Kent Denver Innovative Scholars Program
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Gel Electrophoresis of Fish

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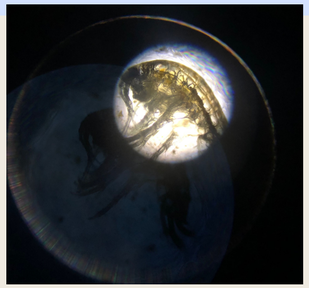
OVERVIEW/HYPOTHESIS

My beginning goal was to be able to capture and correctly identify macroorganisms in the Kent Denver lakes, however that shifted to researching survivability of brine shrimp in different conditions then finally to analyzing gel electrophoresis of fish.

LEARNING CURVE:

My original ideas was Macroorganisms in the Kent Denver lakes and producing a thorough procedure in collecting and labeling said organisms. However, it quickly became too cold outside and the collection process became difficult to execute consistently which lead to extremely variable results with a severe lack in a variety of organisms and amount.

I then moved on to the study of Brine Shrimp concerning pH, NaCl concentration, lighting, and heating requirements. After attempting three different setups in order to increase the overall survival rate of the Brine Shrimp, each one had specific downfalls and a suitable habitat was not sustained.



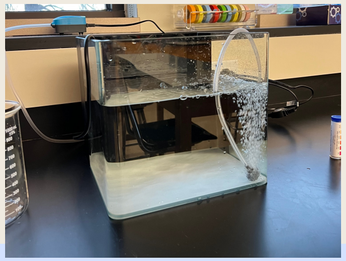


FIGURE 1: (Top 2) Macroorganisms + (Bottom) Final Brine Shrimp Setup

BACKGROUND:

Gel electrophoresis is a laboratory method used to separate mixtures of proteins (in this case) by molecular size and the relative electric charge of the molecule. Smaller charged fragments move faster and farther through the maze of microscopic pores toward the electrode with the opposite charge. Therefore through using gel electrophoresis you can determine the sizes of different proteins and in this case with fish proteins.

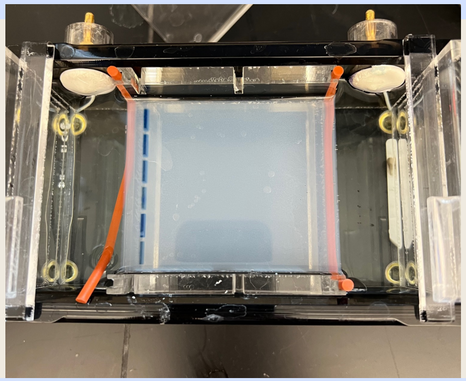


FIGURE 2: Agarose Gel 3% in electrophoresis setup

Tissue Preparation:
Step 1: Go to your favorite local supermarket and get four types of fish in 1cmx1cmx1cm pieces (keep refrigerated).
Step 2: Create the 1X Tris-glycine-SDS running buffer, 1X Protein lysing buffer, 1X protein destaining solution, and 1 3% agarose minigels.
Step 3: Cut 1cm^2 piece of fish and place in mortar and pestle and add 2mL of protein lysing buffer using a graduated pipet. Grind for 1 minute. Then let sample rest for 2 minutes grinding occasionally. Use a new pipet to remove liquid part of sample into a clean and labeled microcentrifuge tube. Clean the mortar and pestle with soap and water. Repeat step 3 for each sample.
Step 4: Cap all microcentrifuge tubes and place into the microcentrifuge. Centrifuge on high speed for 5 minutes.
Step 5: Then use a graduated pipet to transfer 0.5mL of the liquid supernatant from one microcentrifuge tube to a clean labeled microcentrifuge tube. Store in freezer if necessary. Repeat step 5 for all samples.
Step 6: Poke a hole using a dissecting needle in each microcentrifuge tube and place tubes into a floating microcentrifuge rack in a 95 degrees Celsius water bath for 5 minutes.
Step 7: Use a clean graduated pipet to add 5 drops of 6X gel loading solution to each protein sample. Cap each microcentrifuge tube and invert several times to mix the sample. If necessary store in freezer.

PROCEDURE:

Loading, Running, and Staining a Gel:
Step 1: Gently place the agarose gel into the casting tray with the wells toward the cathode (-) end of the unit.
Step 2: Carefully position the gel and tray into the electrophoresis chamber. If gel breaks or cracks or damaged in any way it should not be used.
Step 3: Pour enough Tris-glycine-SDS running buffer into the unit to submerge the entire gel surface to a 2-3 mm.
Step 4: Shake each sample microcentrifuge tube and lightly tap the bottom of each tube on a tabletop to mix the contents. Thaw first if frozen.
Step 5: Withdraw 10-20 microliters of the first sample from the microcentrifuge tube and dispense sample into the first well. Do not puncture the bottom or sides od the well and do not draw liquid back into pipet after dispensing the sample. Repeat for rest of samples and put them all in different wells.
Step 6: Run the gel at around 110V until the first tracking dye is about halfway down the gel then turn off the gel.
Step 7: Wearing gloves, slide the gel off the tray and into the staining tray and gently pour around 40mL of the protein staining solution onto gel. Allow to rest for 2-5 minutes. Pour off stain into proper disposal.
Step 8: Remove the gel from staining solution and wrap in paper towel and then into a resealable bag.
Step 9: Gently pour 150mL of protein destaining solution into the bag and then seal the bag, place in fridge and leave overnight. In 24-48 hours remove the gel and observe the gel bands.

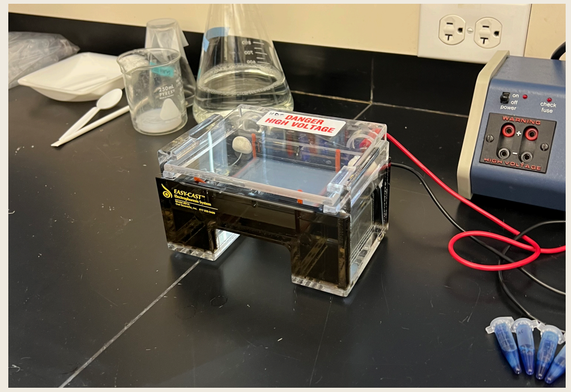


FIGURE 3:Gel electrophoresis and ready microcentrifuge samples to the right

ANALYSIS/RESULTS:

After the gel electrophoresis procedure, there were clear results in seeing some of the different proteins each fish had. However, there was no way to properly distinguish one band of color from another and the albumin (control sample) did not aid in helping to identify the different sizes of proteins in each fish. My gel electrophoresis failed produce any comprehensible data because I did not have a ladder which is used to differentiate the proteins in each chosen fish therefore making each color band of proteins within each fish difficult to distinguish by the exact size.

CONCLUSION

While there were many different processes involved with finding the overall topic of gel electrophoresis with fish, they were extremely beneficial and gifted me the opportunity to expand my knowledge about different science procedures and harden my resolve at problem solving and perseverance. I plan on continuing my area of study by using specific types of sushi from various restaurants/stores that have a range of prices in order to test whether or not their fish is real by using gel electrophoresis.