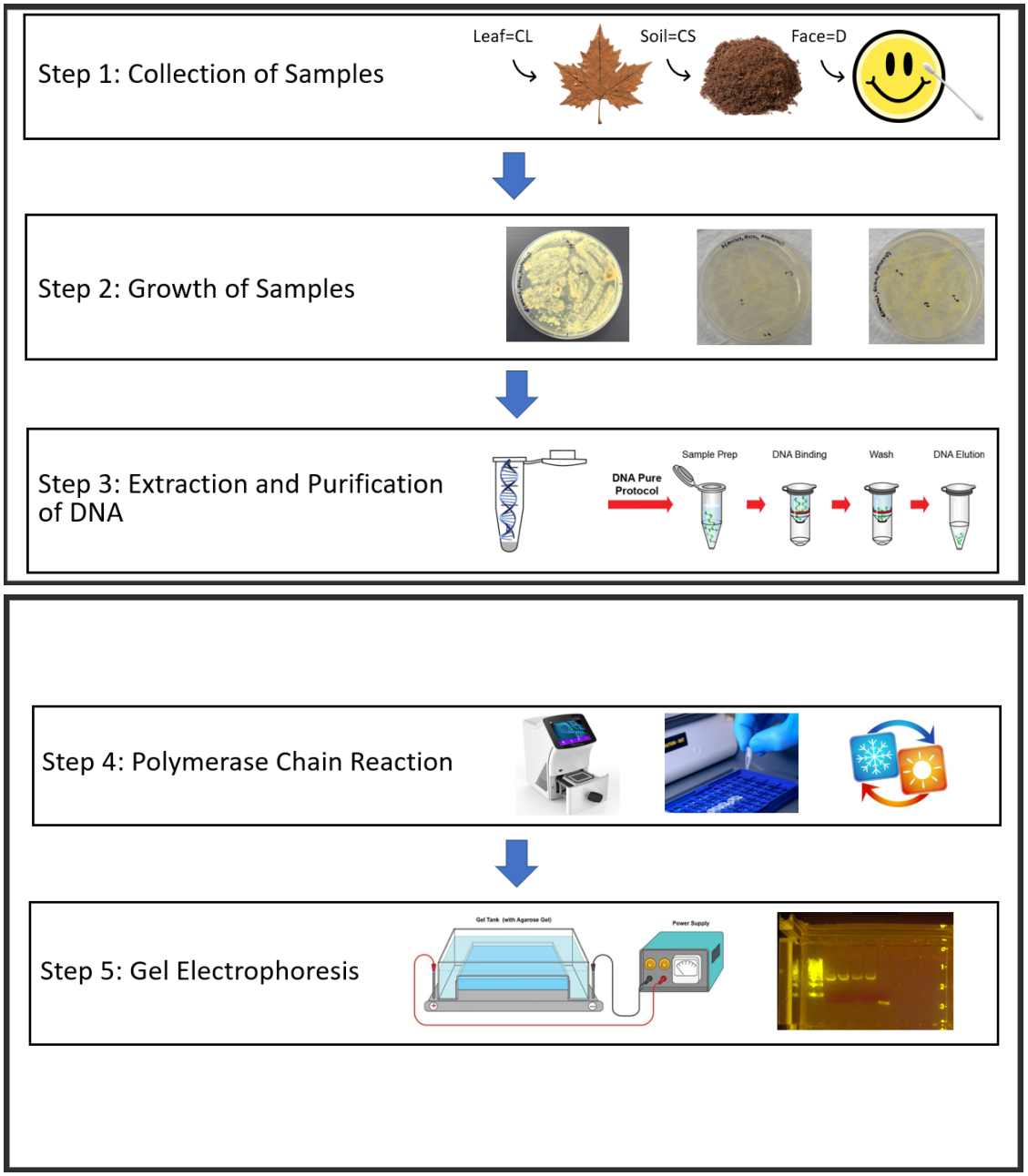
Diagram



Description

Step 1: Collection of Samples	Step 2: Growth of Samples	Step 3: DNA Extraction and Purification	Step 4: Polymerase Chain Reaction	Step 5: Gel Electrophoresis (starting from leftmost well)
CS (soil)	CS*f* (fibrous cell growth, see Figure 4b)	CS*f*	CS*f*	4th well
	CS*l* (light yellow cell growth, see Figure 4a)	CS*l*	CS*l*	2nd well
D (face)	D*y* (dark yellow cell growth, see Figure 4d)	D*y*	D*y*	3rd well
	D*w* (white cell growth, see Figure 4e)	D*w*	D*w*	5th well
CL (leaf)	CL*t* (tan cell growth, see Figure 4c)	CL*t*	CL*t*	6th well

Materials

General Materials:

- Micropipettes (both 200 and 1000 µl variants)
- Micropipette tips (for both variants)
- Underpads
- Paper towel
- 70% Isopropanol alcohol solution
- 50 mL beakers

Collection and Growth of Samples

Materials:

- Sterile inoculation loops
- Cotton-tipped swabs
- 4 Agar petri dishes, one with a layer of Kanamycin

- 1 20g packet of dirt
- 1 leaf
- Distilled water

Purpose of Method: To collect samples to identify the microorganisms and/or growths on those objects and to cultivate the samples' cell colonies.

Procedure:

1. The samples (soil, leaf, and face) were swabbed and mixed with a small amount of distilled water.
2. Sterile inoculation loops were used to streak the three agar test plates while the one with Kanamycin was left untouched as a control.
3. The microbial samples were allowed to grow for four days.

Extraction and Purification of the DNA

Materials:

- 1.5 mL plastic vials
- Solution I for DNA extraction - 50mM Glucose, 25mM Tris-HCl pH 8, 10mM EDTA, H_2O
- Solution II for DNA extraction - 1% SDS, 0.2M NaOH, H_2O
- Solution III for DNA extraction - 3M Na acetate, 2M Glacial Acetic Acid, H_2O
- Dry ice
- Warm water
- Microfuge

Purpose of Method: To isolate and purify the DNA of the cell samples.

Procedure:

1. Samples of differing cell growths (Dy, Dw, CSf, CLt, and CSl) were taken from the agar dishes and placed into vials.
2. These samples were mixed with 300 µl of a solution containing 50mM Glucose, 25mM Tris-HCl with pH 8, 10mM EDTA, and H2O (labelled solution 1).
3. The vials were then incubated by swapping them between dry ice and warm water for three minutes each and for four total cycles.
4. Once incubated, the samples were mixed with 300 µl of a solution containing 1% SDS, 0.2M NaOH, and H2O (labelled solution 2)
5. The samples were left for ~30 minutes.
6. 300 µl of a solution containing 3M Na acetate, 2M Glacial Acetic Acid, and H2O was thoroughly mixed into each sample.
7. The samples were sedimented.
8. After 10 minutes, the supernatant was removed from the sample.
9. 900 µl of isopropanol were then added to each sample.
10. The tubes were shaken to mix the isopropanol in each sample.
11. A microfuge was then used to spin the tubes for 10 minutes.
12. The supernatant was again removed.

13. 500 µl 70% ethanol was added to each sample.
14. A microfuge was then used to spin each for 5 minutes.
15. The supernatant was then carefully removed and each tube was allowed to dry.

Polymerase Chain Reaction

Materials:
- 20 µl DNA primer mix
- 60 µl PCR mix

Purpose of Method: To replicate specific sections of DNA from each cell.

Procedure:
1. Each DNA sample was combined with:
    a. 20µL DNA
    b. 20µL Primer mix, which contained primers for Bacteria (~200 bp), Yeast (~570 bp), and mold (~1.5K bp)
    c. 60µL PCR mix
2. The samples were then run through the PCR process using a thermocycler.
3. The PCR process was repeated 30 times.

Gel Electrophoresis

Materials:
- 10 µl loading dye
- Agarose gel electrophoresis setup
    - Agarose Gel
    - Power source
    - Gel electrophoresis machine

Purpose of Method: To separate the lengths of DNA to identify the types by length.

Procedure:
1. 50 µL of each PCR reaction sample was combined in a different PCR tube with 10 µL of the loading dye.
2. Each mixed product had 10 µL taken out and loaded into an agarose gel setup, along with a DNA ladder.
3. Around 30 minutes of time was allowed for the gel to properly run.
4. Pictures of the finished gel electrophoresis were taken and the number of base pairs in each sample was measured.

Results

While all samples exhibited feeding by bacteria, visibly through yellow, tan, and whitish mass not present before, we noticed the soil had the most bacterial growth within the petri dishes. In the soil sample (Figure 1), there was some form of fibrous growth resembling roots and wide

swaths of material that consists of bright yellow dots and blobs. The face's (Figure 2) cell colonies were lighter in intensity and shape. We observed a fine line of small white dots that varied in size, almost exactly in the orientation that it was rubbed on the dish, with some dots being a darker yellow color. The leaf (Figure 3) had more distinguished colors in the form of blobs and a more singular "line" of material that consists of tan, light, and dark yellow circular blobs in the orientation that it was rubbed on the dish, with a somewhat "oily" texture.

For the electrophoresis results, we saw that CS*l,* D*y,* CS*f*, and D*w* (Figures 4a, 4d, 4b, and 4e respectively) had extremely similar results. Using the reference DNA ladder, we determined that the DNA we extracted consisted of about 1500 base pairs. For CL*t*, or Figure 4c, the strands were able to travel nearly twice the distance in the gel, which corresponds to about 200 base pairs according to the DNA ladder.

Figure 1. CS sample. This photo shows the petri dish containing the soil sample after 4 days of growth.

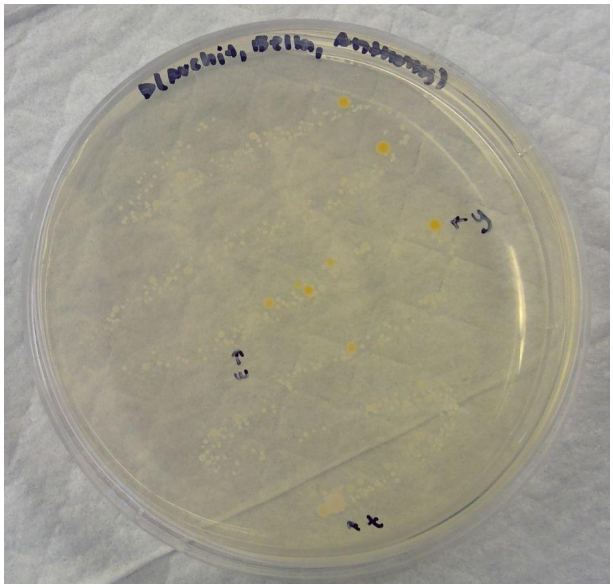


Figure 2. D sample. This photo shows the petri dish containing the sample of Zhang's face after 4 days of growth.

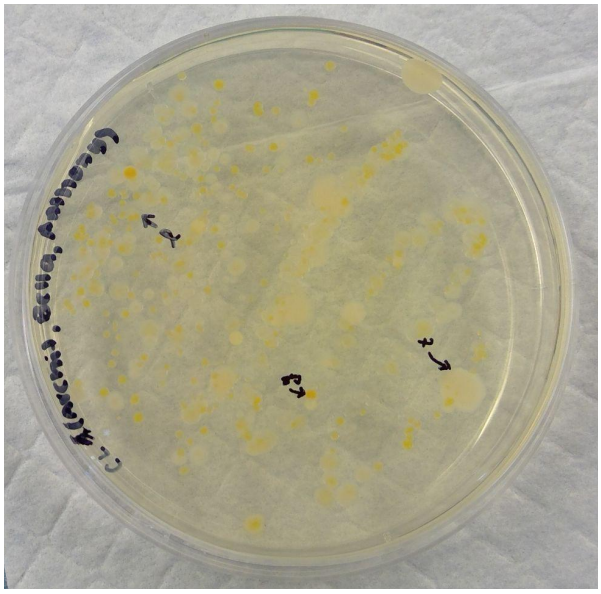


Figure 3. CL sample. This photo shows the petri dish containing the leaf sample after 4 days of growth.

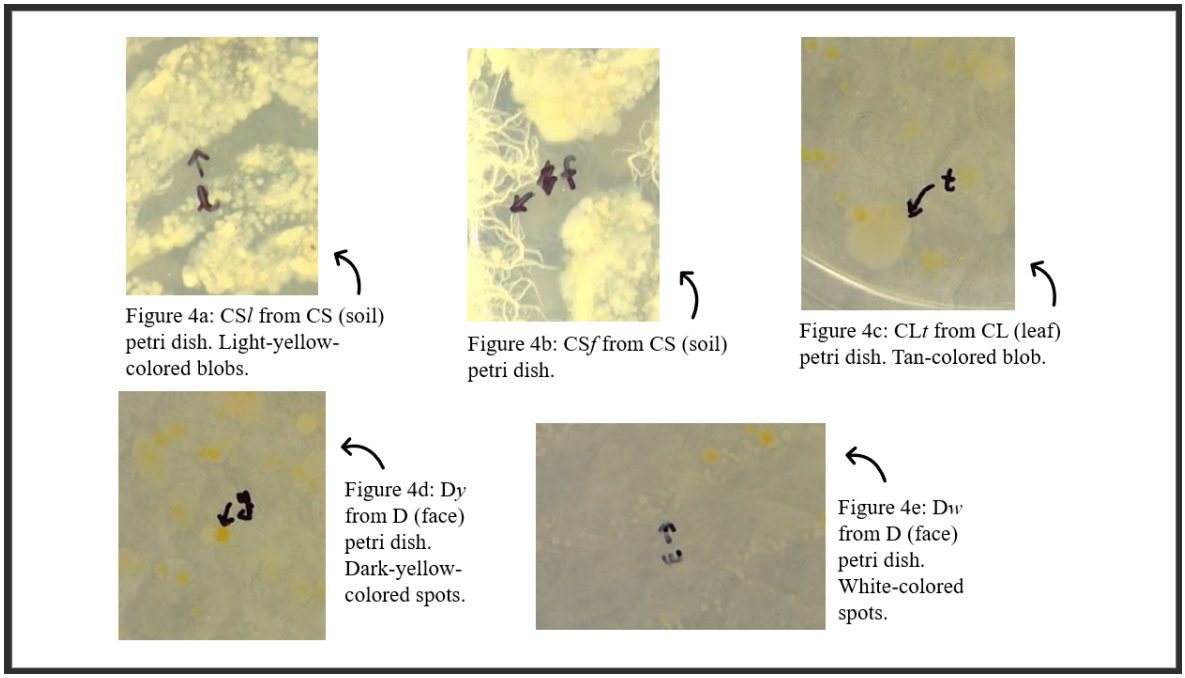


Figure 4a: CS*l* from CS (soil) petri dish. Light-yellow-colored blobs.

Figure 4b: CS*f* from CS (soil) petri dish.

Figure 4c: CL*t* from CL (leaf) petri dish. Tan-colored blob.

Figure 4d: D*y* from D (face) petri dish. Dark-yellow-colored spots.

Figure 4e: D*w* from D (face) petri dish. White-colored spots.

Figure 4. This shows the specific cell colonies we isolated: CS*l,* CS*f,* CL*t,* D*y,* and D*w.*

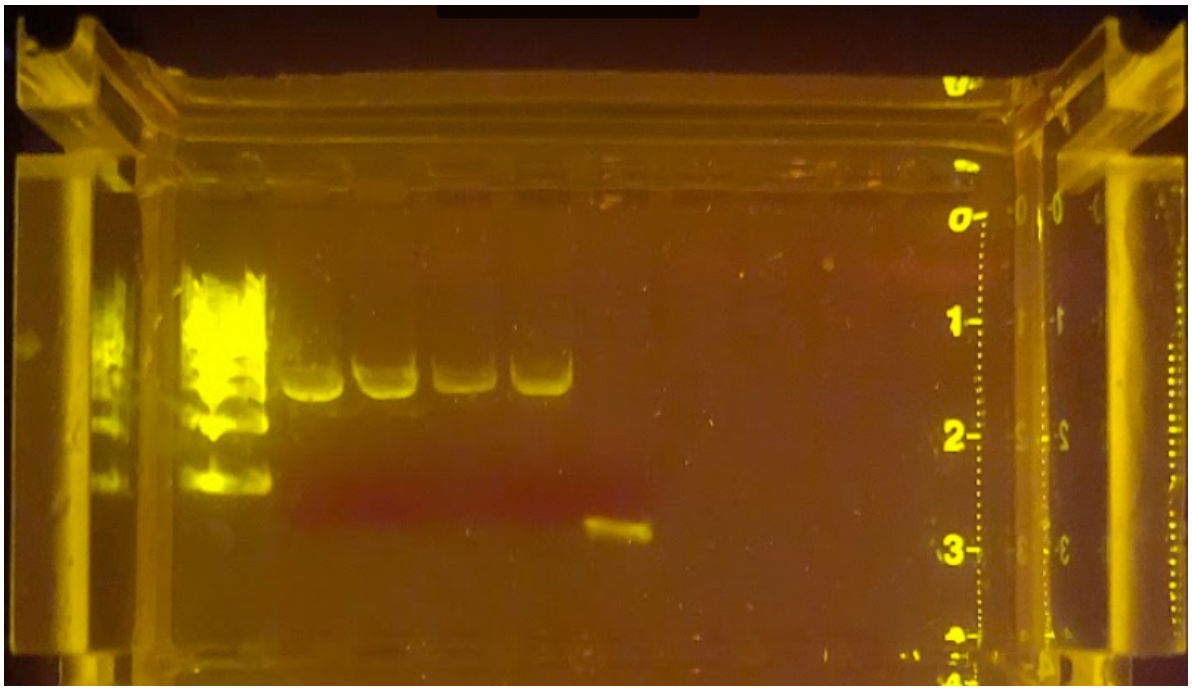


Figure 5. Results of gel electrophoresis. Gel electrophoresis samples in order from left to right: DNA ladder, CS*l,* D*y,* CS*f*, D*w*, CL*t.*

Conclusion

We can see from the gel electrophoresis results that the samples CS*l,* D*y,* CS*f*, D*w* all had a length of around 1.5K bp. According to the primers that we utilized in the polymerase chain reaction, a base pair length of 1.5K is conducive to a bacteria cell's DNA, which means that the samples CS*l,* D*y,* CS*f*, D*w* are all most likely some form of bacteria cell. The final sample, sample CL*t,* had a length of around 200 bp. Because the PCR primers that produced a DNA section length of 200 bp were for mold cells, that means that sample CL*t* is some form of mold.. These results demonstrate that the leaf had mold cells present on its surface and both the soil and leaf samples had bacteria cells present.