In Citrus and In Health

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Abstract

Grapefruits and grapefruit juice possess an enzyme that produces potentially dangerous effects when consumed with specific medications. Therefore, it is possible that citrus fruits closely related to the grapefruit produce similar effects. In order to determine which fruits may be candidates for further research on their effects on medicine, we performed genetic tests to look for similarities between grapefruit and select citrus fruits. In this experiment, the citrus fruits bar coded using chloroplast DNA were: grapefruits, oranges, tangerines, pomelos, and lemons. The results yielded that the genome of pomelos were very similar to that of the grapefruit, closely followed by the orange and the tangerine. The lemon showed the most differences from the grapefruit and would not be the prime candidate for further research. Given that there is only a small number of differences between these citrus fruits and the grapefruit, there is a likelihood that these fruits also affect medications. To investigate this new hypothesis, further investigation can be done on the enzymes of these citruses. In New York, it is common to consume at least one type of citrus, and often more, each day. Therefore, it is critical to conduct further inquiry so as to prevent dangerous consumption of certain citrus fruits with incompatible medication.

Introduction

New York City is home to a variety of citruses, one of which is the grapefruit. Research has shown that grapefruits can help lower cholesterol levels, diminish high blood pressure, strengthen one's immune system, and even help prevent cancer. However, multiple instances in which complications have occurred when individuals ingested grapefruit juice while taking certain medications have been noted. In some cases, these complications turned out to be fatal. Grapefruit juice has been shown to negatively impact the effects that certain drugs have on the human body. In many cases, the effect is an increase in heart rate, even if there is no underlying heart disease present in the individual. Grapefruit juice has also been shown to decrease the activity of a series of cytochrome proteins known as CYP3A4, which are responsible for the breakdown of many drugs and toxins within the body. Thus, the potential buildup of this drug in the bloodstream may occur, causing the patient to overdose.

As part of the Urban Barcoding project, we will be sequencing the genome of the grapefruits, as well as other commonly-eaten citruses. Real-life implications within New York City include the possibility for grapefruits to possess genetic similarities to other citruses such as oranges, tangerines, pomelos, and lemons. If it is indeed proven to be true that there are strong similarities between these citrus fruits and grapefruits, we may have reason to conduct further studies upon the nuclear DNA (proteins) of the chloroplasts of these fruits. Through such additional research, we may be able to determine if indeed these citruses possess the enzyme adversely affect an individual taking certain medications, just as grapefruits have been shown to negatively impact or counteract the effects of certain drugs. For these reasons, it is crucial to conduct research to find similarities between these citruses.

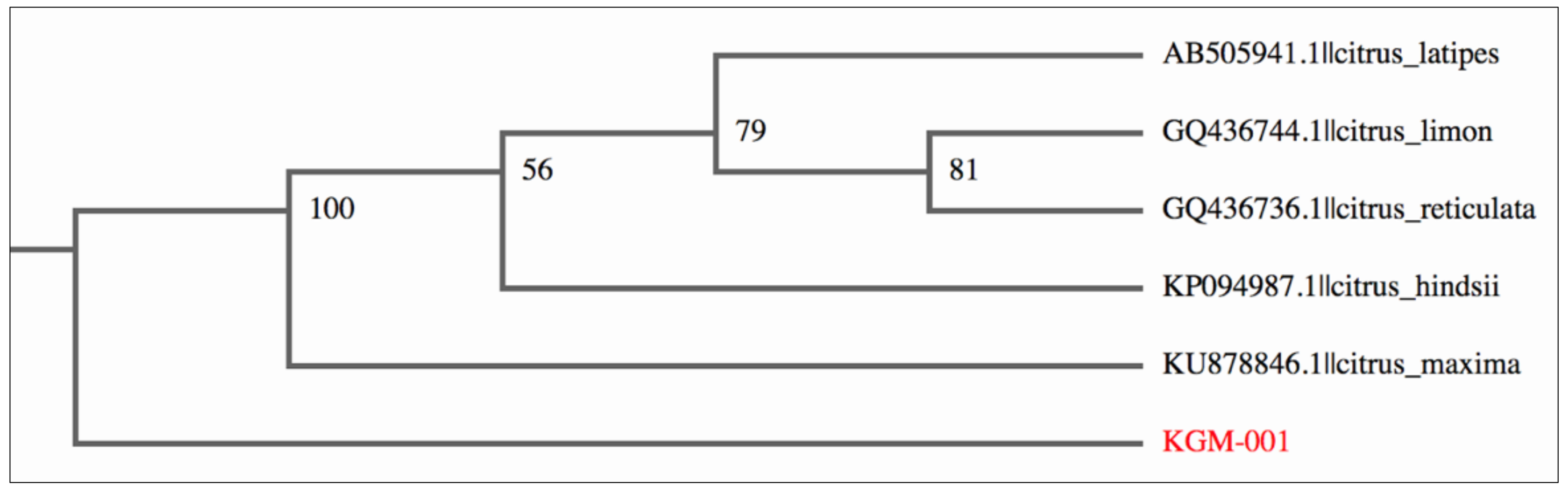
In addition to grapefruits, we will be obtaining samples from oranges, tangerines, pomelos, and lemons. We hypothesize that there will be a high level of similarities (with few differences) between grapefruits and the other citruses tested. If we do indeed detect that there are close similarities between some of the citrus fruits and the grapefruit, then we will have sufficient reason to conduct future research involving the analysis of the nuclear DNA. This way we can test for similarities that may reveal information towards the causation of the drug interaction with this citrus fruit, that doctors have observed.

Materials & Methods

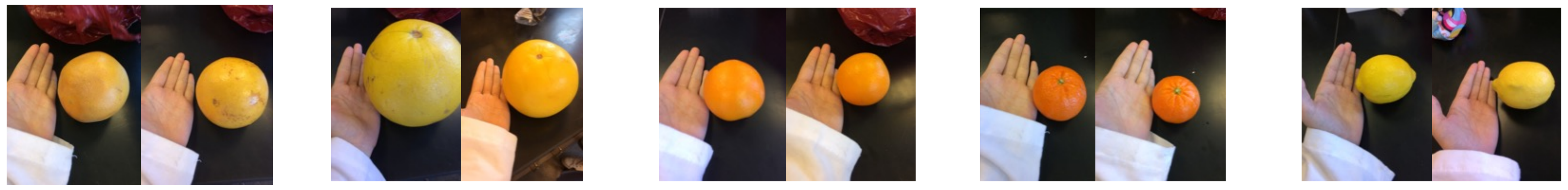
Our group obtained a few of the commonly-utilized citrus fruits in New York from ethnic food markets and from Aaron's Kissena Farm in Queens. We documented the appearance of our various specimens by taking pictures in order to record their visible, or 'phenotypic' attributes. Using a blade or scalpel, we cut a small sample from each fruit (10–20 mg) in order to collect its chloroplasts, which are necessary to our experiment. We labeled and stored the collected tissues of the various citrus fruits in a cooler for preservation of the tissues, and in order to protect the tissues from potentially damaging influences present in the environment. Next, we added 300 μL of lysis solution to each tube, and crushed each of the samples with a pestle for at least two minutes. We heated the tubes at 65 degrees Celsius for ten minutes. We then placed the tubes in a balanced centrifuge and transfer 150 μL of the supernatant to a fresh tube. In order to separate nucleic acids from the extraction solution, 3 μL of silica resin will be added to the tube and mixed well by pipetting up and down. The tubes were then incubated at 57 degrees Celsius for five minutes. Afterward, the tubes were centrifuged for 30 seconds at maximum speed. After pouring out the supernatant, we added 500 μL of wash butter to the pellet and resuspended the silica by pipetting up and down. This step will remove contaminants from the sample while the DNA of the chloroplast will remain bound to the silica. We will then centrifuge the tube at maximum speed for 30 seconds. Once again, we poured out the supernatant and add 500 μL of wash buffer to the pellet and resuspended the silica by pipetting up and down. The tubes will then be placed in a balanced centrifuge for 30 seconds. The supernatant will be poured out, and the remaining supernatant was removed using a micropipette with a fresh tip. To elute the DNA from the silica, we added 100 μL of distilled water to the pellet and mixed well by pipetting. The tubes were incubated at 57 degrees Celsius for five minutes. We then centrifuged the tubes for 30 seconds to pellet the silica resin. Since our DNA was now in the supernatant, we transferred 50 μL of the supernatant to a fresh tube while being very careful not to disturb the pellet while transferring the supernatant. We stored the tubes on ice until we are ready to pipette 2μL from each sample, which we will put into the PCR tubes. Once this was completed, we mixed the samples with loading dye and loaded them into a gel electrophoresis. We ran the gel and compared the samples to ensure proper DNA of the chloroplast isolation. Lastly, we sent off the samples to be sequenced. Once all the data had been collected we were able to make our conclusions and discuss possible further research.

Results

As we expected, many of the citrus fruits that we sequenced possessed few differences to the grapefruit. For the pomelo, we observed a total of one difference when the sequenced chloroplast DNA of the pomelo was compared to that of the grapefruit. When comparing the DNA sequences of the orange and the grapefruit, we observed two differences. In addition, when we compared the chloroplast DNA sequences of the grapefruit and the tangerine, we also observed two differences in the sequenced chloroplast DNA of the two citruses. Interestingly, we also found a number of other citrus fruits common to the New York region that showed many similarities to the grapefruit as well. Such citrus fruits include the as Kaffir lime and kumquats. Between the Kaffir lime and the grapefruit, we found a total of two chloroplast DNA discrepancies. Between the kumquat and the grapefruit, however, we observed a total of zero differences in the sequenced DNA of the two fruits. Between the lemon and the grapefruit, however, we found a significant number of differences that were not seen among other fruits and the grapefruit. When we compared the grapefruit's sequenced chloroplast DNA to that of the lemon, we detected a total of six discrepancies between the two citruses. Therefore, we may conclude that the lemon possessed the largest number of differences to the grapefruit, among those citruses that were compared to the grapefruit. Images of our gel electrophoresis results, as well as a phylogenetic tree of our results are pictured below:



Citrus samples:



Discussion

Through our DNA sequencing, we found that there were quite a few citrus fruits that were genetically similar to the grapefruit. The pomelo, for example, possesses only one difference and is the most closely-related citrus to the grapefruit. However, the orange and the tangerine were also quite similar to the grapefruit, in terms of their chloroplast DNA sequences. Lastly, we discovered that the lemon is the least related to the grapefruit, as it possesses the most differences in its chloroplast DNA, when compared to the grapefruit.

With the result yielded, there is reason to conduct further research to see if certain citrus fruits such as the pomelo, the orange, and the tangerine, produce similar effects as the grapefruit when combined with certain medications. Our results are significant because, it is a widely-known fact that grapefruit, when combined with certain medications, produces certain effects that can be harmful to patients, and in some case, even fatal. Thus, it is crucial to determine whether the ingestion of certain citruses other than grapefruits, is safe along with the consumption of certain medication.

While our hypothesis was proven to be fairly accurate, we did not expect the lemon to be so different in comparison to the other citruses we tested. In total, we found six discrepancies between the sequenced chloroplast DNA of the lemon and that of the grapefruit. In addition, we found the DNA extraction process to be a bit difficult, due to the fact that we were working with very fleshy fruits. The pulp in each of these citruses made the DNA extraction process more difficult; thus, any unclear or incomplete results may be attributed to errors made in the initial DNA extraction process.

In the future, we would like to conduct further research involving the presence of the cytochrome proteins known as CYP3A4, the activity of which grapefruit juice has been known to suppress. These proteins are crucial to the elimination of metabolic wastes and other toxins from the body. If the proper function of these cytochrome proteins is disrupted, the potential buildup of a drug or toxin in the bloodstream may occur, causing harm to the human body. Thus, we suggest that the specific mechanism of suppression of grapefruit juice on these cytochrome proteins be studied in detail.

References

Bailey, David G., J. Malcolm, O. Arnold, and J. David Spence. "Grapefruit Juice-drug Interactions." British Journal of Clinical Pharmacology. Blackwell Science Inc, Aug. 1998. Web. 21 May 2017.

Castillo, Michelle. "Drinking Grapefruit Juice with Some Medications Can Be Deadly, Study Warns." CBS News. CBS Interactive, 27 Nov. 2012. Web. 21 May 2017.

"Common Grapefruit Juice Drug Interactions." Drugs.com. Drugs.com, n.d. Web. 21 May 2017.

"Current and Projected Populations." NYC. N.p., n.d. Web. 21 May 2017.

"Don't Mix Your Meds with These Foods." Don't Mix Your Meds With These Foods - Consumer Reports. N.p., n.d. Web. 21 May 2017.

Morton, Julia F. "Lemon: Citrus Limon." Lemon. N.p.: n.p., n.d. 160-68. Lemon. Web. 21 May 2017.

Rabin, Roni Caryn. "Grapefruit Is a Culprit in More Drug Reactions." The New York Times. The New York Times, 17 Dec. 2012. Web. 21 May 2017.

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