Effect of Soil Type and Chill-Hardening on Antimicrobial Properties of *Aloe vera* on *Staphylococcus epidermidis*

Rachel Weinstein and Allyson Bazer

Biotechnology High School

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Plant Systems: Division IV

Biotechnology High School
5000 Kozloski Road
Freehold, NJ 07728
(732) 431-7208

Abstract:

Purpose: *Aloe vera* is a species that is often used to treat burns and wounds due to its well-known antibacterial properties. In an effort to investigate the methods of achieving the highest antimicrobial efficacy, we tested the effects of different factors on the *Aloe vera*. By determining which factors promote the greatest antibacterial productivity, we can inform consumers, agriculturalists and pharmaceutical companies as to which conditions are best for growing and storing their *Aloe vera*.

Procedure: We tested two different independent variables: soil type and chill-hardening. We began by growing one *Aloe vera* plant in each of the three soil types soil types being tested (compost, sandy soil, and potting soil). Three weeks later, the leaves were removed from the plants. Several additional leaves were removed from the potting soil plant, and two leaves were stored at each of the following temperatures: freezer, refrigerator, and room temperature. We then extracted the gel from the leaves, soaked filter paper discs in each extract, and plated these discs on petri dishes with LB agar and S. epidermidis bacterial broth. To find their antimicrobial efficacy, we measured their zones of inhibition.

Conclusion: We found that the plants grown in compost resulted in the greatest antibacterial efficiency out of all of the extracts. Its zones of inhibition were comparable to those of the ampicillin. As for temperature, after analyzing t-test results, the gel from the leaves kept in the freezer was the most effective.

Introduction and Review of Literature:

The gel from *Aloe vera* plants is commonly used for medicinal purposes in order to treat

burns or wounds. It has antimicrobial, antifungal, as well as antiviral properties (Athiban, p. 247, 2012). Many people grow *Aloe vera* plants in their homes and extract the gel for personal use. Knowing which soil type to grow the plants in and at what temperature the gel should be kept for the greatest antimicrobial activity will help consumers to determine the best conditions to store their plants. Additionally, pharmaceutical companies who are selling the gel can list on their products the best storage temperature.

As previously mentioned, *Aloe vera* has a variety of antibacterial properties. Some of these properties were tested in a 2012 study, in which the antimicrobial productivity of *Aloe vera* was evaluated. The purpose of the experiment was to test if *Aloe vera* could effectively decontaminate gutta percha cones, which are commonly used in dentistry to fill root canals (Athiban, p. 247, 2012). Using the agar diffusion method, scientists measured the zones of inhibition that the *Aloe vera* produced for *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus* against the antimicrobial properties of Sodium hypochlorite, a known decontaminant. The major findings were that *Aloe vera* is a successful decontaminant, and it produced very similar zones of inhibition for all three bacterial species to the Sodium hypochlorite (Athiban, p. 248, 2012). To expand on the results of this experiment, it may be interesting to study the antimicrobial properties of *Aloe vera* on different species, such as *Staphylococcus epidermidis*.

The antibacterial properties of the *Aloe vera* will be tested using *Staphylococcus epidermidis*. *S. epidermidis* typically colonizes the skin and mucous membranes, and *Aloe vera* gel is applied topically to the skin. Therefore, applying the *Aloe vera* gel to *S. epidermidis* will simulate the response that would occur *in vivo* in humans. This bacteria is gram positive and

cocci shaped (Rowlinson, 2006, p. 857). Although it was originally thought to be harmless, recent discoveries have shown that *S. epidermidis* causes nosocomial infections, which are infections that occur in a hospital setting. The bacteria has an affinity to foreign materials, such as prosthetic heart valves found in hospitals, and it forms a biofilm over these devices. As a result, *S. epidermidis* causes 50-70% of "intravenous catheter-related infections" (Rowlinson, 2006, p. 858) in hospital patients.

One of the treatments that the *Aloe vera* leaves will be subjected to prior to experimentation is chill-hardening. This is a technique that involves testing the effects of cold temperatures on plants. Chill-hardening exposes plants to cold temperatures in order to preserve the plant, or to build the plant's tolerance to near freezing temperatures. In a 2013 Polish study, scientists investigated the effects of chill-hardening on *Jatropha curcas* seedlings. *J. curcas* is a chilling sensitive yet high energy plant (Ao, p. 154, 2013). The purpose of the experiment was to investigate whether or not chill-hardening improved the seedlings' tolerance to the cold and if an antioxidant defense system was involved with this new improvement. It was found that longer periods of chill-hardening did decrease the chilling stress and death rate of the seedlings. Additionally, the antioxidant system produced enzymes that significantly impacted the plant's chill-tolerance (Ao, p. 159, 2013). In terms of future work, it may prove worthwhile to study the effects of chill-hardening on different plant species, including *Aloe vera*.

The second type of treatment that the *Aloe vera* leaves will be exposed to is varying soil types. Although the seedlings were germinated in potting soil, they were transferred to different types of soil at the start of experimentation. These soil types include: potting soil, sandy soil, and compost. The compost is known to have antifungal properties, as a result of an experiment

published in 2009 testing the effects of different composts on *Pythium aphanidermatum*. *P. aphanidermatum* is a fungus that causes root rot in plants, specifically tomatoes (Jenana, p. 315, 2009). Three different compost mixtures, consisting of chicken manure, solid olive mill wastes, and a seagrass called *Posidonia oceanica*, were sterilized and then applied to the fungus. All of the composts produced an inhibition rate of between 14-22% (Jenana, p. 319, 2009). In our experiment, we thought that it would be interesting to learn about whether or not the compost had antibacterial properties as well. Also, since the antibacterial properties will be tested from the gel of the *Aloe vera* plant that was growing in the compost, not from the compost itself, the properties may be different. The compost used in this experiment was obtained from a home compost bin containing lettuce, apples, and orange peels. Growing plants in compost is an environmentally friendly alternative to throwing away waste, because it recycles the remnants of one plant in order to promote the growth of another.

After learning about chill-hardening and compost, we were led to our research question. Our research question is: "What is the effect of soil type (potting, compost, and sandy) on the antimicrobial properties of *Aloe vera* on *Staphylococcus epidermidis*? Does chill-hardening enhance or negate these antimicrobial properties?" We predict that the compost will be the most successful in enhancing the antimicrobial properties of *Aloe vera*. We predict that the freezer at 0ºC will be the most effective in promoting antimicrobial properties of *Aloe vera*. Our purpose is to help determine the overall best methods of growing and storing *Aloe vera* leaves.

Materials:

The materials used in this project were: 3 *Aloe vera* plants, compost (obtained from home

compost bin), potting soil, sandy soil (obtained from wooded area in Freehold, New Jersey), 3 pots, 2 grow lights, metric ruler, lab marker, scalpel, scoopula, 6 conical tubes, refrigerator, freezer, *Staphylococcus epidermidis*, standard balance, LB broth base, microwave, water bath, L spreader, micropipet (p1000, p200), ampicillin disks, filter paper discs (40), weigh boats, media bottles, 10 petri dishes, microtubes, autoclave, 70% ethanol, parafilm, microtube rack, two 400 mL flasks, magnetic stirrers, hot plate, hot hands, autoclave, autoclave tape, flame stick.

Methods:

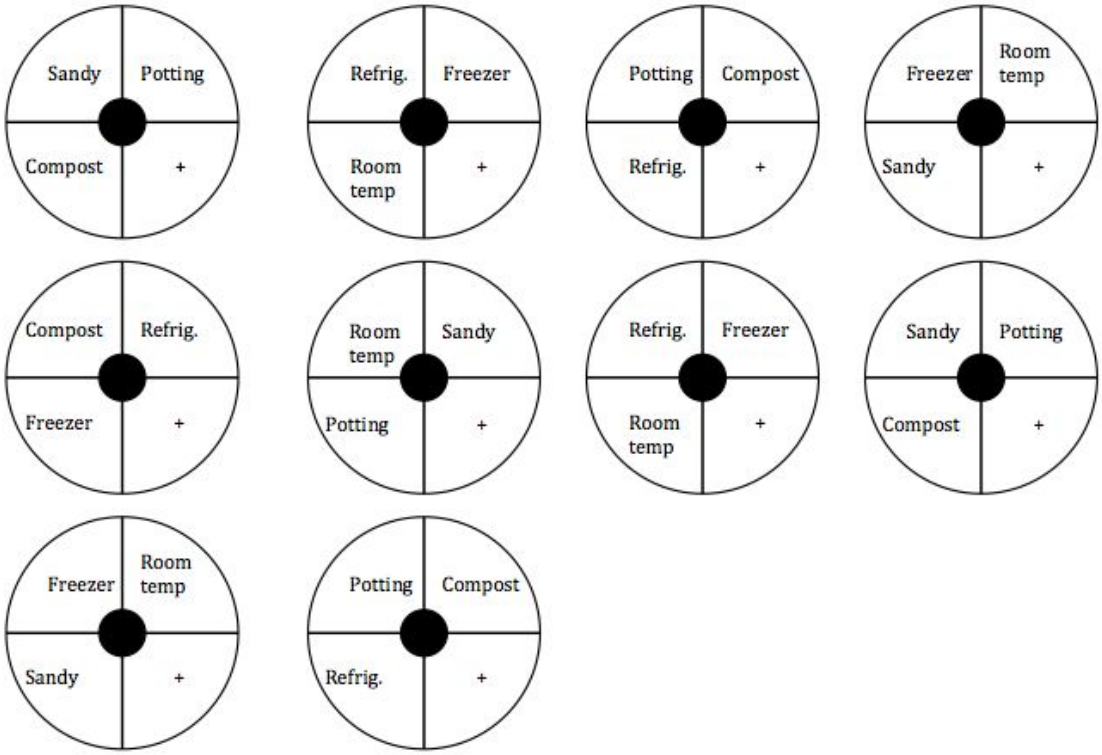
To start, three *Aloe vera* plants were obtained, and three pots of soil were prepared: one containing potting soil, one containing sandy soil, and one containing compost. One plant was put in each of the pots, and they were placed on a table underneath two grow lights. Water was poured into the trays at the base of the each pot as needed to allow the plants free access to the water. After approximately three weeks, two leaves were cut off of each plant, they were wrapped in aluminum foil, and they were stored in an airtight bag in the refrigerator until further experimentation. Three days before experimentation, all of the remaining leaves were cut off of the plant growing in potting soil. The leaves were wrapped in aluminum foil and stored in airtight bags, with two leaves in the freezer, two in the refrigerator, and two kept at room temperature.

To obtain the gel from within the *Aloe vera* leaves, the scalpel was used to slice each leaf in half, lengthwise. A scoopula was used to scoop all of the gel out of the leaves and into the appropriately labeled conical tubes. These conical tubes were stored in the refrigerator, with the exception of the leaves that were to be kept at room temperature and in the freezer, whose gels

were stored accordingly.

Next, ten plates were labeled as shown below, and 25 mL of LB agar was poured into each plate. Once the plates were autoclaved, they were inoculated with *Staphylococcus epidermidis* broth. In the meantime, five filter paper discs were soaked in each of the six extracts and ten filter paper disks were soaked in distilled water for 48 hours. Then, forceps were used to place the filter paper discs in the appropriate locations on each plate. After waiting thirty minutes, the plates were inverted. The plates were stored in an incubator, and the zones of inhibition were measured after 24 and 48 hours. To determine how to prepare the broth and the petri dishes, Daugherty's lab manual was referenced (Daugherty, 2007).

In terms of data collection, the zones of inhibition for each extract on each plate were measured using a metric ruler. The resulting measurements were recorded in millimeters. For statistical analysis, means of each extract's zones of inhibition at 24 and 48 hours were taken, as well as their standard deviations. T-tests comparing all of the extracts to one another, to the controls, and comparing the 24 and 48 hour measurements were conducted.



Independent variables	-Soil type (sandy, potting, or compost) -Temperature (freezer, refrigerator, room temperature)
Dependent variable (quantitative)	-Zone of inhibition area (mm)
Positive control	-Ampicillin
Negative control	-Distilled water
Constants	-Type of *Aloe vera* plants -Method of gel extraction -Method of growing *Aloe vera* plants -Type of bacteria used (*S. epidermidis*)
Number of replicates	5 replicates for each treatment group

Results:

At the end of the experiment, we measured the zones of inhibition for each of the extracts after 24 and 48 hours. These values were averaged, and the resulting values are listed in Table 2.1. The zones of inhibition for all of the extracts were individually greater than the average zones of inhibition for the distilled water. This suggests that the *Aloe vera* does have antibacterial properties, disregarding any further treatment to the gel. The table also shows that the compost resulted in greater zones of inhibition than the positive control (ampicillin). However, the compost had a very large standard deviation, as seen in Graph 1.2. On some of the plates, the compost had very minimal zones of inhibition, while on others it far surpassed the inhibition of the ampicillin. In comparison to the compost, the other treatments had small standard deviations. Out of all of the chill-hardening treatments, the room temperature had the greatest average inhibition, although not by much, but it also had large standard deviations.

We also conducted many t-tests. We compared the treatments to each other, to the controls, as well as comparing the measurements taken at 24 and 48 hours. The only t-tests that resulted in a significant difference in Table 3.1 were comparing compost to other soil types. This suggests that compost truly does enhance the antimicrobial properties of the *Aloe vera*, and it is not due to statistical uncertainties or error. All of the other treatments showed no significant difference, suggesting that any differences between them are merely due to chance.

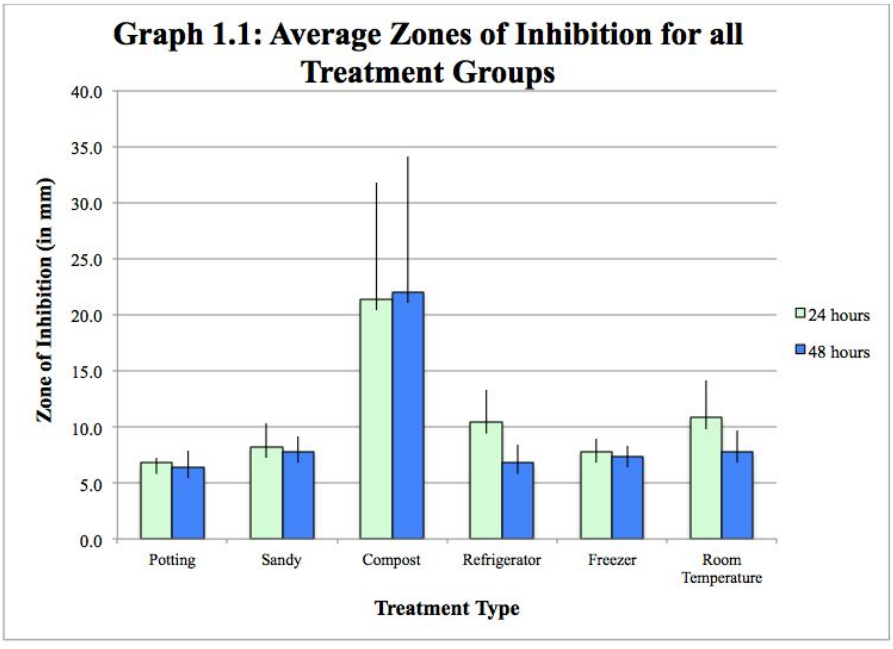
However, when comparing the extracts to the controls, compost showed no significant difference with the ampicillin, suggesting that both treatments are equally effective. In terms of temperature, there was a significant difference between the freezer, refrigerator, and room temperature extracts compared to ampicillin, suggesting that none of these treatments are as effective as the ampicillin would be. As shown in Table 3.3, there is a significant difference

between the freezer and distilled water at 48 hours, which suggests that even though the freezer is not as effective as ampicillin, it is still more effective than no treatment at all. The refrigerator showed a significant difference at 24 hours but not at 48 hours, suggesting that the inhibitory effects of the refrigerator may only be effective for a short period of time. Surprisingly, the room temperature showed no significant difference with the distilled water at 24 or 48 hours.

Table 2.1: Averages (mm)

	24 hours	*48 hours*
Potting	6.8	6.4
Sandy	8.2	7.8
Compost	21.4	22
Refrigerator	10.4	6.8
Freezer	7.8	7.4
Room Temperature	10.8	7.8
dH_2O	6.7	6.1
Ampicillin	20.4	19.4

The data in Table 2.1 shows that compost had the greatest average zones of inhibition. Each value listed in the table is an average of 5 trials.



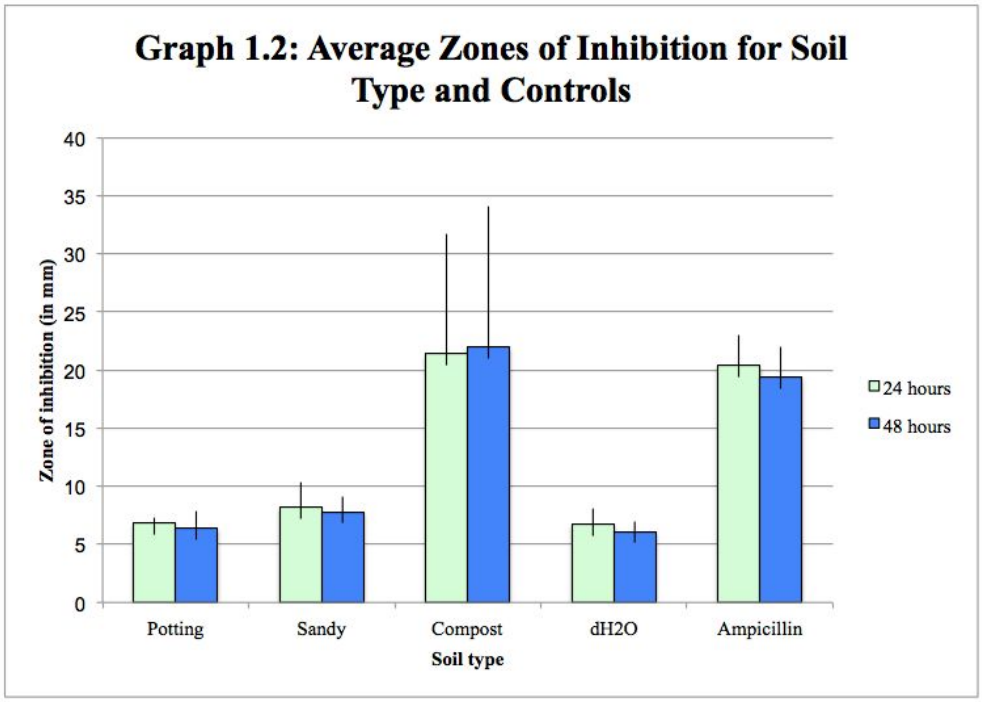
Graph 1.1 displays each of the six extracts' zones of inhibition at both 24 and 48 hours, along with their standard deviation bars.

Table 3.1: T-tests Comparing Treatments	
Comparison	*P-value*
Compost to potting (24 hours)	0.03
Sandy to potting (24 hours)	0.22
Sandy to compost (24 hours)	0.04
Refrigerator to freezer (24 hours)	0.12
Freezer to room temperature (24 hours)	0.12
Refrigerator to room temperature (24 hours)	0.84
Compost to potting (48 hours)	0.04
Sandy to potting (48 hours)	0.16
Sandy to compost (48 hours)	0.06
Refrigerator to freezer (48 hours)	0.50
Freezer to room temperature (48 hours)	0.69
Refrigerator to room temperature (48 hours)	0.40
24-48 hours (potting)	0.59
24-48 hours (sandy)	0.62
24-48 hours (compost)	0.67
24-48 hours (room temperature)	0.07
24-48 hours (refrigerator)	0.10
24-48 hours (freezer)	0.65

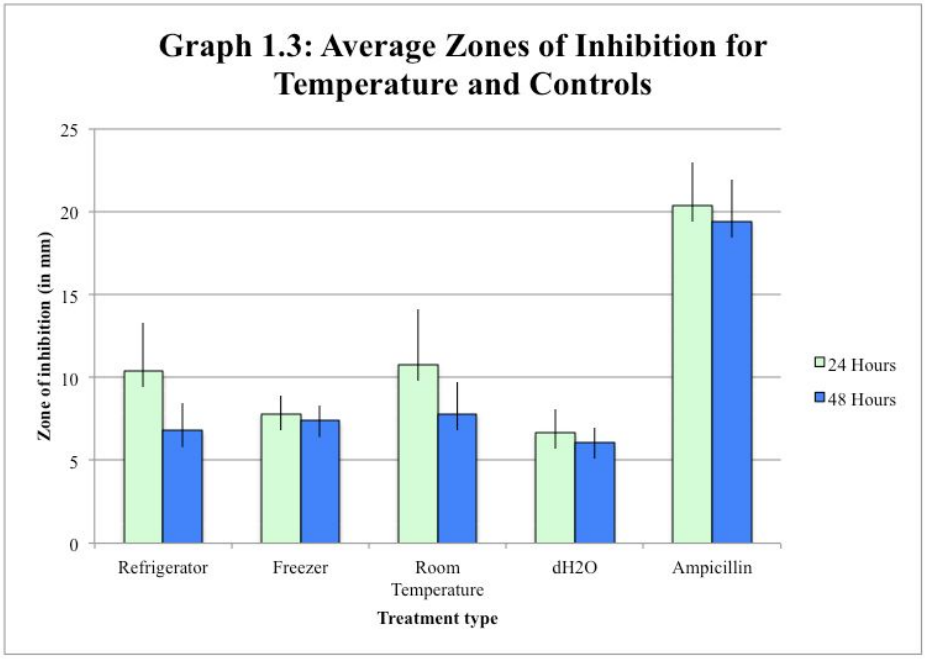
Table 3.2 T-tests Comparing Treatments to Controls (24 hours)						
	Potting	*Sandy*	*Compost*	*Refrigerator*	*Freezer*	*Room Temperature*
dH_2O	0.84	0.21	0.03	0.04	0.27	0.30
Ampicillin	1.69E-08	3.15E-06	0.78	0.00	7.36E-09	5.41E-07

Table 3.3 T-tests Comparing Treatments to Controls (48 hours)						
	Potting	*Sandy*	*Compost*	*Refrigerator*	*Freezer*	*Room Temperature*
dH_2O	0.70	0.04	0.04	0.41	0.00	0.12
Ampicillin	2.63E-08	3.12E-08	0.66	0.00	0.03	1.25E-06

In Tables 3.1, 3.2, and 3.3, the boxes that are shaded in with green represent values with a significant difference. Thier p values were < 0.05.



Graph 1.2 compares the different soil types to the controls. It is apparent that compost was the most effective out of all the soil types, and it is similar to the ampicillin. It is also important to note that the compost's zone of inhibition increased from 24-48 hours.



Graph 1.3 compares the average zones of inhibition for the different temperatures and the controls. Although room temperature appears to be the greatest out of all the temperatures, its standard deviation is the greatest. Note that all of the temperatures' zones of inhibition decrease from 24 to 48 hours.

Discussion:

This experiment proved very successful in allowing us to determine the best methods for growing and storing *Aloe vera* leaves in order to produce the greatest antimicrobial activity in the gel. The first variable being tested was soil type. Compost was very successful in inhibiting the bacterial growth. It produced zones of inhibition that far surpassed the zones of inhibition for potting soil and sandy soil. The compost also showed no statistical difference with ampicillin at both 24 and 48 hours, meaning that it is equally effective with the positive control. The zones of inhibition for the compost grew larger from 24 to 48 hours, suggesting that the effects of the compost are long lasting and not merely transitory. The sandy soil was effective as well, but its zones of inhibition decreased by the second day. It also only showed a statistical difference with the negative control after 24 hours, suggesting that the treatment may not be long lasting. Potting soil produced some inhibition, but it showed no statistical difference with the negative control, and its averages were far lower than those of the other soil types.

One interesting problem that occurred while growing the plants was that a thin layer of white mold grew over the top of the compost. This layer was quickly removed and disposed of, but the fungus may have had some effect on the plant. Despite this, the leaves grown in compost were still very effective in bacterial inhibition. No mold was present on the leaves themselves, and when extracting the gel from the leaves, it was clear and the same consistency as the gel from the other plants.

In terms of which temperature was the most effective, at first glance it may seem that the room temperature gel resulted in the greatest antimicrobial activity versus the freezer and the refrigerator, but further investigation proved this not to be so. The room temperature did not

show significant differences with the distilled water at 24 or 48 hours, suggesting that any differences between the two are merely due to chance. This may seem odd, as the room temperature did have the largest average zones of inhibition over the other two temperatures, but t-tests also take into account standard deviation. The large standard deviation of the room temperature may explain why there was no significant difference between it and the distilled water. After 24 hours, the refrigerator had a significant difference with the distilled water, but it did not at 48 hours. This means that the antimicrobial properties from the refrigerator are only temporary. The freezer, on the other hand, showed a significant difference with the distilled water at 48 hours. This means that its antibacterial properties last longer than those of the refrigerator, and that they are significant. Even though the means of the freezer were less than those of the room temperature, the results of the t-tests show that the freezer is indeed more effective. However, with only five trials for each sample and many other possible errors that may have occurred, we cannot rule out the possibility that these results may not be accurate. Some other sources of error include contamination of the samples, the fungus growing on the compost that was discussed above, and errors with calculations or measurements.

In general, our results did support our hypothesis. The compost was the most effective out

of all of the soil types, as we predicted. The temperatures, on the other hand, were more difficult to analyze. Using the results of the t-tests, the freezer was the most effective. But, it may prove worthwhile to repeat the experiment with more trials. Another improvement to the experiment would be to measure the zones of inhibition at 72 hours as well. This would allow us to tell which of the treatments truly did produce long-lasting results. Other ideas for future work

include testing the effects of compost on plants other than *Aloe vera*, or testing the effects of chill-hardening on a variety of plants.

These results are consistent with the data obtained from other scientists. Similarly to Dr. Ao's 2013 study, chill-hardening did enhance and preserve the properties of the plants (Ao, p. 159, 2013). We learned quite a bit while performing this experiment. We not only answered our research question, but we learned from irregularities that came to our attention during experimentation. One surprising result was that all of the filter paper discs soaked in the room temperature extract, as well as the gel itself, turned a coppery red color after a few days. After a bit of research, we found that *Aloe vera* gel is very prone to oxidative degradation, meaning that unless it is stored in the appropriate conditions, the iron in the gel can react with oxygen (World Health Organization, p. 45, 1999). This reaction often results in a color change like the one that we observed.

This experiment has a variety of real-world applications, both for consumers, agriculturists, and pharmaceutical companies. *Aloe vera* gel is commonly sold at pharmacies, and it is typically marketed as a pain relieving gel. Based on our results, *Aloe vera* gel from leaves grown in compost and then stored in the freezer has been found to have significant antimicrobial properties, and can therefore be marketed as an antibiotic. This antibiotic would be equally effective, if not more effective, than ampicillin at treating nosocomial infections resulting from *S. epidermidis*. This new indication of the product can result in an increased profit for both the pharmaceutical company, and the farmers who work to cultivate the plants. Agriculturists will also benefit in that they can compost their leftover plant products and use them to enhance the properties of *Aloe vera*. This will help the plants to be better suited for the pharmaceutical

market, and it is a natural way of reusing waste materials. As for consumers, pharmaceutical companies can now list on their product the most effective storage temperature. This will help the consumer to get the most effective results from the *Aloe vera* gel.

Our results answered our research question and allowed us to conclude which methods are the best for treating *Aloe vera* leaves. Growing the plant in compost will prove to be beneficial in that it helps to promote recycling and reuse of waste materials. Knowing to store the leaves at cold temperatures will help to provide consumers with the best results when applying *Aloe vera* gel onto their wounds, in that it will minimize the bacterial infections that occur.

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Appendix:

Table 1.1: Raw Data – Zones of Inhibition at 24 Hours (in mm)					
Potting	*Sandy*	*Compost*	*Refrigerator*	*Freezer*	*Room Temp.*
7	10	21	15	8	11
7	10	33	7	8	7
7	7	30	10	9	13
6	9	8	10	8	15
7	5	15	10	6	8

Table 1.2: Raw Data – Zones of Inhibition at 48 Hours (in mm)					
Potting	*Sandy*	*Compost*	*Refrigerator*	*Freezer*	*Room Temp.*
5	8	20	5	8	8
9	10	34	6	8	5
6	7	35	8	6	7
6	7	9	6	7	10
6	7	12	9	8	9

Table 1.3: Raw Data – Positive and Negative Controls at 24 Hours		
Plate #	*dH_2O: - Control (mm)*	*Ampicillin: + Control (mm)*
1	5	23
2	5	19
3	6	20
4	7	25
5	7	20
6	9	23
7	7	17
8	8	20
9	8	20
10	5	17

Table 1.4: Raw Data – Positive and Negative Controls at 48 Hours		
Plate #	*dH_2O: - Control (mm)*	*Ampicillin: + Control (mm)*
1	5	21
2	5	19
3	6	20
4	5	19
5	7	20
6	7	25
7	7	17
8	7	20
9	6	17
10	6	16