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Article

Use of yogurt bacteria as a model surrogate to compare household cleaning solutions

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SUMMARY

With the growing number of pathogens that are responsible for human disease, there is an urgent need to compare the relative efficacy of various household cleaning chemicals. These chemicals play an important role in preventing the spread of disease in the home setting, but there is no uniform way of comparing these cleaning products. In addition, there is no readily available guidance for the consumer while purchasing household cleaning products in terms of their efficacy in destroying bacteria. This research addresses this need by studying the inactivation of yogurt bacteria in the presence of a variety of cleaning products. We found that several of the cleaning products (Citrus II Hospital Germicidal Deodorizing Cleaner, Lysol All-Purpose Cleaner, and Earth Essentials Multipurpose Cleaner) were very effective at rapidly destroying bacteria. On the other hand, several other cleaning products including soap and alcohol-based hand sanitizer were only partially efficacious. Water as a control and one other cleaner were not at all effective at destroying bacteria. We compared the efficacy of cleaning products on bacteria isolated from common public surfaces and found good correlation with the results from the yogurt model. The effective concentration of isopropyl alcohol in hand sanitizer was studied using this model system. The results support our hypothesis that yogurt bacteria are a safe and readily available model system that can be used to rank the relative efficacy of several commonly used household cleaning products, as well as potentially for other studies on bacterial inactivation.

INTRODUCTION

Household cleaning chemicals are widely used to clean surfaces in a home environment and reduce the spread of diseases caused by bacteria and viruses (1, 2). This is particularly important for more at-risk populations such as the very young, the elderly, and the immunocompromised (3). Bacteria are single-celled living organisms, while viruses are much smaller non-living organisms that only grow and reproduce inside of the host cells they infect. However, both bacteria and viruses can cause diseases that are spread by routes of coughing, sneezing, contact with contaminated surfaces, water or food contamination, and skin-to-skin contact. Household cleaning chemicals can reduce the spread of diseases by sanitizing contaminated surfaces (2). The use of household chemicals increased significantly during the COVID pandemic due to the panic related to the disease (4). A shortage of these household cleaning chemicals during the peak of the pandemic in 2020 led consumers to purchase unfamiliar brands of household cleaning products (5). Typically, the average consumer has no way of knowing which products are effective in controlling bacteria and viruses. Even though the COVID pandemic was caused by a virus, most of these household chemicals are also used to reduce the presence of harmful bacteria in home environments.

The COVID pandemic often led to the overuse or improper use of these chemicals (6). Improper use of household cleaning products causes problems of skin disturbances, shortness of breath, and unwanted consumption of bleach (6). Researchers have compared a bleach-based product with an environmentally preferable product with fewer respiratory irritants for inactivation of *Staphylococcus aureus* and *Clostridium difficile* on home surfaces (7). The environmentally preferable product from Seventh Generation Inc., Burlington, VT was equivalent to the bleach-containing product for *Staphylococcus aureus* and *Clostridium difficile* inactivation (7). There are several resources for consumers to identify natural or environmentally safe household cleaning products (8-10). While these resources focus on the safety of the consumer when using these household cleaning products, they ignore the efficacy of these products in destroying pathogens. In fact, there is no single framework to study the relative efficacy of household cleaning products. As a result, consumers are often confused about which chemical to use with very few scientifically based articles to help them (11).

There are only a few published studies comparing the efficacy of home sanitizing chemicals for bacterial inactivation (12-15). The food processing industry has compared various chemicals sanitizers such as lactic acid, peroxyacetic acid, calcium hypochlorite, and ozonated water for cleaning spinach leaves (12). In that study, 2% lactic acid at 55°C was effective in reducing *Salmonella* and *Escherichia coli* (*E. coli*) (12). The poultry industry has compared the efficacy of disinfectants such as a phenolic compound, a quaternary ammonium compound, a nascent oxygen compound, and a compound that contains potassium peroxymonosulfate for cleaning the floor of poultry farms (13). Researchers have compared bleach, thymol, and white vinegar solutions in terms of their efficacy in destroying *Staphylococcus aureus* and *E. coli* on home surfaces (3). Bleach and an environmentally preferable product containing thymol achieved robust disinfection on multiple surfaces (3). Several common household cleaning agents, such as 1% bleach, 10% vinegar, and soap completely inactivated the human influenza virus (14). While comparing several common household chemicals such as vinegar, bleach, soap, and ethanol for the inactivation of the SARS-CoV-2 virus, vinegar was completely ineffective in inactivating SARS-CoV-2, while ethanol, bleach, and soap were effective (15).

There is significant recent interest in studying the antimicrobial properties of certain novel agents and in being able to understand the efficacy of hand sanitizers (16, 17). However, these studies have historically relied upon microbes cultured from common surfaces at home or on bacteria isolated from dirty hands or cell phones (16). This does not enable other researchers to replicate previous results or further extend these studies readily. Thus, there is a need to establish bacterial systems that are relatively easy and robust to use by researchers and provide a commonly available system. In addition, bacteria found on common surfaces can be dangerous to culture on petri plates due to the unknown nature of the bacteria, particularly if the cultured bacteria are not handled with appropriate safety precautions (18). This is often a concern for middle and high school projects, with the result that many science fairs discourage the culture of bacteria from public surfaces.

We sought to establish yogurt bacteria as a readily available and reliable source of bacteria that could be used to study the comparative efficacy of various household cleaning chemicals. Yogurt is manufactured by culturing the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in milk (19). Yogurt is typically made from cow's milk and possesses a gel structure due to the coagulation of proteins by lactic acid produced by the two bacteria species. Yogurt is typically made from a starter culture which is incubated with milk at a temperature of 108-112°F.

In this paper, we hypothesized that the relative sanitization efficacy of various commonly purchased household cleaning chemicals could be compared using readily available yogurt bacteria as a model system. We also hypothesized that during inactivation studies, results using bacteria from swabbing common public surfaces would correlate well with those obtained using yogurt bacteria. Bacterial inactivation data in the presence of cleaning products showed that these products had a range of sanitization efficacies, supporting our hypothesis. We compared inactivation data using yogurt bacteria and bacteria from common public surfaces and found good correlation between the two datasets, also supporting our hypothesis. In addition, we used the yogurt model to study the effect of isopropyl alcohol concentration (the active ingredient in most hand sanitizers) on inactivation of bacteria.

The results shown here demonstrate that yogurt bacteria could be a very useful, safe and readily available model system for all sorts of scientific studies involving bacteria. More specifically, this model system enabled an effective comparison of various household cleaning products and showed good correlations with bacteria obtained from common public surfaces. This model system was also successful in determining the concentration of isopropyl alcohol-based hand sanitizer that could effectively inactivate bacteria.

RESULTS

Effectiveness of cleaning solutions on yogurt bacteria

We used the yogurt bacteria model to test the effectiveness of the different cleaning solutions. We treated the diluted yogurt solution with samples of the various cleaning solutions, placed them on an agar plate, and incubated them. We took photographs of the plates against a dark background using an iPhone to observe the bacterial colonies (**Figure 1A-C**). Each quadrant on an agar plate represents bacteria in a different cleaning solution. We studied a wide range of household cleaning solutions (**Figure 1D**). We counted the number of distinct bacterial colonies in each quadrant of the agar plate and plotted the data (**Figure 2**). Citrus II Hospital Germicidal Cleaner, Lysol All-Purpose Cleaner, and Earth Essentials Multipurpose Cleaner had no colonies of bacteria left. Mrs. Meyer's Clean Day Multi-Surface Cleaner and soap solution had a small but discernable number of colonies of bacteria remaining. Alcohol-based hand sanitizer and Mr. Clean Clean Freak Deep Cleaning Multisurface Spray had some reduction in the number of colonies when compared to water (control) but had numerous colonies still remaining. On the other hand, water (control) and Granite Gold Daily Cleaner had a large

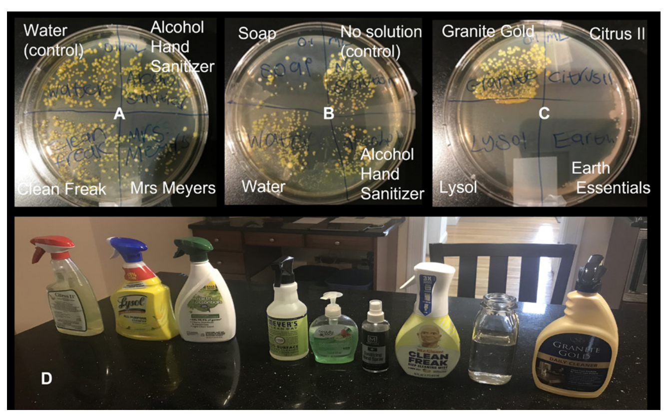

Figure 1: Comparison of the efficacy of various cleaning solutions in deactivating bacteria. Clockwise from the top, left quadrant comparison of A) Water (control), alcohol-based hand sanitizer, Mrs. Meyer's Clean Day, and Clean Freak. B) Soap, no solution (control), alcohol-based hand sanitizer, and water. C) Granite Gold Daily Cleaner, Citrus II Hospital Germicidal Deodorizing Cleaner, Earth Essentials Multipurpose Cleaner, Lysol All-Purpose Cleaner. D) Various household cleaning products compared in this study. Yogurt bacteria were exposed in solution to the various cleaning products for 30 min and then plated on agar.

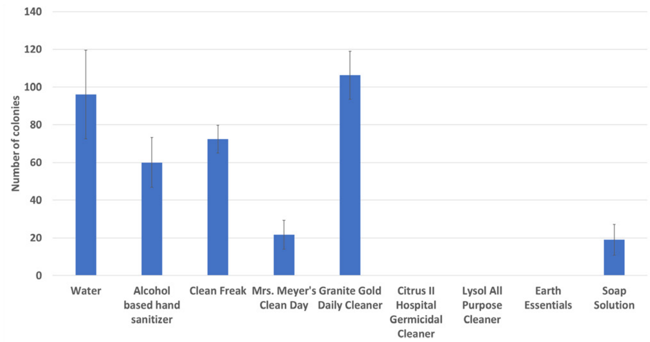

Figure 2: Comparison of the efficacy of various household cleaning products. Bar graph of the number of yogurt bacterial colonies counted after 30 min treatment with various cleaning chemicals. Yogurt bacteria were exposed in solution to the various cleaning products for 30 min and then plated on agar. Error bars for each of the conditions indicate ± 1 standard deviation. A *p*-value of 1.51e-09 was obtained from single factor ANOVA analysis.

number of bacterial colonies still remaining after treatment with the chemical (**Figures 1A-C,2**). These experiments were conducted in triplicate to enable calculation of error bars using ± 1 standard deviation. Statistical significance of the data was determined by single factor ANOVA. A *p*- value of 1.51e-09 (< 0.05) was found. A specific analysis of which datasets were statistically different from the control (water) condition was conducted by the Tukey-Kramer test. All conditions were found to be statistically different from the control (water) condition, except the Granite Gold Daily Cleaner.

Effectiveness of cleaning solutions on school surfaces

We wanted to study bacteria from common public surfaces at school to understand their correlation with the yogurt bacteria system. We swabbed four commonly touched surfaces and cultured their bacteria on an agar plate. This enabled the bacterial count from various common surfaces at school to be visualized (**Figure 3A**). We found the table at the HW Café and knob for the water fountain to be relatively clean because they had 3 colonies and 1 colony of bacteria, respectively. The entrance door and the door handle for restroom were more heavily contaminated because they had 25 and 26 colonies of bacteria, respectively (**Figure 3A**).

Based on our findings of the yogurt model, we selected Citrus II Hospital Germicidal and Lysol All-Purpose Cleaner to test on a pool of bacteria obtained from common school surfaces. In addition, we chose a hand sanitizer solution given the frequency of its use by students and school faculty. We treated the pool of bacteria from common public surfaces in a comparable fashion as yogurt bacteria in this experiment. We studied the effect of these cleaning chemicals on a pool of bacteria from the four common surfaces at school (**Figure 3B**). The control without any cleaning solution had 28 colonies of bacteria. In contrast, a 20% solution of isopropyl alcohol-based hand sanitizer, Citrus II Hospital Germicidal Cleaner, and Lysol All-Purpose Cleaner killed all of the bacteria in 30 minutes (**Figure 3B**).

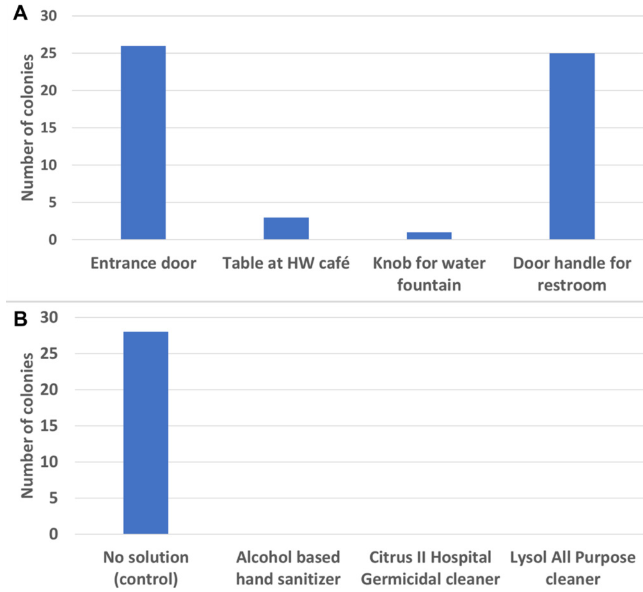

Figure 3: Effect of cleaning products on bacteria from common public surfaces at school. A) Bar graph of the number of colonies observed in swab samples from various common surfaces at school. Swab samples from various surfaces were redissolved in water and plated on agar. B) Bar graph of the number of colonies seen after exposing a pool of bacteria from A to various cleaning products for 30 min. A pool of bacteria from all four surfaces in A were exposed to various cleaning products for 30 min before plating on agar.

Yogurt bacteria deactivation with isopropyl alcohol

Hand sanitizers containing isopropyl alcohol are widely used for cleaning hands following the pandemic (17). The CDC recommends that hand sanitizers should have at least 60% alcohol (20). Sanitizers composed of 100% alcohol is not recommended because it would evaporate too quickly to kill bacteria effectively (21). Thus, we studied the effect of the concentration of isopropyl alcohol on inactivation of yogurt bacteria (**Figure 4**). We used a hand sanitizer solution that contained 70% isopropanol. We diluted the 70% isopropanol solution with water to obtain various final concentrations of isopropanol ranging from 0 to 50%. Then, we mixed these solutions with the yogurt bacteria solution. The 0% isopropanol solution had 115 colonies, 10% isopropanol solution had 112 colonies, 20% isopropanol had 77 colonies, and 30% isopropanol had 15 colonies. The 40% and 50% solutions of isopropanol completely inactivated yogurt bacteria, as no colonies were observed (**Figure 4**).

DISCUSSION

The cleaning efficacy data of the various cleaning solutions fell into four categories (**Figure 1,2**). Two solutions including water (used as a control) and Granite Gold Daily Cleaner were not at all effective in inactivating yogurt bacteria. Colony counts for these chemicals fell in the range of 70-120 residual colonies after treatment with the chemical. In the

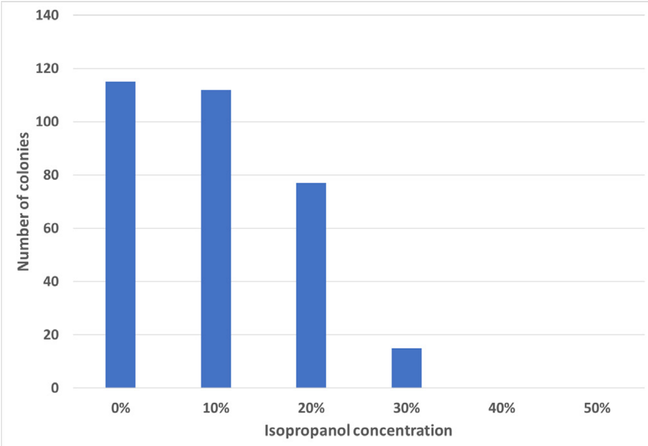

Figure 4: Effect of various concentrations of isopropanol from hand sanitizer solution on inactivation of yogurt bacteria. Bar graph showing the number of bacterial colonies counted after exposing yogurt bacteria in solution to various concentrations of isopropanol from a hand sanitizer solution. Yogurt bacteria were exposed to various concentrations of isopropanol from hand sanitizer solution for 30 min before plating on agar.

second category, we found alcohol-based hand sanitizer and Mr. Clean Clean Freak Deep Cleaning Multisurface Spray to only be slightly effective with 50-80 colonies of bacteria remaining. In the third category, Mrs. Meyer's Clean Day Multi-Surface Cleaner and soap solution to be moderately effective since they had 10-30 colonies of bacteria remaining, respectively. However, in the fourth category, we observed complete inactivation of bacteria with zero colonies observed for Citrus II Hospital Germicidal Cleaner, Lysol All-Purpose Cleaner, and Earth Essentials Multipurpose Cleaner. This final category of cleaning solutions had significantly greater efficacy as compared to the other cleaning solutions.

The small *p*-value for the single factor ANOVA indicates that the data for the different cleaning solutions have a statistically significant difference from each other. The Tukey-Kramer post-hoc procedure helps to determine which specific datasets are statistically different from each other. All cleaning solutions studied were found to be statistically different from the control (water) condition which shows that each of them have a statistically discernable effect on the bacteria. The only exception to this was the Granite Gold Daily Cleaner, which was found not to be statistically different from the control. Hence this solution was not at all effective in deactivating bacteria.

These results have demonstrated a successful methodology for comparing various household cleaning chemicals with each other. The results also suggest that Lysol All-Purpose Cleaner, Citrus II Hospital Germicidal Cleaner, and Earth Essentials Multipurpose Cleaner are some of the most effective household cleaning chemicals. On the other hand, we found several commonly used cleaning products such as soap solution, Mr. Clean Clean Freak Deep Cleaning Multi Surface Spray, Mrs. Meyer's Clean Day Multi-Surface Cleaner and Granite Gold Daily Cleaner, not to be effective in inactivating bacteria. This comparison provides the consumer with a benchmark to determine which product to purchase for home use. Without the results from such a comparative study, there are no benchmarks a consumer could follow for purchasing effective surface cleaners.

Several researchers in the past have relied on swabbing surfaces to obtain bacteria to study (22). That methodology is not always reproducible or safe for experiments due to the unknown nature of the bacterial population on surfaces. However, it is important to study bacteria obtained from common public surfaces to see if the yogurt bacteria could serve as a useful surrogate model. Various common public surfaces at school were swabbed to see if they had a significant bacterial count (**Figure 3A**). Higher bacterial counts were obtained for the entrance door and the door handle for the restroom, as compared to a common use table surface and the knob for the water fountain. The table at the HW Café likely did not have many bacteria because the surfaces in the HW Café were frequently cleaned in accordance with school policy during the pandemic. The knob for the water fountain likely did not have many bacteria because use of the water fountain was discouraged at the time due to COVID-19 control policies. The entrance door and the door handle for the restroom were frequently used by students and school faculty with more limited frequency of cleaning compared to the HW Café surfaces, leading to a higher bacteria colony count.

In general, there was good agreement between the results obtained by using yogurt bacteria in comparison with bacteria from common public surfaces. Both Citrus II Hospital Germicidal Cleaner and Lysol All-Purpose Cleaner destroyed both yogurt bacteria and bacteria from common surfaces (**Figure 3B**). Interestingly, a difference was observed with the alcohol-based hand sanitizer. The alcohol-based hand sanitizer was seen to be effective at even a 20% concentration on bacteria from common public surfaces at school. However, the same concentration of isopropanol did not completely inactivate yogurt bacteria (**Figure 2**).

Subsequently, we further studied the effect of isopropanol concentration from hand sanitizer on yogurt bacteria over a wider range of isopropanol concentrations (**Figure 4**). This data suggests that the number of colonies of bacteria decreased when the percent of isopropyl alcohol was increased. However, the yogurt bacteria were able to survive treatment with up to 30% hand sanitizer. Inactivation of the yogurt bacteria required a higher concentration of isopropanol (40% or higher). This data suggests that there are some differences in alcohol resistance between yogurt bacteria and bacteria from common public surfaces. This is not surprising since these are fundamentally different species of bacteria. Nevertheless, the fact that in general, the cleaning chemicals had a similar effect on yogurt bacteria as compared to bacteria from common public surfaces, demonstrated that the yogurt model was a useful surrogate for our study.

The limitations of this study include the fact that yogurt is a mixture of two different kinds of bacteria and that these bacteria may be present in a wide range of concentrations depending on how the yogurt was produced. Actual numerical counts of bacteria being cultured from yogurt could vary depending on its source. Additionally, achieving a uniform solution of a consistency that can be easily pipetted can be a limitation while working with yogurt. For the purposes of our study, we maintained a consistent procedure in producing the yogurt as detailed in the Methods section. Additionally, a 3% yogurt solution in water was found to be easily pipetted and was generated using a volumetric serial dilution. This procedure served our purpose of achieving a relative comparison of various cleaning solutions well. The quantitation of bacterial colonies is also subject to some subjectivity since only well-separated individual colonies are counted. Nevertheless, relative efficacy of the cleaning products can be easily gained using an approximate count. The study we conducted was limited to bacterial inactivation and did not study viruses, owing to the risks involved in handling pathogenic viruses.

In the future, one could use the yogurt model to test other novel chemicals for their use as household cleaning agents. This would help fulfill the need for environmentally safe and benign household cleaning chemicals that do not harm humans and at the same time are efficacious in destroying bacteria. In addition, this model can be used to compare the efficacy of the large diversity of household cleaning products that are available and create a useful database for consumers. In general, the yogurt bacteria system can potentially be a useful, readily available and safe surrogate system for scientific studies on bacterial growth and inactivation.

MATERIALS AND METHODS

Preparation of yogurt and yogurt solutions

Bacteria in yogurt were used as a model to study what can kill the bacteria. The yogurt was made by incubating 1 L of 1% fat milk (Stonyfield 1% milk fat organic milk) mixed with 3 tablespoons of yogurt starter for 3 h at 110°F using an Instant Pot System. A 30% v/v solution of homemade yogurt was made in water by pouring 3 mL of yogurt into a conical tube and diluting with 7 mL of deionized water. This 30% solution was then further diluted to create a 3% stock solution for the experiments.

Comparison of cleaning chemicals by inactivation of yogurt bacteria

4 mL of the 3% yogurt solution was pipetted into a 15 mL conical tube. 0.1 mL of various cleaning solutions were then added to each tube. The cleaning solutions that were compared were: water (as a control), 70% isopropyl alcohol-based hand sanitizer (Purell Advanced hand sanitizer), soap solution (Softsoap liquid soap), Mr. Clean Clean Freak Deep Cleaning Multi Surface Spray, Mrs. Meyer's Clean Day Multi-Surface Cleaner, Granite Gold Daily Cleaner, Citrus II Hospital Germicidal Deodorizing Cleaner, Lysol All-Purpose Cleaner, and Earth Essentials Multipurpose Cleaner.

The tubes were mixed and allowed to sit at room temperature for 30 minutes. Following this, 100 µL of each solution was pipetted onto each quadrant of an agar plate (EZ Bioresearch, pre-poured LB agar plate) and spread using a sterile swab. The plates were incubated at 95°F for 5 days in an incubator. Five days was experimentally established as a suitable incubation period to maximize the number of visible colonies on the agar plate. At the end of the incubation period, colonies on the agar plates were counted visually to determine the number of colonies.

Comparison with bacteria from common public surfaces

Bacteria from common surfaces at school were collected using a wet sterile swab. The surfaces swabbed included an entrance door, a table at Homework Café, a knob for the water fountain, and a door handle for the restroom. The wet sterile swab was rubbed on these surfaces for approximately 20s and then immersed in 5 mL of water purified using a Milli Q IQ water purification system. The sterile swabs were agitated and squeezed against the walls of the conical tube to ensure successful transfer of the bacteria. Each of these solutions from the various surfaces were cultured on agar plates by transferring 100 µL of each solution onto each quadrant of the agar plate and spread using a sterile swab. The plates were incubated at 95°F for 5 days in an incubator. In addition to separately culturing the bacteria from the four surfaces, a combined representative bacterial pool of the common surfaces was created by mixing equal volumes of each of the bacterial solutions that were made above. 4 mL of this mixed bacterial solution was pipetted into conical tubes and 0.1 mL of various cleaning solutions were added. The tube was mixed and allowed to sit at room temperature for 30 minutes. Following this, 100 µL of each solution was pipetted onto each quadrant of the agar plate and spread using a sterile swab. The plates were incubated at 95°F for 5 days in an incubator.

Effective concentration of hand sanitizer for bacterial inactivation

To determine the percent of alcohol needed to deactivate yogurt bacteria, 1 mL of a 3% yogurt solution was pipetted into a conical tube. Various volumes of hand sanitizer solution containing 70% isopropyl alcohol were added to each of the tubes to achieve a final isopropanol concentration between 0 and 50%. The tubes were mixed and allowed to stand for 30 minutes at room temperature. Then, 100 µL of the solution was plated onto each quadrant of the agar plate and spread using a sterile swab. The plates were incubated at 95°F for 5 days in an incubator.

Statistical analysis

Statistical analysis of the data was conducted by single factor ANOVA using Microsoft Excel. Post-hoc analysis of the data was conducted by the Tukey-Kramer method.

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