Efficacy of Pure Bioscience Surface Disinfectant on Varying Bacterial Strains

A Senior Project
presented to
the Faculty of the Biological Sciences Department
College of Science and Mathematics
California Polytechnic State University, San Luis Obispo

By

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June 2023

Abstract

Agriculture represents a crucial aspect of the acquisition of food resources in modern society. Consequently, it is a paramount undertaking to process crops devoid of any pathogenic agents. A cleaning product named Pure Hard Surface Disinfectant was presented by Pure Biosciences, acclaiming its capacity to destroy bacteria and fungi, while impeding their proliferation for an extended duration following application. This pilot project sought to quantify the efficacy of Hard Surface Disinfectant. Empirical observations conducted thus far have concentrated on surveying the immediate effects of the principal ingredient, silver ion, on diverse bacterial strains. Future prospects for this project include a shift in focus towards evaluating Pure's residual properties at 24, 48, and 72 hour intervals subsequent to its application. Further testing of Pure on agricultural machinery is an additional prospect that can be anticipated. After two quarters of data collection, the experimental endeavors yielded noteworthy reductions in the populations of various bacterial species, including *Listeria monocytogenes*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Provided that forthcoming trials testing Pure surface disinfectant within the laboratory and on farm equipment are successful, it is promising that Pure may obtain certification from the Food and Drug Administration (FDA) and become a commercially available product.

Introduction

In today's global community, the rapid transmission of infectious diseases causes a significant threat to public health. According to the World Health Organization's annual report, an estimated 600 million people worldwide contract foodborne illnesses after consuming contaminated food (World Health Organization, 2023). Pathogenic organisms responsible for such ailments have the potential to survive and proliferate on a variety of surfaces encountered throughout daily-life. As such, it is paramount that effective disinfecting routines are employed in order to prevent the spread of infection.

Pure Hard Surface Disinfectant, a product created by Pure Biosciences, has gained astounding recognition for its ability to eliminate a broad spectrum of microorganisms. This disinfectant contains the patented ingredient Silver Dihydrogen Citrate (SDC), which is an electrolytically generated source of stabilized ionic silver. SDC exhibits its microorganism-killing capabilities through two distinct modes of action. For one, the silver ions in SDC possess the ability to target and inactivate the structural and metabolic membrane of proteins in microorganisms. This disruption of vital proteins within the microbial membrane can lead to microbial death. Secondly, SDC may also be perceived as a nutrient source by microorganisms, enabling silver ions to penetrate microbial cells and denature DNA. Consequently, this function impairs the microorganism's ability to replicate, ultimately impairing its reproduction abilities and resulting in its own eventual death. The founding company has marketed the product as having a long-shelf life, remarkable disinfectant kill time of 30-120 seconds, and the ability to reduce risk of cross contamination (PURE Bioscience, 2023). While the product has already become quite notable, the scientific evaluation of its efficacy and residual effects on various bacterial strains.

The purpose of this study was to assess the effectiveness of Pure Hard Surface Disinfectant in eliminating pathogenic microorganisms on hard surfaces. Multiple strains of bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* were utilized to determine the efficacy of the product. The bacterial strains selected were representative of microorganisms consistently found during the acquisition of food resources.

S. aureus is a significant foodborne pathogenic bacterium that has contributed to many foodborne illnesses each year. The bacterium is frequently found in the nasal passages and on the skin of humans, in turn, making food quite susceptible to being contaminated. *S. aureus* produces toxins whose ingestion is the direct cause of staphylococcal food poisoning. Unfortunately, these toxins are heat-stable, allowing them to withstand high temperatures, and making them extremely difficult to eliminate from contaminated food. When food contaminated with *S. aureus* is consumed, these toxins cause a range of gastrointestinal symptoms, including abdominal cramps, nausea, vomiting, and diarrhea. Other symptoms experienced may include headache and fever, and in the most severe cases dehydration, electrolyte loss, muscle weakness, and low blood pressure. Foods typically contaminated with *S. aureus* include custard, cream-filled pastry, milk, processed meats, and fish (Gotfried, 2023).

L. monocytogenes is a species of foodborne pathogenic bacteria that is regarded as a major causative agent for foodborne illness and even life-threatening infections in the human population. This bacterium is known to thrive in moist environments, soil, water, and decomposing plant matter and animals. Consequently, agricultural crops are particularly susceptible to contamination by this bacterium, and proliferation occurs when food is harvested, processed, prepared, and packed. When food contaminated with *L. monocytogenes* is consumed, a disease called listeriosis may be contracted. Former listeriosis outbreaks in the United States have been traced back to unpasteurized milks and cheeses, ice cream, raw vegetables or fruits, undercooked poultry or deli meats, and raw or smoked fish. (Food and Drug Administration, 2022).

P. aeruginosa is a bacteria commonly present in healthcare settings through contaminated surfaces, equipment, and unsanitized hands (WebMD, 2022). However, despite this bacterium being a leading opportunistic human pathogen, it also poses a significant risk to food safety. This bacterium has high adaptability, a rapid reproduction rate, and low nutrient and humidity requirements for growth. As such, *P. aeruginosa* inhabits a number of environments, including soil, water, and plant, animal and human hosts. Former food safety incidents have found this bacterium in tap water, dairy products, meat products, and (Xu, 2019)

As each of the aforementioned bacteria posed a serious risk in food processing facilities or general public settings, the development of effective antimicrobial agents to control bacterial growth on treatable surfaces is crucial to ensuring food safety and minimizing public health risks. This study aims to investigate the efficacy of Pure Hard Surface Disinfectant and the paired Quaternary ammonia based coating in reducing *Staphylococcus aureus*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* populations.

Materials and Methods

The objective of the efficacy test was to evaluate the effectiveness of Pure Hard Surface and a Quaternary ammonia based coating in combating Listeria monocytogenes (L. *monocytogenes*) on a treatable surface.

An *L. monocytogenes* culture was prepared overnight in Brain Heart Infusion broth under optimal conditions. The culture was grown until the logarithmic growth phase was reached, ensuring maximum viability and population density. The cellular density per milliliter of the *L. monocytogenes* culture was quantified using optimal density (OD) 600. To achieve the desired inoculation load of 6 log, the *L. monocytogenes* culture was serially diluted. A concentration of 6 log in a 1 mL centrifuge tube was the recommended amount to enable gentle pelletization. The supernatant was carefully removed, and the culture was resuspended in 200 mL of buffer for inoculation of the coupon surface. The autoclaved coupon surface was inoculated with the prepared culture of 6 log cells of *L. monocytogenes*. A careful technique was utilized to ensure even distribution of the culture on the surface within the defined testing area. The inoculated culture was then allowed to sit until fully dried. This experimental process was repeated six times, and included the testing of control coupons, two coupons misted with Pure Hard Surface Disinfectant, and two coupons with combined misting of Pure Hard Surface Disinfectant and a Quaternary ammonia based coating.

This design allowed for a comparison of the efficacy of the experimental treatment coupons in comparison to the untreated control coupons. For the first treatment group which tested the efficacy of Pure Hard Surface Disinfectant alone, a misting apparatus was utilized to thoroughly wet the coupons with the product on each of the treatment coupons. Misting was conducted at a distance of 1.5 feet from the coupon surface with an approximate rate of 2 feet per 5 seconds conducted via two sweeps. The wet coupons were then allowed to sit until dry, and were subsequently treated with 100 mL of D/E Neutralizing Buffer that was pipetted onto the coupon surface. This buffer is a liquid medium that serves to remove the bacterial strain from the coupon's surface and help distinguish between bacteriostatic and bactericidal activity. 100 mL of the resulting solution on the coupon was then pipetted and transferred onto D/E Neutralizing Agar. The Agar was then incubated in a controlled environment for 48 hours.

For the treatment group containing Pure Hard Surface and the Quaternary ammonia based coating, the misting apparatus was used to first apply the disinfectant, and then allowed to fully dry. After the coupon was fully dry, the coating was misted onto the coupon and allowed to sit until fully dry. Following the drying period for the coating, 100 mL of Neutralizing Buffer was pipetted into the coupon surface to remove the bacterial strain from the surface. Similar to the other treatment group, 100 mL of the resulting solution on the coupon was then pipetted and transferred onto D/E Neutralizing Agar. The Agar was then incubated in a controlled environment for 48 hours.

Control samples remained untreated and served as a baseline for comparison. These samples underwent the same aforementioned Neutralizing Buffer protocol, and were also incubated for a 48 hour period. Following the incubation period of all treatment groups, the efficacy of the coupons was compared. This was determined by computing the colony-forming-units (CFUs) and calculating the reduction in bacterial strains in comparison to the control samples. **Figure 1** displays the experimental set up for the three treatment groups of coupons. The procedure was also conducted with strains of *S. aureus* and *P. aeruginosa*.

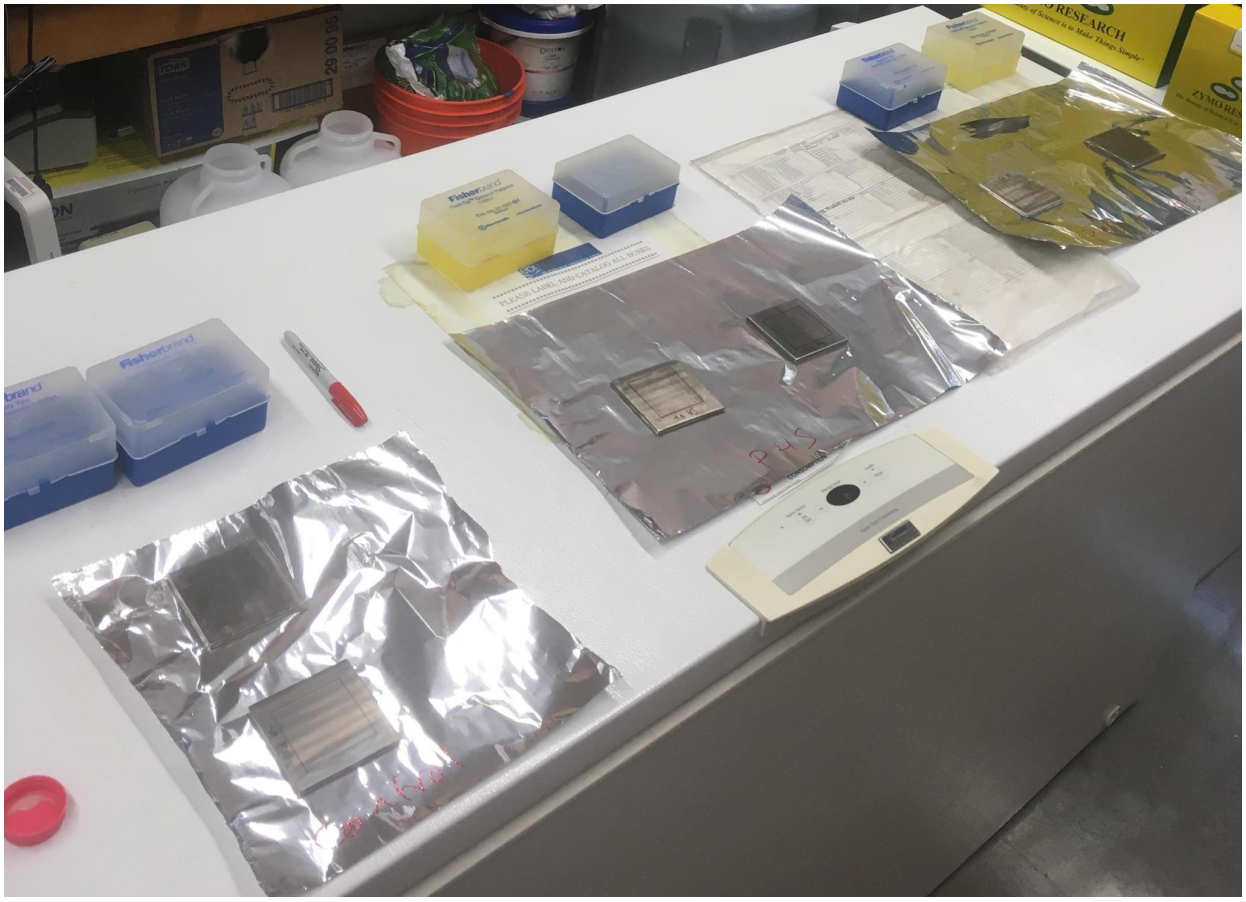

Figure 1. Efficacy test experimental setup. Six coupons were placed on sterile aluminum foil to simulate real-world applications for Pure Hard Surface Disinfectant. Two coupons were designated to the three conditions: the control, Pure Hard Surface Disinfectant alone, and Pure Hard Surface Disinfectant combined with the Quaternary ammonia based coating.

Results

The results were obtained from the enumeration of *L. monocytogenes*, *S. aureus*, and *P. aeruginosa* on the D/E Neutralizing agar plates. CFUs were approximated at inoculum density as well as after the incubation period where average survivors were quantified. The efficacy of Pure

Hard Surface Disinfectant and Quaternary ammonia coating was determined by calculating the percent reduction between approximate inoculum density and average survivors. Moreover, CFUs were compared between groups in order to observe the efficacy between treated samples and untreated controls.

The results exhibited significant reductions in colony forming units for each of the bacterial strains. The inoculum density of *Staphylococcus aureus* approximated 1.22E+08 for the control group, Pure Hard Surface Disinfectant alone, and Pure combined with Quaternary ammonia coating. The average CFUs or survivors calculated for the control group remained 1.22E+08 with a 0% reduction, and served as the baseline for the treatment groups. The average CFUs for Pure Hard Surface Disinfectant was 59 survivors, and the average CFUs for Pure combined with Quaternary ammonia coating was 0.5 survivors. As shown in **table 1**, the D/E Neutralizing agar plates inoculated with *Staphylococcus aureus* showed a 99.99% reduction for both Pure Hard Surface Disinfectant alone and Pure combined Quaternary ammonia coating treatment group.

The D/E Neutralizing agar plates inoculated with *Listeria monocytogene* also showed a 99.99% reduction in both Pure Hard Surface Disinfectant alone and Pure combined with Quaternary ammonia coating. Looking at **table 2**, each of the treatment groups began with an inoculum density of 1.23E+08. While the control group experienced a 0% reduction rate and the average CFU approximated 1.22E+08 survivors, Pure alone and Pure combined with Quaternary ammonia coating showed a 99.99% reduction rate. Pure Hard Surface Disinfectant alone had an average of 0.07 survivors after inoculation and Pure combined with Quaternary ammonia coating had 1.31 survivors.

The D/E Neutralizing agar plates inoculated with *Pseudomonas aeruginosa* showed a 100% reduction in both Pure Hard Surface Disinfectant alone and Pure combined with Quaternary ammonia coating. Looking at **table 2**, each of the treatment groups began with an inoculum density of 1.69E+08. The control group once again experienced a 0% reduction rate and the average CFU approximated 1.69E+08 survivors. On the other hand, Pure alone and Pure combined with Quaternary ammonia coating showed a 100% reduction rate. Each of these treatment groups had a total of 0 survivors.

Sample	Approximate Inoculum Density	Average Survivors (CFU)	Percent Reduction (%)
D/E control	1.22E+08	1.22E+08	0%
Pure	1.22E+08	59	99.99%

Pure + Coating	1.22E+08	0.5	99.99%

Table 1. Efficacy of Pure Hard Surface Disinfectant alone and with Quaternary ammonia coating on *Staphylococcus aureus* population. The approximate inoculum density for the control and each of the treatment groups was compared to the average surviving colony forming units in order to determine the percent reduction for each condition. Percent reduction values are comparable to the disinfectant's efficacy with low percent reductions indicating poor efficacy and high percent reductions indicating success.

Sample	Approximate Inoculum Density	Average Survivors (CFU)	Percent Reduction (%)
D/E control	1.23E+08	1.23E+08	0%
Pure	1.23E+08	0.07	99.99%
Pure + Coating	1.23E+08	1.31	99.99%

Table 2. Efficacy of Pure Hard Surface Disinfectant alone and with Quaternary ammonia coating on *Listeria monocytogenes* population. The approximate inoculum density for the control and each of the treatment groups was compared to the average surviving colony forming units in order to determine the percent reduction for each condition. Percent reduction values are comparable to the disinfectant's efficacy with low percent reductions indicating poor efficacy and high percent reductions indicating success.

Sample	Approximate Inoculum Density	Average Survivors (CFU)	Percent Reduction (%)
D/E control	1.69E+08	1.69E+08	0%
Pure	1.69E+08	0	100%
Pure + Coating	1.69E+08	0	100%

Table 3. Efficacy of Pure Hard Surface Disinfectant alone and with Quaternary ammonia coating on *Pseudomonas aeruginosa* population. The approximate inoculum density for the control and each of the treatment groups was compared to the average surviving colony forming units in order to determine the percent reduction for each condition. Percent reduction values are comparable to the disinfectant's efficacy with low percent reductions indicating poor efficacy and high percent reductions indicating success.

Conclusion

Pure Hard Surface Disinfectant presents itself as a promising solution for combating the growth and proliferation of pathogenic microorganisms on hard surfaces. The results showed significant reductions in the colony forming units of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. following misting of Pure alone and in combination with the Quaternary ammonia based coating, demonstrating that Pure Biosciences' product is a broad spectrum antimicrobial. For *Listeria monocytogenes* and *Staphylococcus aureus* inoculated coupons, both Pure Hard Surface Disinfectant alone and in combating with the coating achieved a 99.99% reduction in colony forming units in comparison to the control samples for each bacterial strain. The *Pseudomonas aeruginosa* trials demonstrated even more success with both treatment groups reaching a complete 100% reduction in bacterial survivors in comparison to the control samples. These findings indicate that Pure Hard Surface Disinfectant is a strong contender for real-world applications and demonstrate its high potential as a disinfectant in both agricultural settings and food processing facilities.

Future prospects for this research project include further evaluation of Pure Hard Surface Disinfectant's residual properties, testing the effects of the disinfectant on yeast viability staining, and running experimental trials on agricultural machinery. Methodology for the residual kill experiment would observe the effects of inoculating coupons with a bacterial strain subsequent to misting the coupons with Pure Hard Surface Disinfectant alone or Pure combined with the Quaternary ammonia based coating. Residual properties would be tested at different time intervals, including 24, 48, and 72 hours after the product's application.

Methodology for the yeast viability experiment would include an initial protocol similar to the one utilized in this efficacy test, however, the study would focus on yeast cells as opposed to bacterial strains. Furthermore, the procedure would include the application of a chemical stain called methylene blue to the yeast. Methylene blue is often used in research to investigate cellular processes. When applied to yeast cells, the chemical readily permeates the cellular membrane due to its small molecular size and lipophilic properties. The function of the chemical specific to this prospective experiment would be to distinguish between living and dead yeast cells based on their metabolic activity. Live yeast cells are actively engaging in metabolic activity, and therefore, possess reducing systems that convert methylene blue into its oxidized form. This reduced form does not exhibit strong staining properties, thus transforming the yeast cell to a colorless state. In contrast, dead yeast cells lack metabolic activity and consequently do not reduce methylene blue. As such, the original oxidized form of methylene blue retains its staining ability, allowing for dead cells to retain a blue hue. The protocol for the experiment would include microscopic investigation and quantification of yeast cells by use of a counting chamber. Viability counts would be conducted to determine the survival of yeast cells following Pure and coating applications. The formula, **viability [%] = (Total counted cells – total**

counted dead cells) / total counted cells x 100, would be utilized to compute viability for each of the treatments and the control group (Geneq Inc.).

The final prospect for this research project would seek to take testing beyond the laboratory and focus on real-world applications. An excellent approach to achieving this objective would be to conduct testing on agricultural machinery, to assess the efficacy in reducing bacterial populations in the intended circumstances. **Figure 2** displays a harvester from an agricultural food processing site in Santa Maria, California. Future research prospects would seek to test the efficacy of Pure Biosciences' products on the harvester conveyor belt and trays seen on this piece of agricultural machinery. Provided that the forthcoming research prospects for Pure Hard Surface Disinfectant yield positive results, there is a strong potential for this product to obtain official certification from the Food and Drug Administration. The success achieved during this project has served as an advancement towards achieving this goal, as well as an advancement in public health protection.

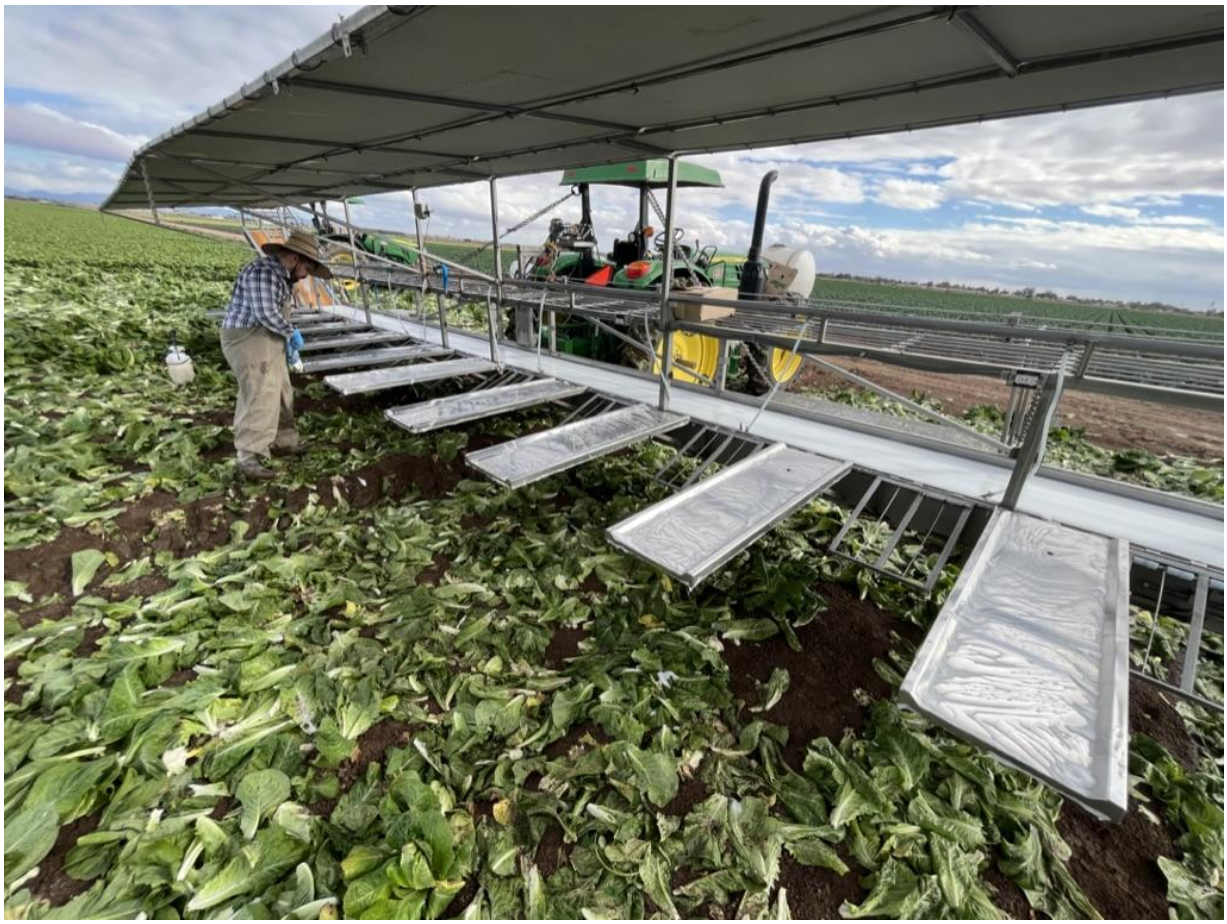

Figure 2. Harvester from an agricultural food processing site in Santa Maria, California. The coupons utilized in the efficacy test serve to simulate the harvester conveyor belt and trays that compose this piece of agricultural machinery.

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