



Probiotics on Microbial Growth - Suppress or Stimulate?

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INTRODUCTION

When one thinks of ‘microbial diseases’, one usually thinks of bacterial diseases or viruses; however, other types of microbial diseases are present such as Aspergillosis, an infection caused by *Aspergillus* mold that leads to coughing, wheezing, and fever (Cleveland Clinic Medical Professional 2019). Now, it is evident with the COVID-19 pandemic that no disease should be ignored. The study of microorganisms can be done over short time periods, as each microbial cell reproduces into two daughter cells leading to exponential growth (Sargen, 2020), and this is partly why controlling microbial growth in food transportation and storage is so important. Given that the growth of bacteria is devastating for food storage, understanding factors that influence this growth is important. One such factor may be the presence of probiotic bacteria, which are living bacteria that can offer health benefits, such as manipulating harmful microbial communities and suppressing the growth of pathogens (Hemarajata & Versalovic, 2013). Fermented vegetables like kimchi typically contain probiotics (Han, 2020), while fresh vegetables like cabbage do not. Given the recent focus on microbial diseases, investigations into the effect that probiotic inoculation has on delaying microbial growth are warranted and may inform anti-microbial practices. To do this, mold growth on fresh vegetables will be compared to mold growth on fermented vegetables, which are enriched for probiotic bacteria. Given that fermented vegetables contain probiotics, it is expected that fresh vegetables will better support growth of mold than fermented vegetables.

MATERIALS AND METHODS

A peeled orange was placed in an open-air container for 7 days. After seven days, three 8cm x 8cm samples of kimchi and three 8cm x 8cm samples of fresh lettuce were each placed onto a 10cm x 10cm flat dish. Small, 1cm x 1cm pieces of mold were measured with a ruler and then extracted from the open-air container with a spoon and exacto knife and placed onto each of the vegetables. 30mL of water was then added to each plate to ensure the sample was moist. Then, to avoid evaporation, two tissues were placed on the sides of each plate, followed by the plates being wrapped with plastic wrap with holes to allow for air flow. The dishes were left to sit in a dark room for six days, with photos taken daily. After taking each photo, the areas of the mold of each trial were measured with a ruler and analyzed

RESULTS

Growth of mold was observed on all trials of kimchi across the entire experimental timeline (Figure 1). The average area of the mold on kimchi in cm² was 1.00, 2.11, 3.61, 4.95, 21.45, and 64.00 incrementally from day 1 to day 6. The rate of growth also accelerated during the experiment, ranging from 1.11 cm² between the first and second days of data collection, to 42.55 cm² between the final two days. In addition, the data points for the mold on kimchi in the processed graph are extremely close to the exponential trend line ($r^2 = 0.99$). Conversely, there was no observable growth of mold (cm²) in any of the trials of cabbage throughout 6 days.

DISCUSSION

It was hypothesized that the growth of mold will see an increase on both types of vegetables, but a greater exponential increase every day when put onto fresh vegetables without probiotics than when put onto fermented vegetables. However, the results did not support this prediction. For microbes to flourish, there needs to be ample nutrients in their surroundings (Sargen, 2020). The ingredients for kimchi include cabbage, salt, water, grated garlic, granulated sugar, Korean red pepper flakes, and Korean radish (Han, 2020); given the observed growth of mold on kimchi and the lack of growth seen on fresh cabbage, the findings may suggest kimchi is a more favourable environment for mold. Another possible explanation may be that the interaction between probiotics and other microorganisms is more complex than initially thought. Despite the hypothesis that probiotics will suppress all microbial growth, including mold growth, it seems likely that probiotics don't uniformly suppress microbial growth, but instead play multiple roles including stimulating or suppressing them. This may also explain why they are able to manipulate microbial communities to help gut health (Hemarajata & Versalovic, 2013). Since the observed growth closely mimicked exponential growth which would only be seen in perfect growing conditions for mold, the findings suggest that the probiotics in kimchi likely do not have any anti-mold effects and instead may even stimulate the growth of microbes like mold.



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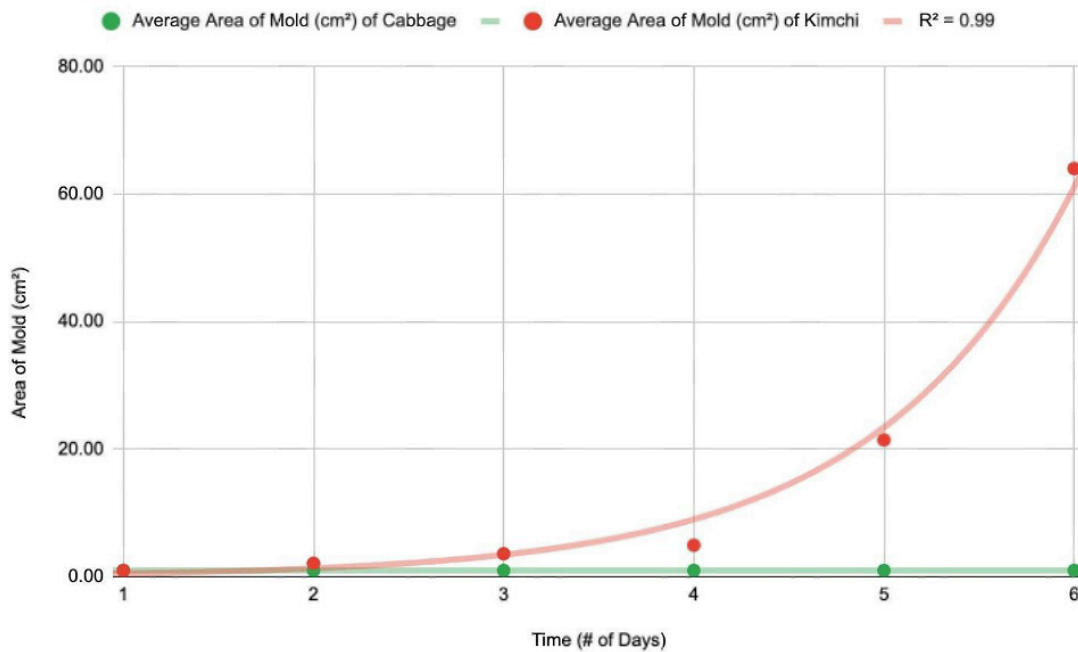


Figure 1: Mold Growth. Processed graph showing the relationship between days growing and mold growth on cabbage (green) and kimchi (red).

The kimchi trials were relatively precise and controlled. For instance, the range between the three trials for the area of mold (in cm²) on Kimchi on day 5 is 2.75, which represents 13% of the total measurement on this day (21.45 cm²). This precision increases the reliability of the results of the experiment, and with that, possible extrapolations and interpolations of the data.

One possible improvement to the experimental method would be the inclusion of control groups; experimental conditions for both kimchi and cabbage where no mold was transferred to them prior to the experiment beginning. This would allow the assessment of whether mold grows naturally on either of these media. Another possible improvement would be to slice off pieces of kimchi and cabbage much larger than 8cm x 8cm, like 16cm x 16cm, so that growth would not be limited by the size of the media.

Additional experiments that investigate how probiotics suppress or stimulate microbial growth could be expanded to other forms of microbial life, including bacteria, as the results suggest probiotics may yield different effects depending on the environment. Instead of comparing the results of the growth of mold on two contrasting vegetables (kimchi and cabbage), the results of the growth of two contrasting microbes, yeast and bacteria, on both kimchi and on an empty plate or petri dish could be compared.

As mold can cause harmful diseases to humans, it is essential to expand our understanding on what conditions suppress or support mold growth, such as the effect of probiotics on microorganisms like mold. As our understanding of this increases, researchers may discover possible solutions to food spoilage or approaches to manage diseases caused by mold and other microorganisms.

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My name is Jonghoon (Kevin) Lim, and I will be in grade 10 to start the 2021-2022 school year at Glenlyon Norfolk School in Victoria, BC. Born in South Korea and having lived in America and Canada, I have grown accustomed to many unique cultures that have shaped me. Some subjects I have always enjoyed include science, math, and computer engineering, while my favourite activities to pass time include reading articles, playing sports, coding, and playing the cello. Now, I hope to start my scientific endeavours with the Canadian Science Fair Journal and help make the world a better place.

