Effect of Varying Salt Concentrations on DNA Extraction from Strawberries and Bananas

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Abstract

A variety of methods have been used for DNA extraction. This experiment aims to understand the effects of salt concentration of a solution with a mixture of salt, water, and dish detergent on DNA yield of fruits. Prior research shows that strawberries and bananas yield a greater amount of DNA than other fruits, which will provide a more accurate comparative analysis. 40 grams of bananas and strawberries were mixed with salt water at varying concentrations (0, 0.0208, 0.0417, 0.0625, 0.0833), dish soap, and rubbing alcohol to allow for the precipitation of DNA into a measurable form. The results demonstrated relative alignment with the given hypothesis, which is that the yield of extraction increases with salt concentration, which is due to the ability of salt to neutralize DNA's negative charge, separate it from the cell, and dissociate it from the water. An unexpected outcome of this research was the unusually high DNA yield from the mixture with 0% salt concentration. Since this trial was conducted in New Delhi, India, while the other trials were conducted on the mainland USA, it is possible that geographical differences in the chemical makeup of water, rubbing alcohol, or dish detergent allowed for DNA precipitation without the use of salt. Future research should be conducted to understand how salt may be unnecessary in the process of DNA extraction.

Keywords

DNA extraction, DNA purification, DNA isolation, salt concentration, bananas, strawberries

Introduction

Prior research on this subject has revealed a variety of methods for DNA purification, ranging from solid-phase purification to capillary electrophoresis (Shin JH, 2012). The process of DNA extraction occurs when something is used to lyse a cell open and separate DNA. For our purposes, we used a solution with a mixture of salt, water, and dish detergent. DNA is soluble in water but insoluble in the presence of salt and alcohol. Salt helps to neutralize the charge of DNA's sugar-phosphate backbone and make it less hydrophilic, allowing it to dissociate from water more easily (Jina Heikrujam et al., 2020). Additionally, it stabilizes the DNA molecule after the proteins have been stripped from it. By adding these to the water, the DNA precipitates into white pellets that are visible. DNA extraction uses physical and chemical bonds from a sample that separates DNA from cell membranes, proteins, and cellular components. Additional background research also showed us that the fruit must be fresh and organically grown, without the use of any pesticides or chemicals, to ensure purity of the DNA. Additionally, a 2009 California Science Fair project showed that under-ripe bananas have the most intact DNA, so under-ripe fruits will be purchased for the purposes of our experiment (Erich N. Herzig, 2009) . We predict that a higher salt concentration will yield more DNA because it will neutralize more DNA and separate it from the organism, and strawberries will yield more DNA than bananas because they have more chromosomes in each cell.

Methods

Purification Medium:
The purification medium from which the DNA was extracted came from different parts of the world–the American west coast, American east coast, and north India–but the organisms used were organic and underripe, as to maximize the yield of DNA. The strawberries were kept refrigerated, while the bananas were kept at room temperature, but the time period between obtaining the fruits and conducting data collection was not significant enough for this difference to play a role in the difference in their yields. A maximum yield is typically obtained through freezing the fruits prior to extraction.

DNA Extraction Protocol:
Precisely 40 grams of each fruit was weighed out, and gently mashed in a Ziploc bag in order to prepare for the extraction process without harming the DNA. Then approximately 100g of salt water (with varying concentrations) was heated to 100o F, because extraction typically yields the greatest amount of DNA in a range of 95o-105o (Issaq HJ et al., 2000). The saline solution was added to the Ziploc bag, and mixed with the mashed fruit. Salt neutralizes the charge of DNA, and makes it less hydrophilic. It also removes the proteins bound to DNA and keeps them dissolved in water (North Carolina Community College, n.d.). From here, about 80 mL of the mixture was weighed out into a beaker, and 1⁄2 a teaspoon of dish soap was mixed into it. Soap contains a compound that removes fats and proteins, and hence has the ability to lyse cell walls, which is why dish soap or detergent is a common ingredient in cell lysis solutions. The dish soap pulls apart the membranes, releasing the DNA (North Carolina Community College, n.d.). At this point, the mixture was ready to be put into the graduated cylinder, and was strained through a coffee filter inside of a funnel. The purpose of this was to block any larger chunks from entering the graduated cylinder and interfering with the extraction process, but this generally should not be an issue. After adding approximately 80 mL of mixture to the cylinder, it was gently stirred. Then, 20 mL of 91 % isopropyl alcohol was added to the cylinder. The purpose of rubbing alcohol–especially when cold–is to allow the DNA to clump together and form a visible white precipitate (Let's Talk Science, 2013). After letting this sit for approximately 5-10 minutes, the DNA was collected with a popsicle stick, and placed into a Ziploc bag. It was later weighed on an analytical balance, which was tared with an empty Ziploc bag. This procedure was conducted with varying salt concentrations in 1⁄2 a cup of water:
• 0 tsp. (0 %) – control
• 0.5 tsps. (2.08 %)
• 1 tsp. (4.17 %)
• 1.5 tsps. (6.25 %)
• 2 tsps. (8.33 %)

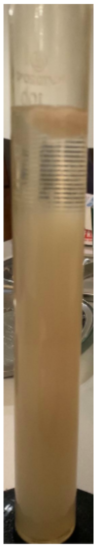

Figure 1: Precipitated DNA in Graduated Cylinder

Results

The below table and graph depict the results from 8 trials of DNA extraction, with varying salt concentrations as listed above.

Salt Concentration	Banana DNA Yield (g)	Strawberry DNA Yield (g)
0	1.4	0.47
0.0208	0.487	0.0193
0.0417	0.62	0.031
0.0625	0.5	0.2
0.0833	0.76	0.24

Figure 2: Table of Results

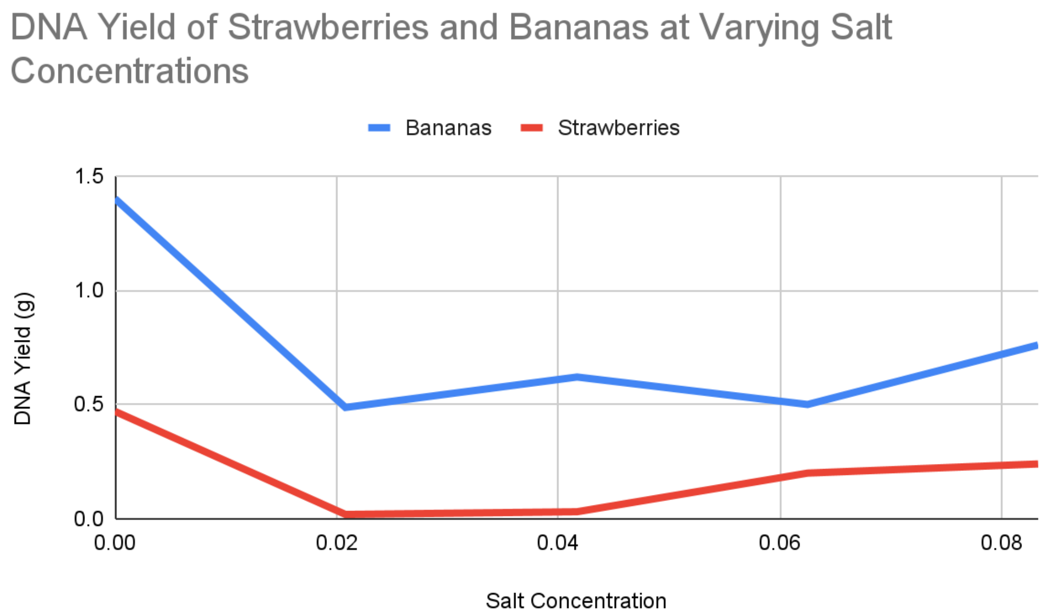

Figure 3: Graph of Results

Discussion

The results of the trials displayed a general trend of the DNA yield increasing with salt concentration, and bananas having a significantly higher yield than strawberries. DNA extraction is made possible by precipitating the DNA out of a solution with water. Since water has a relatively weak positive charge, this is accomplished by providing a stronger positively charged ion in the solution. Sodium is the perfect candidate for this. After the DNA dissociates from the water, it binds to sodium (as they have opposing charges) and precipitates (Stacy Taylor, 2009). This reasoning supports the experimental data, as a higher availability of sodium allowed for a greater amount of DNA to dissociate and precipitate.

The data begins to misalign with our hypothesis when analyzing the results of the control trials. In both strawberries and bananas, the control yielded a greater amount of DNA, despite having no sodium to allow for precipitation to occur. Although initially perplexed by these results, a repeat of the control yielded similar results (the results of the original are displayed above). We are still unsure as to the exact cause of such a discrepancy, but it is likely due to the contents of the water solution. The control trial was run in New Delhi, India, while the experimental trials were done in the United States. It is also possible that the difference in DNA yield was due to the variety of bananas used. The 91% isopropyl alcohol used by the involved researchers was a different color, suggesting a different chemical makeup. Additionally, as tap water was used by all parties, there was likely contamination in the water, especially in India. The level of contamination may also differ between each location, affecting the results. Although further testing is still required in order to narrow down a conclusion, we hypothesize that either the water or the isopropyl alcohol in India contained another chemical component with a positive charge that was able to neutralize the negatively charged DNA. Additionally, it is also possible that the isopropyl alcohol alone allowed for such a high rate of extraction. Although DNA is not soluble in the alcohol, since the alcohol is miscible in water, so as more is added, the solubility of DNA increases (NileRed, 2017).

The second half of our hypothesis was disproved, with bananas yielding significantly more DNA than strawberries. The initial prediction that strawberries would allow for more DNA to precipitate stemmed from the fact that strawberries are octoploid, meaning that their cells have eight duplicate copies of each chromosome (Folta et al., 2019). On the other hand, cultivated bananas are diploid, triploid, or tetraploid hybrids, meaning that their cells have two, three, or four sets of DNA. This makes a total chromosome count for strawberries 56 and the total chromosome count for bananas 33. This higher availability of DNA led us to believe that strawberries would yield significantly more than bananas, but the opposite occurred. This is likely due to our procedure. Since we stored the fruits in a cold environment, it led to the strawberries being overly firm, making it difficult to mash them in preparation for the extraction process. As such, the strawberries were likely not in a form that allowed for optimal extraction. Though the bananas were kept in a similar environment, they were still in a state that allowed for the cells to be exposed through physical mashing.

Future research on the subject of DNA extraction should be repeated in order to more accurately identify the causes behind some of the unexpected results of this experiment. Research that looks into the reason that a salt concentration of 0% yielded the highest amount of DNA could provide insights into the influence of regional discrepancies on biological research, and possibly reveal widely held misconceptions.

Conclusion

In this study, we aimed to explore the effects of salt concentration and the type of fruit on DNA yield of bananas and strawberries. It was predicted that the DNA yield would increase with high salt concentration and the bananas would yield the most DNA. Though the DNA yield generally increases when salt concentration was greater than 0, the greatest DNA yield was at the salt concentration of 0. Although our prediction had been supported by the non control trials, the results of the control were different from our predictions. Additionally, through all the trials, bananas had a higher DNA yield than strawberry, disproving our second prediction. For each trial, a saline solution was created for each trial using 0, 0.5, 1, 1.5, or 2 teaspoons of salt, heated to 100 degrees Fahrenheit and added to the gently mashed fruits. ½ teaspoon of dish soap was then added and mixed. 80 mL of the mixture was then strained through a coffee filter into a graduated cylinder and 20 mL of 91% isopropyl alcohol was added. After letting sit for 5-10 minutes, the DNA precipitate was collected and weighed. There were several flaws in our procedure. The alcohol used by the researchers were different colors, suggesting differences in chemical makeup. Additionally, each trial was held in different locations, calling into question the differences of the level of added substances in the tap water, such as minerals. Furthermore, as the strawberries were stored in a cold environment, they were overly firm, making them harder to mash than the bananas and less ideal for extraction. This experiment shows that generally, as salt concentration increases from 0.0208 to 0.0833, the DNA yield increases. Additionally, this experiment shows nuances in DNA extraction that should be further researched, such as the importance of regional differences in the materials for DNA extraction. Additionally, we recommend further research on the causes of the unexpected results of this experiment, such as the effect of amount of mashing on DNA yield.

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