New York City Can Keep the Vampires Away: Investigating the Types of Garlic Sold in NYC Supermarkets

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Abstract

Garlic, or Allium sativum, is an edible bulb used most commonly as a flavor in foods, however it can also be used as a dietary supplement for antibacterial purposes. Garlic has two large subspecies characterized by the absence or presence of a firm central stalk—hardneck and softneck—each of which have subspecies of their own. The goal of this experiment was to see what specific subspecies of garlic is most commonly sold at supermarkets in New York City, and which of these subspecies has the highest antibacterial properties. To see which subspecies of garlic is most commonly sold, we bought random garlic samples from various New York City supermarkets, and DNA barcoded them. To test the antibacterial properties of each sample, we performed a disk diffusion assay. We discovered that specific subspecies of garlic are not classified as their own species, but rather as garlic in general. However, due to morphological observations, we concluded that softneck garlic is more commonly sold in supermarkets. As for which subspecies of garlic has the best antibacterial properties, we found that a fresh sample of hardneck garlic had significantly stronger antibacterial properties than older samples and than softneck samples.

Introduction

Garlic, or Allium sativum, is an edible bulb belonging to the lily family. The bulb grows beneath the ground and is comprised of multiple sections called cloves, each one encased in a paper-like membrane. Garlic is most commonly used to flavor foods, and it is also one of the earliest documented plants to be used medically. Garlic has been found in ancient Egyptian pyramids and ancient Greek temples, was used by Native Americans to cure flu-like symptoms, and was used in the 1800's as a stimulant, expectorant, and tonic. Today, garlic is often used as a dietary supplement for a variety of medicinal purposes.

There are two major subspecies of garlic grown: softneck garlic (A. sativum sativum) and hardneck garlic (A. sativum ophioscorodon). Softneck garlic is characterized by its pliable, soft stalk protruding from the top of the garlic, formed by a layering of the garlic's paper-like membranes that grow up past the top of the bulb. Softneck garlic typically contains several layers of cloves surrounding the center of its bulb and has many varieties, the two most prominent being silverskin garlic and artichoke garlic. Hardneck garlic is characterized by a firm stalk protruding from the top of the bulb. There are three main types of hardneck garlic: rocambole garlic, porcelain garlic, and purple stripe garlic.

Garlic's strong smell and taste are related to its antibiotic properties as both come from the production of a sulfur containing molecule allicin ($C_6H_{10}OS_2$). The two components that go into making allicin are alliin ($C_6H_{11}NO_3S$) and alliinase. In the garlic plant cell, alliin is found in the cytoplasm and alliinase is found in a different part of the cell, in structures called vacuoles. When fresh garlic is cut, alliin and alliinase come into contact with each other and react. Alliinase is an enzyme, so it catalyzes the chemical reaction of alliin breaking apart into allicin.

In this experiment, the goal was to see what subspecies of hardneck and softneck garlics are most commonly sold at supermarkets in New York City, as well as to see if seemingly different subspecies of garlic actually had any major genetic differences. Our secondary goal was to see which subspecies of garlic have the best antibacterial properties.

Materials & Methods

Samples of garlic were collected at supermarkets throughout New York City. We paid little attention to what subspecies each sample resembled, but instead simply purchased whichever types of garlic the supermarket had available. Some of the supermarkets we collected samples from include D'agostino, Whole Foods, and Westside Market.

DNA extraction, PCR amplification, and gel electrophoresis protocols were provided by the Urban Barcode Project and performed in the Biology lab at Friends Seminary. We cut off a small piece of a clove of each sample for each extraction. The larger the amount of garlic we used for the sample, the more difficult it was to separate the garlic fibers from the DNA itself. Additionally, we extracted DNA from one sample twice, as a safety measure in case the DNA extraction was ineffective for one of the extractions.We used seaweed as a positive control, as another UBP group in our school was also using it as a positive control. Plant primers (rbcl) were used in the amplification step. Amplified sequences were electrophoresed to verify proper amplification of the sequence of interest and, if noticeable after electrophoresis, were sent for sequencing.

To complete the antibiotic experiment, we performed a disk diffusion assay, the protocols of which were provided by Gold Biotech and performed in the Biology lab at Friends Seminary. We seeded an E. coli bacterial strain in Luria broth for 24 hours, then prepared plate cultures on Luria agar plates.We then placed sterile filter paper discs which had been dipped in various oil-based garlic extracts made from the garlic samples we had used in the DNA barcoding experiment, a blank disk as a control, and chloramphenicol antibiotic disks to compare the garlic-infused disks to onto the agar plates. To make the garlic extract, we pulverized the peeled garlic in a food processor, then transferred the garlic pulp into coffee filters and squeezed the extract out. To sterilize the extract, we filtered it through a pipet filter. We let the bacteria grow on the agar plates with the antibacterial disks for at least 24 hours, before measuring the zones of inhibition around each respective disk. Photographs were taken for record keeping.

Figure 1
The above photo shows the hardneck garlic agar plate. Sections 2 and 5 correspond to the garlic-soaked discs, while sections 3 and 6 correspond to the chloramphenicol antibacterial discs. Section 1 was left empty, and a blank disc was placed in section 4 as a disc control.

Results

	Identification Numbers	Hardneck / Softneck	Date Purchased	Identified Subspecies by DNA Barcoding
Sample 1	DET-001 DET-002	Softneck	January 19th, 2017	Failed to extract DNA
Sample 2	DET-003 DET-004	Softneck	January 23rd, 2017	Allium sativum
Sample 3	DET-005 DET-006	Softneck	January 23rd, 2017	Allium sativum
Sample 4	DET-007 DET-008	Hardneck	February 2nd, 2017	Allium sativum
Sample 5	DET-009	Hardneck	February 3rd, 2017	Allium sativum
Sample 6	DET-010	Softneck	February 3rd, 2017	Allium sativum

Table 1
The table above shows the number of each sample of garlic, the identification number of each sample's separate sample, the date it was purchased, the subspecies of garlic it was identified as, and the zone of inhibition it created.

Sample #:	Zone of Inhibition for (mm):			
	Garlic Disc 1	**Garlic Disc 2**	**Antibiotic** (chloramphenicol) **Disc 1**	**Antibiotic** (chloramphenicol) **Disc 2**
Sample 1	9.6	9	15	19
Sample 2	11.5	11.5	19	18.5
Sample 3	11.5	11	20	19
Sample 4	9.5	9	20	19
Sample 5	13	13	19	18.5
Hardneck Sample	19	18	20	18

Table 2
The above table shows the zone of inhibition found for the two trials of each garlic-extract soaked disc and the two trials of the antibiotic disk for each sample used. The same antibiotic was used for each sample of garlic. The hardneck sample shown was used in the experiment as we made an oil-based extract from a sample of hardneck garlic purchased in a supermarket to practice making oil extracts, and we decided to use the extract in the experiment.

Figure 2
The map to the left displays the locations of the various samples collected throughout the city Each number on each pin corresponds to the sample number. Because Samples 5 and 6 were purchased from the same location, the pin is not visible for Sample 5, as it is hidden behind Sample 6's pin.

Discussion

Once we received our results and BLASTed them through DNA Subway, each of our samples matched 100% with three different species—Allium sativum, Allium cepa, and Allium dregeanum—as all three of the species have almost the same rbcL DNA sequence. However, because the latter two species are not garlic, we were able to eliminate those. That left us with all five of our samples matching with Allium sativum. We then realized that we amplified a region of the DNA common to all subspecies of garlic, preventing us from seeing the differences between the DNA of the subspecies. We were able to determine whether or not the most commonly sold garlic in New York City was hardneck or softneck, however, due to general morphological characteristics of our samples. Due to these physical observations, we determined that softneck garlic is the most common type of garlic sold in NYC supermarkets.

After conducting the DNA barcoding element of our experiment, we shifted our attention to the antibacterial component of our project. After performing a disk diffusion assay, we observed the results of our experiment. The clear zones of inhibition surrounding each disk that was saturated with one of our samples of garlic extract evidence that garlic does have antibacterial properties. On average, our garlic samples were about 65% as effective as the chloramphenicol antibiotic we also tested. While all of our samples displayed antibacterial properties, samples one and four were only about 49% as effective as the antibiotic, while our sample of hardneck had the largest zone of inhibition, being 100% as effective as chloramphenicol. Sample four's relatively small zone of inhibition is likely the result of our marginally unsuccessful extraction. We only had half a clove of sample four with which to make extract, and, as a result, we did not have enough extract to filter. We decided to test the sample anyway, but our results were likely skewed due to lack of filtration. Sample one, which also had relatively small zones of inhibition, was the only sample that we observed to be purple stripe garlic. It is possible that purple stripe garlic has weaker antibacterial properties. Sample one was also our oldest sample, while our hardneck sample was the freshest sample. This observation implies that the freshness of garlic may impact its antibacterial properties. Sample five, also harneck, was the second most recently purchased, and it had the second largest zones of inhibition. This strengthens the idea that freshness and subspecies (preferably hardneck) impact the antibacterial strength of garlic.

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