Evolution Modelling through SDS-PAGE of Fish Muscle Protocol using BIORAD Kit

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(SDS-Page of Fish Muscle Protocol, found in Activity 7.4 of *Biotechnology, A Laboratory Skills Course*[6])

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Abstract

The origins and ancestry of species were considered few of many questions in life. However, as science progresses, evolution proves itself to be more valid. Through a proteomics influenced protocol, the evolutionary model of ancestry will be constructed. In this protocol, vertical gel electrophoresis will be utilized on six muscles of unknown fish species. Protein from the fish will be extracted and will be loaded onto the vertical gel electrophoresis. The results of the gel will then be compared and further analyzed. The results will ultimately help compare the relationships of the species. Through the results, inferences can be made that most of the species share a common ancestor. Thus, the protocol will support the evolutionary model of common ancestor. [120]

Introduction

Ever since 1859 and the publication of *On the Origins of Species*, Charles Darwin's theory of evolution has become ever so controversial. Through many generations prior and after, no evidence was given on the origin of species. In the theory of evolution, Charles Darwin talks about the origins of species and common ancestry through evolution. For a while, Darwin's claims were mere guesses. However, through the use of proteomics, Darwin's theory becomes more credible.

Proteomic is a study of proteome and heavily involves proteins. Proteomic is often used to process and identify fish species. This is because the amino acid sequences found in proteins are unique to different species. Furthermore, the unique sequence allows for identification of common ancestry.

In the research, *Proteomics for the authentication of fish species,* conducted by Maria Fiorella Mazzeo, proteomics is focused with the purpose fish species authentication. By analyzing the protein of fishes, industries and markets are able to identify species of fish and prevent fraud.

By reinforcing the presence of proteins and amino acid chains within different species, it can be stated that evolution is valid. In a simple protocol, a simple example of proteomics will be exercised.

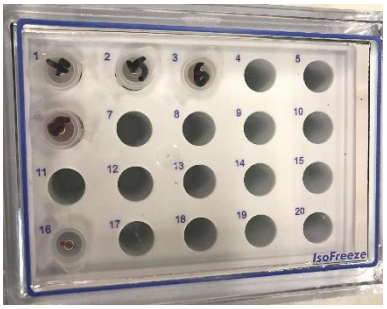
The purpose of this protocol is primarily to focus on the evolutionary relationships of six different fish species. This will be done by extracting the protein samples of the fish samples and running it under a vertical gel electrophoresis. The gel will then be compared and similarities will be drawn. Since all the samples are fishes, all the samples will share at least one band – that one band will represent the common ancestor.

Methods and Materials

The Methods and Materials of SDS-Page of Fish Muscle Protocol, found in Activity 7.4 of *Biotechnology, A Laboratory Skills Course*[6].

The protocol consists of three major sections, which include: Laemmli Extraction of Proteins, Electrophoresis of Test Samples, and Gel Drying and Analysis.

Before starting with the protocol, appropriate materials and equipment were gathered. The primary laboratory apparatuses, micropipettes, were set up. First, six of 1.5 ml microcentrifuge and screwcap tubes were labelled appropriately (A-F). Then, 250 µl of the Laemmli sample buffer (LSB) was added to the 6 microcentrifuge tubes. After the LSB was successfully transferred, muscle from the fish was cut to approximately 2mm. The 6 different fish muscles were then transferred to their respected microcentrifuge tubes. The tubes were then flicked around 15 times to mix the LSB with the fish muscle. After flicking, the microcentrifuge tubes were then incubated at room temperature for 5 minutes. After incubation, the



buffer from the microcentrifuge tubes were transferred into the screwcap tubes, respectively (**Muscle was not transferred**). The protein extracts within the screwcap tubes were then heated in a 95℃ water bath for 5 minutes. The protein extracts were then placed into bench top coolers.

In the next section of the protocol involves the use of the vertical gel electrophoresis chamber. First, Mini-PROTEAN TGX gel was prepared by removing the comb (the Mini-PROTEAN TGX gel was prepared by BioRad™). The inner chamber was then filled with 1x TGS. Before loading the samples, the fish muscle samples and actin and myosin standard were heated to 95℃ for five minutes. Then the samples were loaded as shown below:

Figure 1 – **Bench Top Cooler with LSB and muscle samples 3, 4, 5 &6.**

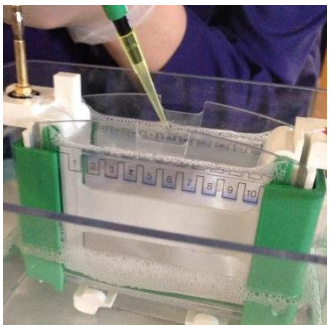


Figure 2 – **Loading of the vertical gel electrophoresis in the sequence stated by 'Table 1'**

Table 1 - **Protein Distance Migrated Relative to Molecular Mass of Precision Plus Protein Kaleidoscope**

Lane	Volume (μl)	Sample
1	*empty*	*empty*
2	*empty*	*empty*
3	5	Precision Plus Protein kaleidoscope standards (Std)
4	10	Fish sample A
5	10	Fish sample B
6	10	Fish sample C
7	10	Fish sample D
8	10	Fish sample E
9	10	Fish sample F
10	10	Actin and Myosin standard (AM)

After loading the samples, the power ran for 30 minutes at 200V. When finished running, the gel was placed into a gel staining tray and was rinsed in tap water for 5 minutes. Then, the gel was stained with 50 ml of Bio-Safe Coomassie stain. Once the blue stains were formed, a picture of the gel was taken.

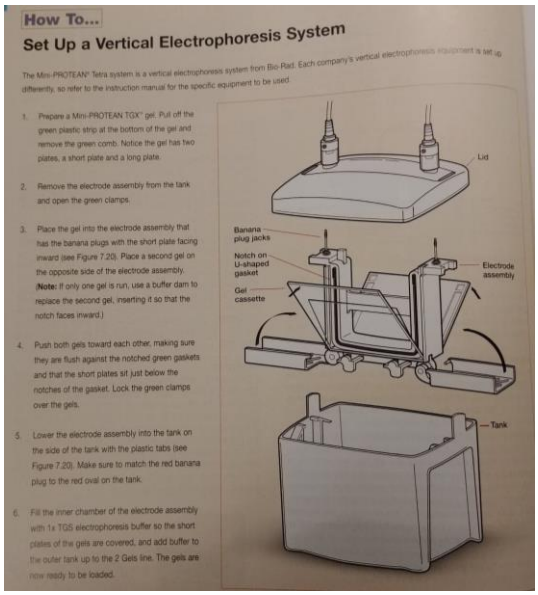


Figure 3 – **Instructions on how to set up a vertical electrophoresis system, found in Activity 7.4 of *Biotechnology, A Laboratory Skills Course*[6]**

Results

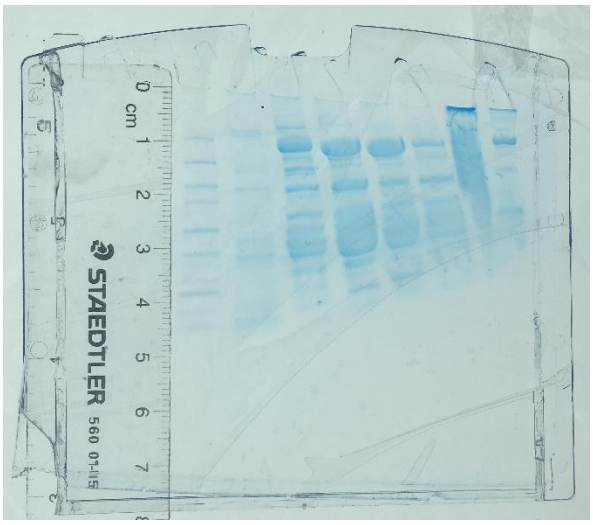


Figure 4 – **The gel sample with visible blue bands after staining by Bio-Safe Coomassie Stain.**

As shown by 'Figure 4', the distance for the bands was measured. The distance can be measured from any point on the gel as long as it is consistent. This is because, the data that will be calculated, is relative. The distances will be used in finding relations between the 6 fish samples. Since the bands were faint and hard to see, the image was edited for a better results.

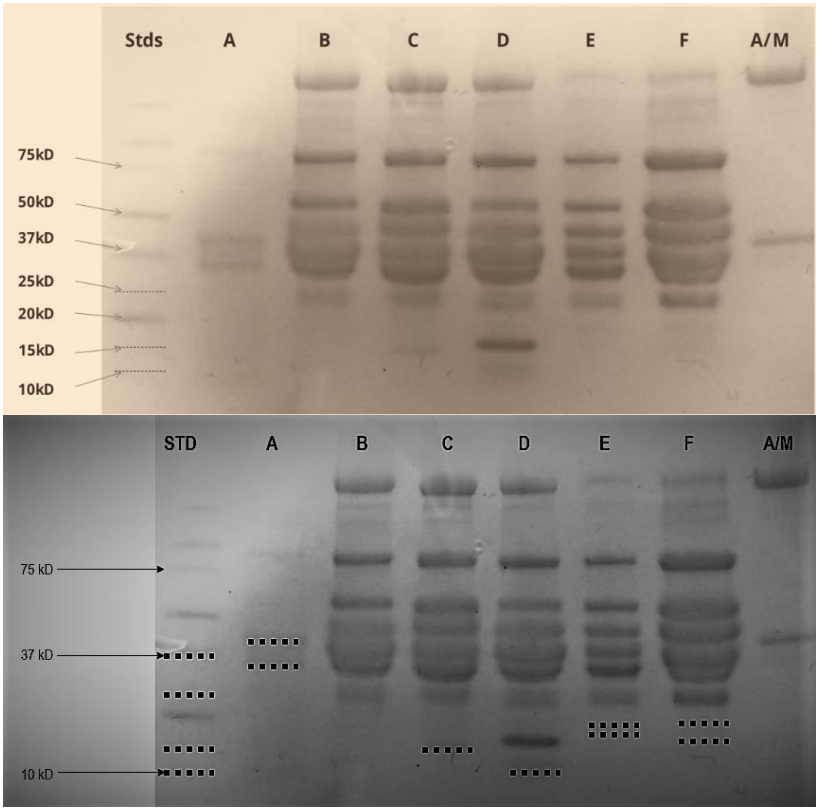


Figure 5 – **Two filtered pictures of gel analysis for the 6 unknown fish samples (A-F) and the actin/myosin bands (A/M)**

Using digital imaging and photoshop, the bands of the gel were made more visible. The images of the gel were labelled accordingly and the protein molecular mass were labelled. Using the filtered pictures, the protein molecular mass and their distance for the Precision Plus Protein Kaleidoscope Standard (STD) was determined. The distance and the protein molecular mass were then organized into a table.

Table 2 - **Protein Distance Migrated Relative to Molecular Mass of Precision Plus Protein Kaleidoscope**

Precision Plus Protein Kaleidoscope Standard (STD)	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	12	250
	21	150
	29	100
	34	75
	45	50
	53	37
	62	25
	72	20
	74	15
	80	10

The organized data recorded in the table was used to graph the standard curve of a semi-log graph.

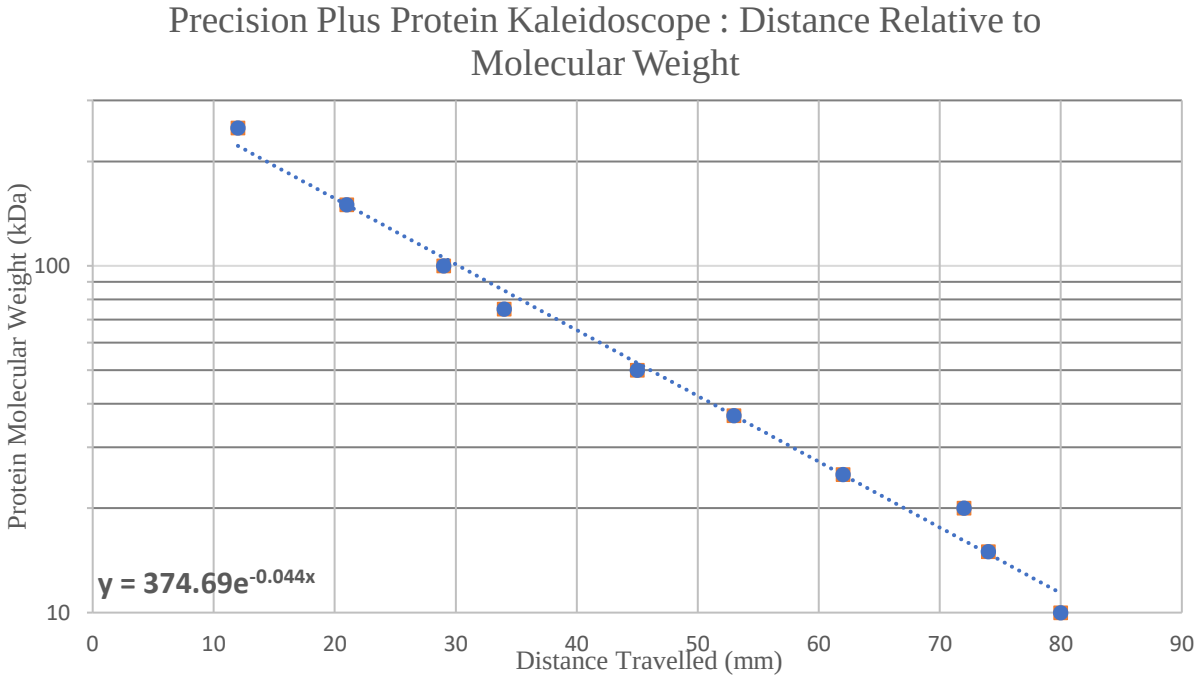


Figure 6 – **Semi Log Graph for the Precision Plus Protein Kaleidoscope. The distance of the proteins are relative to their molecular weight.**

The semi-log graph of the Precision Plus Protein Kaleidoscope standard (STD) then can be used to find the protein molecular mass (kDa) of the remaining samples. The protein molecular mass can be calculated by the equation of the trend line: $y=374.69e^{-0.044x}$.

Table 3 - **Protein Distance Migrated Relative to Molecular Mass of Fish Sample A**

Fish Sample 1	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	52	38.0
	57	30.5

Table 4 - **Protein Distance Migrated Relative to Molecular Mass of Fish Sample B**

Fish Sample 2	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	43	56.5
	49	43.4
	53	36.4
	59	27.9
	64	22.4

Table 5 - **Protein Distance Migrated Relative to Molecular Mass of Fish Sample C**

Fish Sample 3	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	44	54.0
	49	43.4
	53	36.4
	59	27.9
	64	22.4
	75	13.8

Table 6 - **Protein Distance Migrated Relative to Molecular Mass of Fish Sample D**

Fish Sample 4	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	43	56.5
	49	43.4
	54	34.8
	58	29.2
	64	22.4
	72	15.8
	80	11.1

Table 7 - **Protein Distance Migrated Relative to Molecular Mass of Fish Sample E**

Fish Sample 5	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	43	56.5
	49	43.4
	54	34.8
	58	29.2
	64	22.4
	70	17.2
	72	15.8

Table 8 - **Protein Distance Migrated Relative to Molecular Mass of Fish Sample F**

Fish Sample 6	Distance Migrated (mm)	Protein Molecular Mass (kDa)
	44	54.0
	49	43.4
	56	31.9
	59	27.9
	64	22.4
	70	17.2
	72	15.8

Using the data a table containing the summary of the proteins present in the fishes can be constructed.

Table 9 - **Summary of various proteins that are present in 6 unknown, different fish species**

Distance Migrated (mm)	Protein Molecular Mass (kDa)	Fish A	Fish B	Fish C	Fish D	Fish E	Fish F
43	56.5		X		X	X	
44	54.0			X			X
49	43.4		X	X	X	X	X
52	38.0	X					
53	36.4		X	X			
54	34.8				X	X	
56	31.9						X
57	30.5	X					
58	29.2				X	X	
59	27.9		X	X			X
64	22.4		X	X	X	X	X
70	17.2					X	X
72	15.8				X	X	X
75	13.8			X			
80	11.1				X		
	COUNT	2	5	6	7	7	7

From ‘Table 9’, information on which samples share the same bands is shown. Using this information, a matrix can be made to compare which samples have the most similarities.

Table 10 – **Shared Characteristic Matrix for Six Different Fish Species.** Bands common among fish species were counted and recorded

	Fish A	Fish B	Fish C	Fish D	Fish E	Fish F
Fish A	**2**					
Fish B		**5**	**4**	**3**	**3**	**2**
Fish C			**6**	**2**	**2**	**2**
Fish D				**7**	**6**	**3**
Fish E					**7**	**4**
Fish F						**7**

From the matrix, it can be concluded that Fish D and Fish E are the most common as they share 6 common bands.. It can also be concluded that Fish A is the least common out of all the samples with no similarities to other samples. Also, Fish B and Fish C are shown to be closely related as they share 4 bands in common. The remaining Fish F is seen be to moderately common to all species. From this data, a cladogram is constructed, illustrating the evolutionary model of common species.

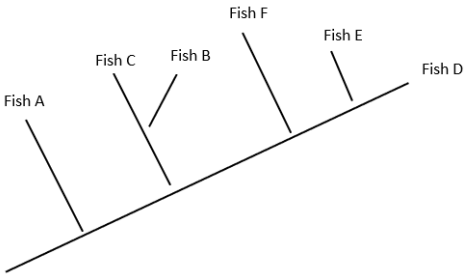


Figure 7 – **The cladogram of the 6 different fish species, constructed from data collected from gel electrophoresis**

From the constructed cladogram, it can be analyzed that Fish E and Fish D are the most recent common species. Fish C and B are also very common related. Fish F is moderately related to Fishes C,D,B,E, thus falls in-between the two sister groups. Fish A is not common to any species, therefore it is placed at the very far left.

Discussion

As concluded from the results, Fish E and Fish D share the most common band under the actin band. Furthermore, Fish A has the least common bands among all the samples. Through the constructed cladogram, the evolutionary relationship between the gels is shown. This relationship was not shown on the gels. The cladogram represents the gel bands that were studied and compared.

By utilizing proteomics, the study of proteomes[7], species, and their evolutionary species are proven. As shown in this experiment, proteins extracted from different fish species are compared. The key factor in determining the relationships comes from the proteins. Proteins are macromolecules that are synthesized from amino acids. Amino acid sequences are unique to their corresponding species and can show similarity between common ancestors[8]. For example, between humans and chimpanzees, the amino acid sequence for the ubiquitous protein, Cytochrome C, is identical[9]. Furthermore, the amino acid sequence of humans compared to other mammals only show slight variations. This shows that humans and mammals all come from the same ancestor while the chimpanzees are their most common ancestor. Likewise, proteomics is used between fish samples to determine their ancestry.

A possible argument may come from the fact that the proteins have similar structures but varying genetic sequences. The similar structures could question the species as being an example of convergent evolution rather than common ancestry. As Douglas Theobald, a professor at Brandeis University, once stated, "All the classic evidence for common ancestry is qualitative and is based on shared similarities."[10] Just by phenotypes, it is hard to determine since almost all the fishes have similar structures. The fishes have common characteristics, such as jawless, vertebra, and also where the gills are located[11]. However, through proteomics, the common amino acids sequences prove common ancestry over convergent evolution. Moreover, this does not mean that fishes that are not commonly related in having drastically different phenotypes. After all, evolution proves that all species have common ancestors.

Based on the results and research, conclusions have been made that the corresponding fish species from A to F are: Octopus, Salmon, Blue Fin Tuna, Asgard, Snapper, and Albacore tuna.

One common error in this protocol may result from the misuse of the vertical gel electrophoresis. When pouring, the 1x TGS should be poured from the inner chamber out. Another common error can result from contamination. Proteins are ultimately made of RNAs. RNAs are less stable (than DNA) as they contain ribose, as opposed to deoxyribose[12]. RNA can be easily destroyed in the lab through contamination.

If this protocol were to be performed again, more emphasis would be placed on lab sanitation for better results.

References

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