Statistical and Cellular Analysis of Rhizopus stolonifer Growth as Affected by Potassium Sorbate Concentration

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Abstract

Known as black bread mold, R. stolonifer is a fungus commonly found in contaminated food products such as meat, preserved, and/or baked goods. Potassium sorbate is a common food preservative used to prevent fungi, mold, and mycotoxin growth by damaging the cell membrane or altering proteins in the cell. Based on background research on potassium sorbate growth prevention, it was hypothesized that a 25% concentration of potassium sorbate will most efficiently prevent R. stolonifer growth compared to lower concentrations. R. stolonifer was grown on potato dextrose agar with eight levels of potassium sorbate concentrations. During a two week period, fungal growth was observed, photographed, and the height and width of each sample were recorded. At the end of two weeks, the fungi were stained using lactophenol cotton blue dye and observed under a microscope. Qualitative observation at the cellular level showed healthy R. stolonifer solely in the control group (see Figure 1). When comparing the fungi samples across the 5% and 30% concentration groups, large areas of dead mass dominated their cellular makeup. At the end of two weeks, the 1% concentration sample group had an observed increase of 1.383 cm2 in total area, a significant decrease compared to the control group. Between the 5% and 30% concentration sample groups, there were minor changes in total area with no change exceeding 0.125 cm2. Between the 1% and 5% experimental groups, there was a significant decrease in fungal area growth, and a similarly large decrease between the 10% and 15% concentration fungi samples. Thus, it can be concluded that it is unnecessary to exceed a 1% concentration of potassium sorbate to prevent fungal growth.

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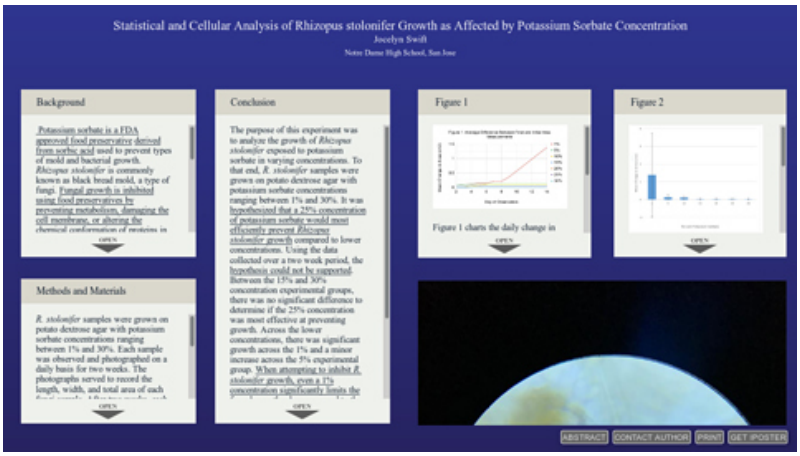

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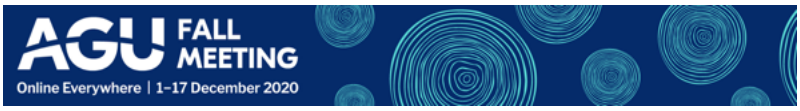


BACKGROUND

Potassium sorbate is a FDA approved food preservative derived from sorbic acid used to prevent types of mold and bacterial growth. *Rhizopus stolonifer* is commonly known as black bread mold, a type of fungi. Fungal growth is inhibited using food preservatives by preventing metabolism, damaging the cell membrane, or altering the chemical conformation of proteins in the cell (Ray 1992 (https://meridian.allenpress.com/jfp/article/45/10/953/188835/Preventing-Growth-of-Potentially-Toxic-Molds-Using)). In a previous experiment performed at West Virginia University, *Rhizopus sp* growth was efficiently limited by potassium sorbate concentration present in peeps, a marshmallow candy (Klein 2019 (https://www.nytimes.com/2019/03/29/science/marshmallow-peeps-fungus.html)). Potassium sorbate is widely used at a concentration of 0.3% of the food products weight (United States Department of Agriculture 2002 (https://www.ams.usda.gov/sites/default/files/media/P%20Sor%20technical%20advisory%
By performing the experiment in potato dextrose agar, the ideal growth medium for *R. stolonifer* is juxtaposed with a commonly used, effective inhibitor of growth.

METHODS AND MATERIALS

R. stolonifer samples were grown on potato dextrose agar with potassium sorbate ($C_6H_7KO_2$) concentrations ranging between 1% and 30%. Each sample was observed and photographed on a daily basis for two weeks. The photographs served to record the length, width, and total area of each fungi sample. After two weeks, each *R. stolonifer* sample was mounted on microscope slides using Lactophenol Cotton Blue and photographed under 25x, 40x, and 50x magnification using a microscope. The microscopic photographs were used to qualitatively record the cellular condition. All data was statistically analyzed.

CONCLUSION

The purpose of this experiment was to analyze the growth of *Rhizopus stolonifer* exposed to potassium sorbate in varying concentrations. To that end, *R. stolonifer* samples were grown on potato dextrose agar with potassium sorbate concentrations ranging between 1% and 30%. It was hypothesized that a 25% concentration of potassium sorbate would most efficiently prevent *Rhizopus stolonifer* growth compared to lower concentrations. Using the data collected over a two week period, the hypothesis could not be supported. Between the 15% and 30% concentration experimental groups, there was no significant difference to determine if the 25% concentration was most effective at preventing growth. Across the lower concentrations, there was significant growth across the 1% and a minor increase across the 5% experimental group. When attempting to inhibit *R. stolonifer* growth, even a 1% concentration significantly limits the fungal growth when compared to the control. Between the 1% and 5% concentration, once again a significant decrease in fungal area was observed. To prevent fungal growth, it is unnecessary to exceed a 1% concentration. For future work, the experiment should be run again with a larger sample pool of concentrations between 0.01% and 1.0%. Based off this experiment and the guidance of Dr. Kasson at WVU, a paper was written and currently pending publication in the Journal of Microbiology & Biology Education.

FIGURE 1: AVERAGE DIFFERENCE BETWEEN FINAL AND INITIAL AREA MEASUREMENTS

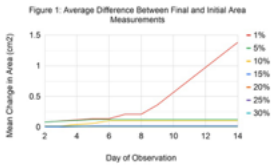


Figure 1 charts the daily change in area of the experimental fungi groups using data from Table 1. Excluding the control, which increased by 37.43 cm² to the maximum area available in the petri dish. The 1% concentration had the largest experimental growth, increasing by an area of 0.155 cm². All other experimental groups had similar levels of area growth.

FIGURE 2: OVERALL CHANGE IN AVERAGE AREA OF EXPERIMENTAL GROUPS

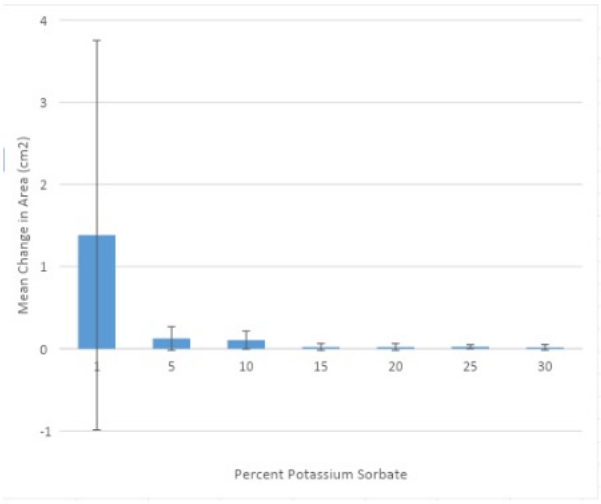
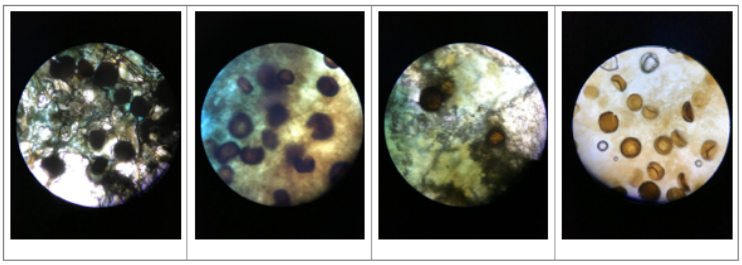


Figure 2 details the difference between the final and initial sample area of the experimental group fungi using the data from Table 2. The relatively equal area change between the experimental groups does support either the hypothesis or a clear rebuttal. The control group had a total area change of 37.43 cm² and is not included in the above graph. Error bars represent ± 2SEM.

ABSTRACT

Known as black bread mold, *R. stolonifer* is a fungus commonly found in contaminated food products such as meat, preserved, and/or baked goods. Potassium sorbate is a common food preservative used to prevent fungi, mold, and mycotoxin growth by damaging the cell membrane or altering proteins in the cell. Based on background research on potassium sorbate growth prevention, it was hypothesized that a 25% concentration of potassium sorbate will most efficiently prevent *R. stolonifer* growth compared to lower concentrations. *R. stolonifer* was grown on potato dextrose agar with eight levels of potassium sorbate concentrations. During a two week period, fungal growth was observed, photographed, and the height and width of each sample were recorded. At the end of two weeks, the fungi were stained using lactophenol cotton blue dye and observed under a microscope. Qualitative observation at the cellular level showed healthy *R. stolonifer* solely in the control group (see Figure 1). When comparing the fungi samples across the 5% and 30% concentration groups, large areas of dead mass dominated their cellular makeup. At the end of two weeks, the 1% concentration sample group had an observed increase of 1.383 cm^2 in total area, a significant decrease compared to the control group. Between the 5% and 30% concentration sample groups, there were minor changes in total area with no change exceeding 0.125 cm^2. Between the 1% and 5% experimental groups, there was a significant decrease in fungal area growth, and a similarly large decrease between the 10% and 15% concentration fungi samples. Thus, it can be concluded that it is unnecessary to exceed a 1% concentration of potassium sorbate to prevent fungal growth.

Figure 1: Fungi Sample of R. stolonifer at 25x Magnification



From left to right, Figure 1 contains samples of control, 1%, 5%, and 30% concentration fungi samples. In comparison to the control, the experimental fungi samples show damaged spores and disrupted growth.

(https://agu.confex.com/data/abstract/agu/fm20/8/1/Paper_668518_abstract_640651_0.png)