BIOL 342 TERM PROJECT: IMPACTS OF VARIOUS CLEANING REAGENTS ON MOLD GROWTH

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ABSTRACT

Bread is an ideal environment for mold growth due to its high moisture content and optimal pH level. Past research has investigated the effects that cleaning agents have on killing mold-growth inducing spores on various surfaces. It was hypothesized that mold growth on bread would be affected when the surface in contact with the bread was wiped with a cleaning product prior. No condition had consistent mold growth presence among all samples, however Control 1 (bread only) and Lysol® treatment had ⅔ bread slices with mold growth.The highest percent coverage of mold was from sample 1 of Control 1(bread only), at 81.05%. Besides when no growth was detected, the lowest mold coverage was from sample 2 of the bleach treatment (36.74%). The highest mean growth rate was from Control 1 (no wiping) at 11.8%. The lowest growth rate was from the bleach treatment, at 7.26%. Although trends suggest that bleach was the most effective treatment, statistical analysis found no statistical significance among growth rate as $p>0.05$ ($p=0.637$). Overall, our study did not support our hypothesis, as there was no difference in mold growth and rates between treatments.

INTRODUCTION

The following study aims to investigate the effects of various cleaning products on mold growth in bread that was exposed to the surface it cleaned. The high moisture content in bread and the pH level is ideal for the germination and growth of various molds (Magan et al., 2012). Mold is a type of fungi, with thousands of different spores that can commonly be found on bread. Spores, which are cast off by mature fungi, are present in the air and on all surfaces. Once a spore has landed on the surface of the bread, it begins to multiply quickly and form a colony (Reynolds, 2019). Past research has shown that various cleaning products are able to kill mold spores when applied to surfaces, therefore reducing the chance of spore exposure and inevitable mold growth. Therefore, it was hypothesized that the use of the cleaning agents on surfaces exposed to breads would have an effect on the amount of mold growth. Bleach washing has been found to be a successful method of reducing spore levels on a wide array of surfaces, including wood (Wilson et al., 2004). Sargripanti & Bonifacino found that water cleaning did not effectively kill spores on contaminated surfaces (1999). According to the brand website, Lysol® disinfectant wipes are commonly advertised to ”Kill 99.9% of Germs,” (2022), however it does not specify if it is effective against killing spores and reducing mold growth. Evaluating how various cleaning products impact mold growth in this experiment gives valuable insight on the effectiveness of both store-bought or home-made products. Based on previous research, it was predicted that treatments exposed to cleaning products such as bleach and Lysol® will have less mold growth compared to the controls.

METHODS

A loaf of bread without preservatives was obtained from a Safeway bakery. Fifteen slices were separated into five groups of three slices, for two controls and three treatments. Using four tables in one of the classrooms of UBC's Biology Sciences building, four of the five groups of bread were exposed to the table surfaces. The first control was bread only. The second control was bread

Fig 1. Example picture of a daily picture in normal light conditions. Normal light Day 1 picture of Control 1 (Bread Only) Sample 1 on a board with a scale.

wiped on the table surface without any treatment. Each of the three pieces of bread were swiped from top to bottom on one side in three separate paths along the table. The first treatment was bread exposed to a table surface cleaned with distilled water. The second treatment was bread exposed to a table surface cleaned with Lysol® wipes. The third treatment was bread exposed to a table surface cleaned with a homemade blend (1:1 bleach and distilled water). The swiping process of bread for all three treatments follows the same technique used in the second control. All fifteen slices were placed into a Ziploc® bag using gloves.

Fig 2. Example image of bread sample in dark lighting conditions. Image of Control 1 Sample 1 under dark lighting condition.

Data collection was to be performed for consecutive days until at least one sample of bread was observed to have 100% coverage of mold growth in the area of interest, which was the front side of the bread where the Ziploc® was labeled. A scale was drawn on a board for size reference of bread and each sample was placed on the board for daily pictures to obtain possible progressive growth of mold (Fig 1). Observations on mold growth particularly in the appearance (for example: color) was recorded in order to help in mold identification. There were two types of lighting conditions for pictures: normal and dark. Normal light conditions were pictures of bread on the board with the scale in a lit room. Comparatively, a dark set-up was performed in a room with the lights off, a lamp turned on for indirect light, an open top box with phone flashlight centered through the bottom, a see-through plastic cover on top of box, and a bread sample placed on top of a plastic cover centered with a phone flashlight. The pictures in a

Fig 3. Picture of how all bread samples stored in a box.

dark lighting showed mold growth with stronger contrast which allowed for the visualization of smaller specs of mold which was difficult to see in normal light conditions (Fig 2). Additionally, pictures in normal light conditions as well as dark conditions allowed for clarification of mold growth or lack thereof (particularly internal growth of mold in bread). All bread samples were stored together in a box in the center of the room (Fig 3). After daily pictures and observations were obtained, mold identification was then performed using a microscope.The microscope allowed for a magnified view of mold which could be compared to different types of bread mold characteristics (looking at stock and hyphae). Data was collected over 16 days roughly before a sample reached 100% due to health risks concerning a strong smell of mold. Microscopic identification was performed on day 18.

Percent coverage of mold on bread was obtained digitally using ImageJ (Rasband, 2018). The data obtained was used to obtain a growth rate graph (% Coverage Vs. Days) for each treatment. After clarifying linearity, a slope for each sample was then obtained and utilized in statistical tests (one-way ANOVA tests) to see if there was significance between treatments correlating to the growth of mold.

RESULTS

The experiment was conducted for a total of 16 days. During this time, seven of the 15 slices developed mold growth. Control 1 and Lysol® had two slices with mold growth. Control 2, distilled water, and bleach all had one slice with mold growth. Control 1 sample 1 started to grow mold on day 11. Control 1 sample 2 started to grow mold on day 9. Control 2 sample 1 started to grow mold on day 11. The distilled water treatment sample 3 started to grow mold on day 10. The bleach mixture treatment sample 2 started to grow mold on day 11. The Lysol® treatment sample 2 started to grow mold on day 9. The Lysol® treatment sample 3 started to grow mold on day 10. Mold growth was calculated through percent coverage. The order of percent coverage of the bread samples with mold from smallest to largest was as follows:

Bleach Sample 2 - 36.74%
Water Sample 3 - 58.79%
Lysol Sample 3 - 64.28%
Control 2 Sample 1 - 75.31%
Lysol Sample 2 - 75.48%
Control 1 Sample 2 - 78.04%
Control 1 Sample 1 - 81.05%

Figure 4: Percent coverages from smallest to largest

The growth rate of each sample containing mold was calculated by graphing out the percent coverage from the first day of mold growth to day 16. To make it easier to view, the x-axis of each graph starts on the first day mold growth is recorded.

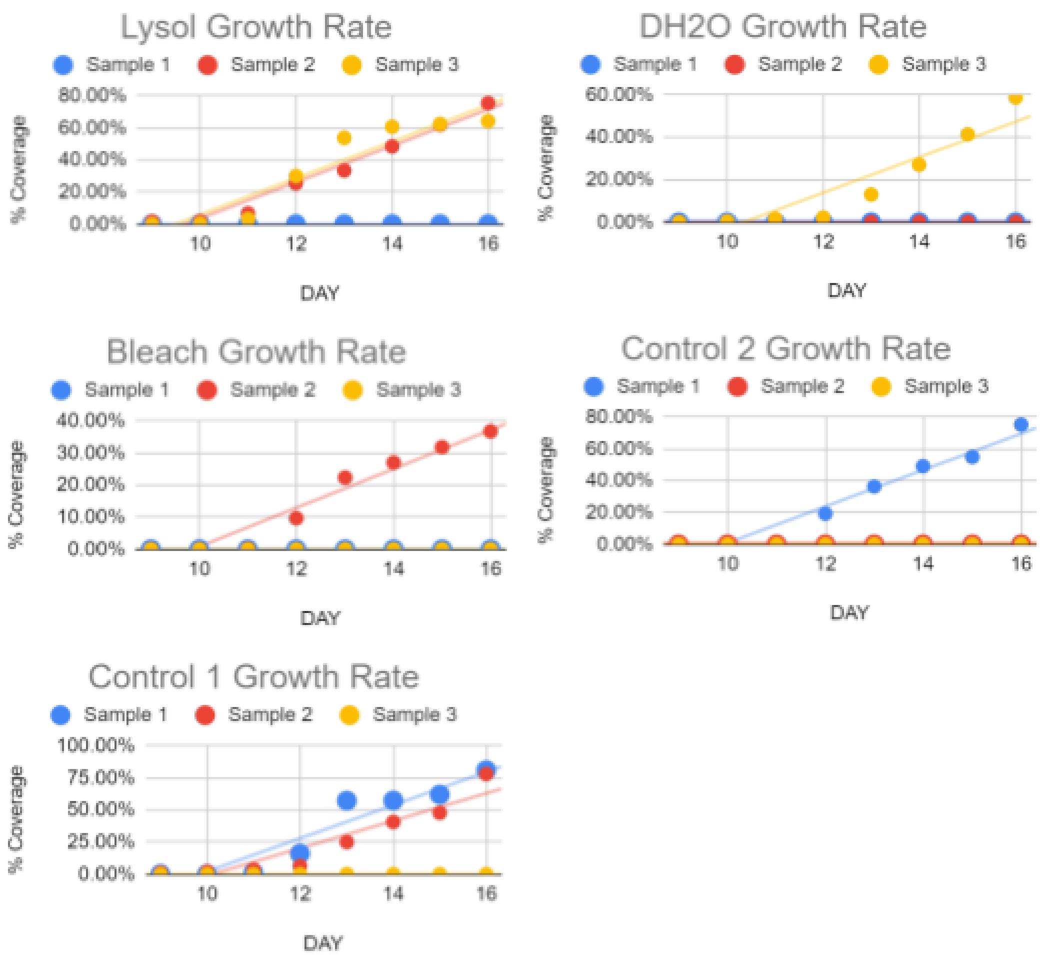

Figure 5: Graphs of growth rates for each control and treatment

From this, the slope (average growth rate) was calculated for each sample.

	Control 1	Control 2	DH2O	Bleach	Lysol
Sample 1	0.129	0.115	0	0	0
Sample 2	0.108	0	0	0.0607	0.113
Sample 3	0	0	0.0838	0	0.114

Figure 6: Average growth rates for all three samples of each control and treatment

These growth rate values were then used to calculate the p value using a one-way ANOVA test. The p value was calculated using all 5 groups with 3 values in each group. The p value was 0.637.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	4	0.008919	0.00223	0.6545	0.637
Error (within groups)	10	0.03407	0.003407		
Total	14	0.04299	0.00307		

Groups:	Control 1	Control 2	dH2O	Bleach	Lysol
Data:	0.129 0.107 0.000	0.115 0.000 0.000	0.000 0.000 0.0838	0.000 0.0607 0.000	0.000 0.113 0.114

Figure 7: One-way ANOVA test - data and resulting p-value

DISCUSSION

Food mold originates from fungal spores within bioaerosols that, when deposited, can tolerate a wide range of environmental conditions and lead to spoilage (Magan *et.al*, 2012). Antimicrobial chemicals are required to inactivate or delay mold activity and growth (Magan *et.al*, 2012), with those deemed safe for consumption being based on propionic, sorbic, and acetic acids (Magan *et.al*, 2012). Benzalkonium chloride (C17H30ClN) and sodium hypochlorite (NaOCl) found in Lysol® and bleach respectively have also been studied for their antifungal properties. While Basaran (2011) established C17H30ClN as a promising fungicide against the growth and/or spore germination of food molds aspergillus and penicillium, Reynolds et. al (2012) reported low concentration (2.4%) NaOCl to have similar effects, leaving molds nonculturable after five-minute treatments. Furthermore, the goal was to assess the antifungal properties of household cleaning products, Lysol® and bleach, on bread mold growth.

Different rates of mold growth were measured amongst identical pieces of bread that were subjected to different controls and treatments. ⅔ samples of Control 1 group (non-wipe, treatment absent) showed an average 11.8% daily growth rate, which was considered high given that the maximum number of slices displaying mold growth among each treatment was ⅔. Surprisingly, the Lysol® group had the third highest mean daily growth rate at 11.35% for ⅔ samples, suggesting that either C17H30ClN is ineffective towards preventing mold growth, or that the Lysol® group was subjected to sources of error. On the contrary, the Control 2 group (wiped, treatment absent) showed mold growth in only ⅓ samples, but with the highest rate of 14%. The remaining two groups (DH2O and bleach) displayed mold growth in ⅓ of their samples, with average mold growth rates of 9.99% and 7.26% respectively. While this suggests that bleach was an effective treatment in slowing mold growth, water did not differ in effectiveness. The latter does not support initial predictions given that moisture is required for abundant mold growth (Scott, 1957, p.84).

An observational analysis confirmed that aspergillus and penicillins were the most (and only) abundant species (McGregor, 2018). Mold contamination was described as dark green and/or white, powdery and fuzzy congregations that often began at the crusts. There were times that the mold was found in tiny segregated dots rather than full continuous patches. Other mold structures that were observed under microscopy included penicillin septae which were seen in Control 1 Sample #1 and yellow hyphae in Lysol® Sample #2.

A one-way ANOVA test suggested that the differences in mean growth rates and mold coverage amongst all groups inclusive of all samples were not statistically significant. Given that the P value was 0.637 ($p>0.05$), there is no evidence that any treatment is more or less effective than the other.

These results call for a reflection of sources of error and methods of improvement by which to conduct future studies. What was initially observed as 100% mold coverage in multiple bread slices was later measured as ≤ 81%. Although ImageJ (Rasband, 2018) was an accurate measurement tool that allowed for digital tracing over contaminated areas, there is room for improvement in navigating its settings for more accurate analyses. In terms of mold identification via microscopy, it was difficult to pinpoint each species phenotypically, and so DNA or rRNA sequencing would be required for more accurate identification. Regarding application methods, there were many ways that each bread was potentially subjected to external variables. The same pair of gloves was utilized for every sample, which potentially led to cross-contamination of treatment solutions and/or fungal spores between samples. Plausibly, the table segments on which each sample was wiped were unequally contaminated, depending on whether some areas were utilized more than others prior to the experiment. Since each slice was stored in sealed sandwich-sized Ziploc® bags, this may have prevented mold spores from the external space to deposit onto the bread, and so storing them in a more open setting may lead to more observable growth. Further studies should consider applying each treatment directly to the bread slice itself rather than a table surface, to include more slices, and to occasionally spray each slice with water to ensure that the bare-minimum moisture requirement for mold growth is fulfilled.

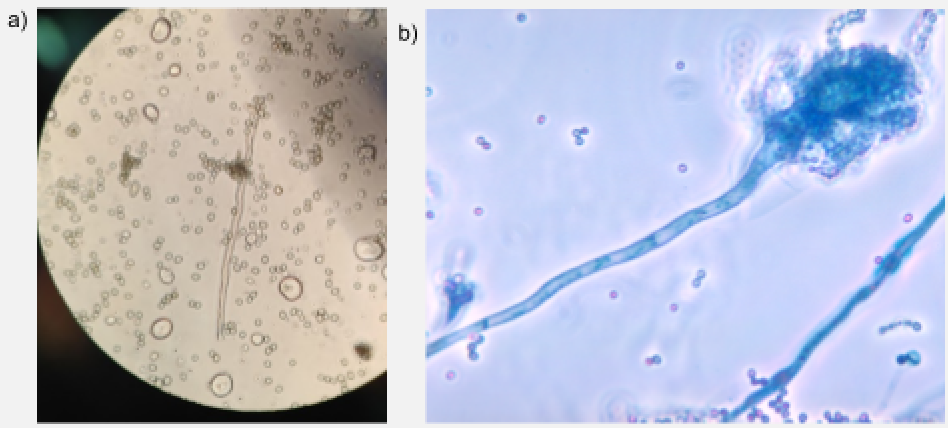

Fig 8. Microscopic images of *Aspergillus* mold. a) Microscopic image of mold from DH2O Sample 3.
b) Microscopic image of *Aspergillus fumigatus* for comparison (Ajello, 1963).

CONCLUSION

No significant differences were found in mold growth rates and coverage under non-wipe and wipe controls, water, Lysol® or bleach. Given multiple sources of error under this study, further trials are encouraged to verify whether the absence of antifungal properties in Lysol ® and bleach are due to said errors, or to both treatments being naturally ineffective towards preventing mold contamination. With many prior studies having already established significant discrepancies in mold growth under the presence and absence of moisture, it would be worthwhile to explore mold growth under different types of bread or foods, different temperatures, or performing a closer analysis on the optimal growth conditions of different mold species.

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Works Cited

Basaran, P. (2011). Inhibition effect of benzalkonium chloride treatment on growth of common food contaminating fungal species. *Journal of Food Science and Technology*, *48*(4), 515–519. https://doi.org/10.1007/s13197-011-0268-5 Retrieved December 11, 2022, from https://doi.org/10.1016/s0065-2628(08)60247-5

CDC, & Ajello, L. (1963). *Details - public health image library(phil)*. Centers for Disease Control and Prevention. Retrieved November 21, 2022, from https://phil.cdc.gov/details.aspx?pid=4297

Magan, N., Aldred, D., & Arroyo, M. (2012). Mold prevention in bread. *Breadmaking*, 541–560. Retrieved November 20, 2022, from https://doi.org/10.1016/b978-0-08-102519-2.00018-9

McGregor, J. (2020, March 14). *Bread Mold: How to identify types of mold*. Science Trends. Retrieved November 20, 2022, from https://sciencetrends.com/bread-mold-how-to-identify-types-of-mold/

Sagripanti, J.-L., & Bonifacino, A.. (1999). Bacterial Spores Survive Treatment with Commercial Sterilants and Disinfectants. Applied and Environmental Microbiology, 65(9), 4255–4260. Retrieved November 20, 2022, from https://doi.org/10.1128/aem.65.9.4255-4260.1999

Same protection: Lysol® Canada. Lysol® Canada. (2022). Retrieved November 20, 2022, from https://www.lysol.ca/en/biodegradable/biodegradable-wipes/

Scott, W. J. (1957). Water relations of food spoilage microorganisms. *Advances in Food* Research Volume 7, 84. Retrieved November 20, 2022, from https://doi.org/10.1016/s0065-2628(08)60247-5

Rasband, W.S. (2018). ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA, Retrieved November 15, 2022, from https://imagej.nih.gov/ij/ Retrieved November 20, 2022, from https://doi.org/10.1016/s0065-2628(08)60247-5

Reynolds, K. A., Boone, S., Bright, K. R., & Gerba, C. P. (2012). Occurrence of household mold and efficacy of sodium hypochlorite disinfectant. *Journal of Occupational and Environmental Hygiene*, *9*(11), 663–669. Retrieved December 11, 2022, from https://doi.org/10.1080/15459624.2012.724650

Reynolds, L. (2019, March 2). How does mold grow on bread? Sciencing. Retrieved November 20, 2022, from https://sciencing.com/mold-grow-bread-5403099.html

Wilson, S. C., Brasel, T. L., Carriker, C. G., Fortenberry, G. D., Fogle, M. R., Martin, J. M., Wu, C., Andriychuk, L. A., Karunasena, E., & Straus, D. C.. (2004). An Investigation into Techniques for Cleaning Mold-Contaminated Home Contents. *Journal of Occupational and Environmental Hygiene*, *1*(7), 442–447. Retrieved November 20, 2022, from https://doi.org/10.1080/15459620490462823