FUNGAL CONTAMINATION IN YOGURT AND ITS POTENTIAL ASSOCIATION WITH VARIOUS PACKAGING MATERIALS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of Food Science

by

Priti Chintalapati

August 2023

ABSTRACT

Dairy products contribute to 17% of total waste generated at the consumer and retail levels, with microbial spoilage being a major factor. Cultured dairy products like yogurt, cream cheese, and buttermilk are produced through bacterial fermentation and are susceptible to contamination by spoilage microbes during processing and production. Spoilage can be influenced by intrinsic factors such as product pH and extrinsic factors like packaging type.

In this study, I aimed to quantify the microbial load and identify fungal spoilage on recycled and non-recycled yogurt packaging. Four different yogurt container samples were tested, two were constructed using recycled packaging materials while the remaining two containers were made from non-recycled heavy-duty cardboard packaging materials. A total of 48 containers were processed; they were homogenized, plated on Malt Extract Agar (MEA) at different dilutions, and then incubated for 8-10 days at 77°F. Plate counts and descriptions of distinct morphologies for each sample were recorded. It was concluded that most samples showed low counts (~1 mold colony) at the lowest dilution factor (10^0) and yogurt containers made from recycled packaging materials exhibited a higher percentage of fungal contamination compared to non-recycled containers.

BIOGRAPHICAL SKETCH

Priti Chintalapati graduated from St. Francis College for Women, Hyderabad India with the Degree of Bachelor of Science majoring in Microbiology, Biochemistry, Chemistry in April 2022. She started her Professional master's degree in Food Science (MFS) at Cornell University in August 2022 and graduated in August 2023.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my advisor Dr. Abigail Snyder, mentor Meg Shi, the Snyder Lab, and Department of Food Science at Cornell University for their encouragement and support throughout my academic journey. I am also deeply grateful to my family and friends for their unwavering support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Bp: Base pairs

BLAST: Basic local alignment search tool

DNA: Deoxyribonucleic acid

DRBC: Dichloran Rose-Bengal Chloramphenicol

EtBr: Ethidium bromide

FSL: Food safety lab

H2O: Water

ITS: Internal transcribed space

LAB: Lactic acid bacteria

LSU: Large subunit

MEA: Malt extract agar

NCBI: National center for biotechnology information

PBS: Phosphate buffered saline

PCR: Polymerase chain reaction

RPM: Revolutions per minute

SSU: Small subunit

TBE: Tris/Borate/EDTA

LIST OF SYMBOLS

mL: Milliliter

µL: Microliter

µM: Micromolar

°F: Fahrenheit

°C: Celsius

1. INTRODUCTION

Cultured dairy products are those that have gone through the fermentation process, resulting in changes to their flavor, texture [1]. Yogurt, sour cream, kefir, buttermilk, and several forms of cheese are examples of cultured dairy products. In addition to sensory modifications, the fermentation process has a positive impact on the nutritional composition of the dairy product. It can enhance digestion by boosting the growth of healthy gut bacteria and increasing the absorption of certain minerals like calcium [2]. However, the interplay of processing, packaging, storage conditions can endanger conditions conducive to microbial spoilage, subsequently compromising the overall product quality [3]. Dairy products exhibit relatively lower susceptibility to fungal spoilage compared to perishable items due to refrigerated storage, heat treatment and their fermented nature which fosters competitive microbiota, acidic pH, and presence of organic acids. Nevertheless, despite these factors, a substantial number of fungal species can persist and thrive in dairy products [4]. The most common types of spoilage in cultured dairy products include the growth of undesirable Lactic acid bacteria (LAB), proliferation of yeasts and contamination by molds [5]. Mold spoilage commonly arises from the proliferation of aerial fungal spores readily disseminated within the dairy processing facility [6]. The presence of a wide range of metabolic by-products, creating off-odors and flavors, as well as observable changes in color or texture, indicates fungal spoilage of dairy products [7].

A multitude of contemporary preservation strategies exist for extending the shelf life of food products among which packaging plays a crucial role. Packaging fulfills diverse fundamental functions such as safeguarding food from environmental influences, curtailing microbial growth and retarding the decline in food quality [8]. Numerous materials have been identified and

developed for application as food packaging materials. These encompass a diverse range of substances including paper, paperboard, glass, metals such as tin and aluminum, plastics, and composites [9]. The incorporation of recycled packaging in the food industry is essential to advance sustainability objectives and mitigate environmental repercussions. It can also be economically advantageous using less resources which leads to cost savings. However, it is essential that the recycled packaging is of high-quality and meets the food safety standards to avoid potential risks of contamination [10].

In recent years, various food safety outbreaks pollutants migrating from packaging materials has been brought to the consumers notice and some of them even at moderate levels can be detrimental [11]. Due to the phenomenon of processing and functional additives including mineral oil, phthalates and other substances migrating from recycled paper into food products, the use of recycled paperboard materials does not comply with the standards required for direct contact food packaging applications [12].

The primary objective of this study is to investigate the presence of the fungal microorganisms on the outer label affixed to the yogurt containers. Specifically, the study aims to address three main research questions. 1. To determine whether the outer label of the yogurt container is contaminated with fungal microorganisms. 2. To assess whether the extent of the fungal contamination and the diversity of the fungal genera vary depending on the type of packaging material used. 3. To look into the possibility of a relationship between the fungal contamination of the containers and yogurt spoilage. For instance, if the packaging material leads to substantial growth of a certain fungal genus (e.g., *Penicillium*) but the yogurt itself does not contain *Penicillium* according to the yogurt

sample database (Shi, in progress) it would suggest a lower likelihood of contamination originating from the packaging material. The study aims to provide insights into the potential sources of fungal contamination in the outer label of the yogurt packaging and its impact on yogurt quality.

Sanger sequencing represents a pioneering and fundamental technique within the domain of molecular biology dedicated to elucidating the nucleotide sequence of DNA [13]. Despite the advent of low-throughput sequencing technologies, sanger sequencing retains its significance as an indispensable tool for validating DNA sequences and exploring specific DNA regions [14].

The ITS (Internal Transcribed Spacer) DNA regions in fungi are short stretches of genetic material located between ribosomal RNA genes. These regions have proven to be invaluable in fungal research due to their high variability among species [15]. The genetic variation found in ITS regions allows for accurate identification and classification of fungi and provides insights into fungal diversity and evolution making them crucial in modern fungal research [16].

2. METHODOLOGY

2.1 Sample collection and processing

Yogurt containers with four types of label material were received in 2022 winter from a cultured dairy processing facility in upstate New York. Specifically, two were constructed using recycled packaging materials while the remaining two container labels were made from non-recycled heavy-duty cardboard packaging materials. A total of 48 containers, 12 of each label material were processed. The labels affixed to each container were detached and transferred into individual 720 mL filter bags (Whirl-Pak® Nasco, Fort Atkinson, WI) each containing 100 mL diluent (autoclaved distilled H_2O). The Whirl Pak bags, with the label contents, were then positioned within a stomacher (Stomacher® 400 Circulator Lab Blender, Seward Technology Centre, West Sussex) and subjected to 30 minutes of homogenisation at 230 RPM (Revolutions Per Minute). This was employed to facilitate the breakdown of the labels and attain a homogenous suspension. Following homogenisation, a series of four consecutive serial dilutions were conducted where 100 µL of the resulting homogenous suspension from each bag was meticulously pipetted into separate 1.5 mL Eppendorf tubes with 900 µL of Phosphate Buffered Saline (PBS) and vortexed for 5 seconds. 100 µL of the prepared solution from each serial dilution tube along with 100 µL from the undiluted sample was pipetted onto Malt Extract Agar (MEA) plates and incubated at 77°F (25°C) for a duration of 8-10 days. After the incubation period, the distinct morphological colonies were sub-cultured onto new MEA plates and incubated at 77°F (25°C) for a duration of 4 days to obtain pure cultures. The distinct morphological characteristics observed on each plate were recorded (Table 1) and samples were glycerol stock (25% concentration) preserved at -80°C for future analysis.

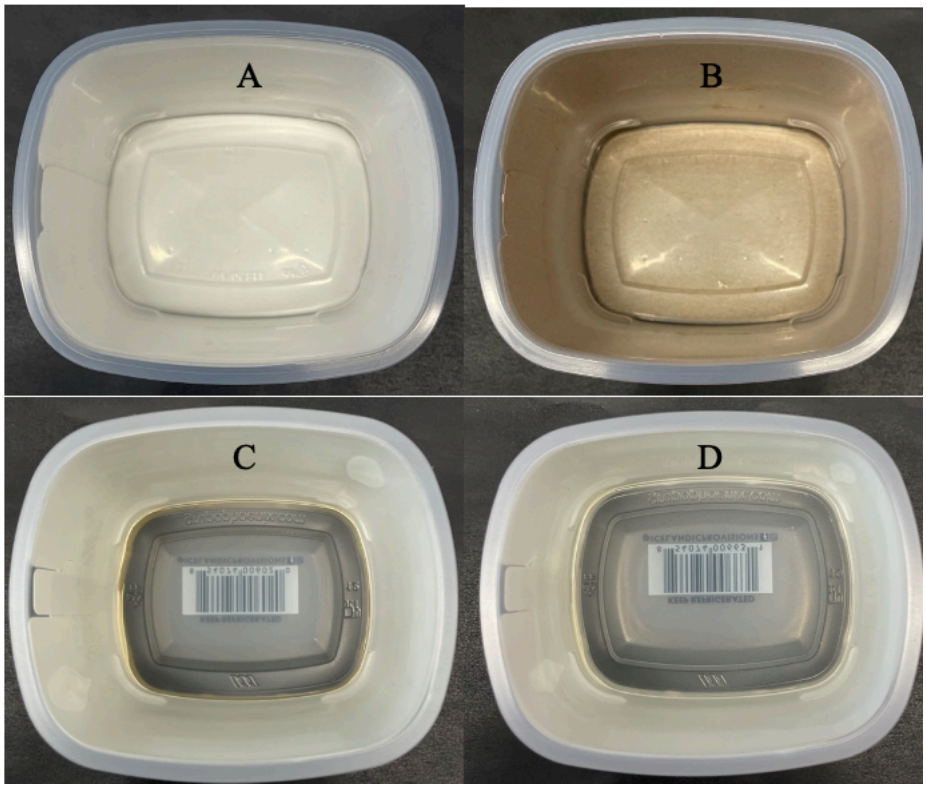


Figure 1: *The above image shows the yogurt containers with the different types of label materials. A) Recycled packaging 1; B) Recycled packaging 2; C) Non-recycled packaging 1; D) Non-recycled packaging 2*

2.2 DNA extraction

DNeasy® PowerSoil® Pro Kit (Qiagen, Hilden, Germany) was used to perform DNA extraction for the pure culture MEA plates selected (n=19). The protocol was adhered to without deviations but minor modifications. In the final step, for the elution of DNA from the filter membrane, a volume of 40 µL of distilled H_2O was introduced as a substitute for the C6 solution. Subsequently, nucleotide DNA concentration was measured using the NanoDrop® 2000c UV-Vis Spectrophotometer (Thermo Scientific Wilmington, DE, USA) through the process of nanodrop analysis.

2.3 Internal Transcribed Spacer Polymerase Chain Reaction (ITS PCR)

The experimental procedure developed by the Food Safety Lab and Milk Quality Improvement Program at Cornell University was employed. A Master mix was prepared of the components listed in the table below (Table 2) by pipetting each component into a sterile 1.5 ml microcentrifuge tube. The volume of the Master mix was calculated to incorporate one positive and one negative controls. In a 96-well PCR plate, 48 µL of the prepared Master mix was dispensed into individual wells corresponding to each lysate following the addition of 2 µL of lysate. The Applied Biosystems 2720 Thermal cycler (Foster city, CA, USA) was set up using the parameters in the table below (Table 3).

Reagent	**Volume per reaction (μL)**	**Final reaction concentration**
Sterile ddH_2O	28.5	
GoTaq Green 5x PCR Buffer	10	
dNTPs, 1mM each	5	200 µM
$MgCl_2$, 25mM	2	2 mM
ITS 4 Primer 10 μM	1	0.4 µM
ITS 5 Primer 10 μM	1	0.4 µM
GoTaq Flexi Polymerase	0.5	
Total	48.0 µL	

Table 2: *Components and volume of the master mix used for ITS PCR*

Time (minutes: seconds)	Temperature (℃)	Number of cycles
5:00	95	1
1:00	95	35
1:00	56	
1:00	72	
10:00	72	1
∞	4	1

Table 3: *Thermal cycler conditions for ITS PCR*

2.4 PCR product purification

The GeneJET PCR Purification kit (Thermo fisher Scientific, Lithuania) was utilized for the purification process. Following the completion of PCR, 50 µL of Binding buffer was added to the reaction mixture. 100 µL of the resulting solution was transferred into a GeneJET purification column and centrifuged at 15000 RPM for 1 minute. Next, 700 µL of Wash Buffer was introduced to the GeneJET purification column and centrifuged at 15000 RPM for 1 minute. The flow-through was discarded and an additional centrifugation was carried out for 1 minute to ensure complete removal of any remaining impurities. Subsequently, 50 µL of Elution Buffer was added to the column, followed by centrifugation for 1 minute. At this stage, the purified PCR product is ready for further analysis.

2.5 Gel electrophoresis and imaging

1 µL of Blue/Orange, 6X loading dye (Promega, USA) was added to 5 µL of PCR products. A prepared agarose gel (1.5%) was submerged into the chamber containing 0.5x Tris/Borate/EDTA

(TBE) buffer with the samples migrating from the negative electrode towards the positive electrode. In the first well, 3 µL of pGEM DNA ladder was introduced. 3 µL of PCR product was loaded into each respective lane. The PCR products underwent electrophoresis for a duration of 35 minutes at 90 volts. The gel from the electrophoresis chamber was transferred to Ethidium Bromide (EtBr) (0.0005%) staining solution and allowed to stain for 1 minute. The gel was them removed from EtBr solution and placed in a container with fresh tap water and allowed to de-stain for 1 hour. Following the de-staining process, the gel was viewed and analysed on the Molecular Imager® Gel Doc™ XR+ (Bio-Rad, Hercules, California) with Image Lab™ software.

2.6 Sanger sequencing

In a 96-well plate, the following volumes were added. 12.5µL of ddH2O, 2.5µL of primer, and 3µL of purified PCR product. Two sanger sequencing samples were prepared for each PCR product, one forward amplification (ITS 5 primer: GGA AGT AAA AGT CGT AAC AAG G) and one reverse amplification (ITS 4 primer: TCC TCC GCT TAT TGA TAT GC). The samples were then sent to the Biotechnology Resource Centre at Cornell University for sanger sequencing.

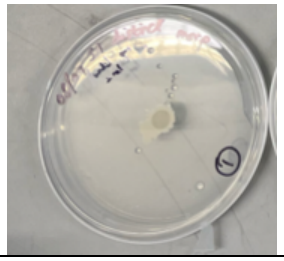
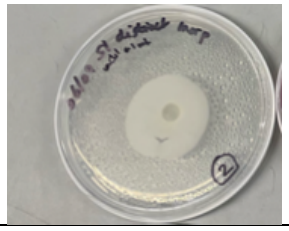
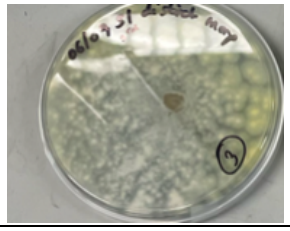
2.7 ITS sanger sequencing results analysis

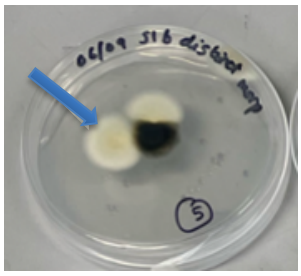
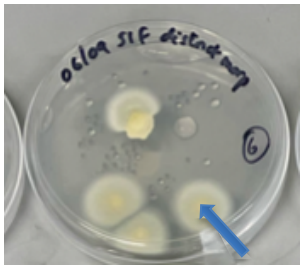
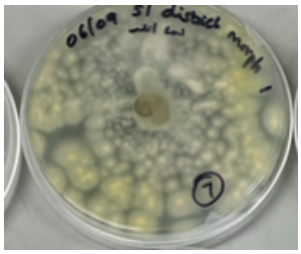
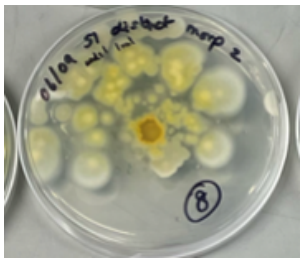
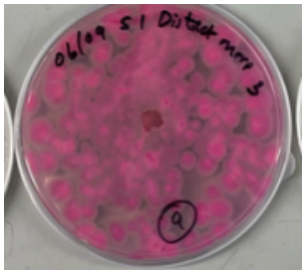
Geneious Prime® version 2023.0.4 was employed for the analysis of Sanger sequencing data. Initially, the data underwent a trimming process to eliminate low- quality sequences from raw reads. Subsequently, a de novo assembly was performed for batch alignment generating a consensus sequence for each pair of reads. The consensus sequences were subjected to Basic Local Alignment Search Tool (BLAST) on June 2023, against National Center for Biotechnology Information (NCBI) ITS database for the identification of the organism on genus level.

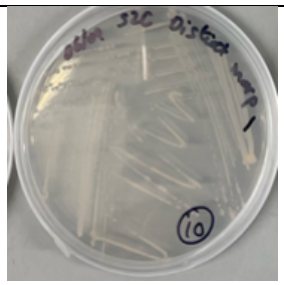
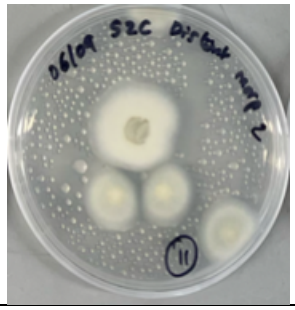
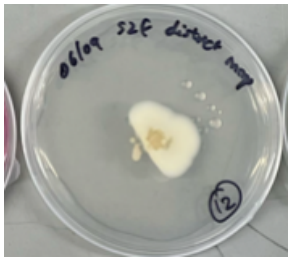
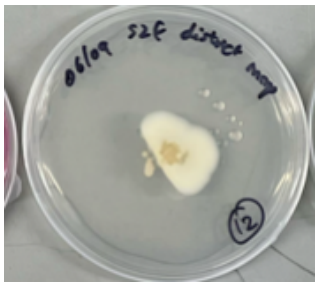
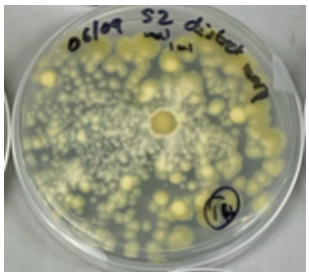
3. RESULTS AND DISCUSSION

3.1 Distinct morphologies and sanger sequencing results

The Distinct morphological characteristics of all 19 samples are recorded as below along with their genus which was determined from BLAST.

Sample ID	Type of Packaging	FSL ID	Genus (NCBI) Database	Colony Morphology Description	Picture
Sample 1	Recycled packaging 1	FSL S13-254	*Penicillium*	Medium sized white lobate like mold colony	
Sample 2	Recycled packaging 1	FSL S13-255	*Penicillium*	Large white mold colony	
Sample 3	Recycled packaging 1	FSL S13-238	*Penicillium*	Penicillium like dark green sporous mold colonies	
Sample 4	Recycled packaging 1	FSL S13-239	*Aspergillus*	Medium sized white lobate like mold colony with light yellowish center	

Sample 5	Recycled packaging 1	FSL S13–240	*Penicillium*	Medium sized white mold colony with light greenish yellow center	
Sample 6	Recycled packaging 1	FSL S13–241	*Penicillium*	Medium sized sporous white mold colony with green yellow center	
Sample 7	Recycled packaging 1	FSL S13–242	*Penicillium*	Penicillium like dark green sporous mold colonies	
Sample 8	Recycled packaging 1	FSL S13–243	*Penicillium*	Penicillium like dark green sporous mold colonies	
Sample 9	Recycled packaging 1	FSL S13–244	*Penicillium*	Penicillium like dark green sporous mold colonies on DRBC medium	

Sample 10	Recycled packaging 2	FSL S13–245	*Cystobasidium*	Small salmon pink yeast colony	
Sample 11	Recycled packaging 2	FSL S13–246	*Penicillium*	Medium sized white mold colony with green center	
Sample 12	Recycled packaging 2	FSL S13–247	*Aspergillus*	Medium sized white lobate like mold colony	
Sample 13	Recycled packaging 2	FSL S13–248	*Penicillium*	Medium sized white coral like mold colony	
Sample 14	Recycled packaging 2	FSL S13–249	*Penicillium*	Penicillium like green mold with yellow color outer and brownish center colonies	

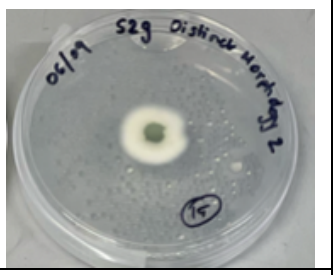
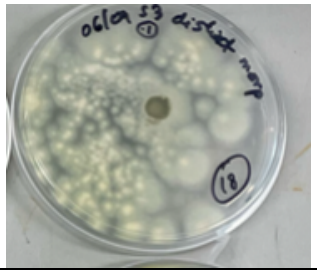
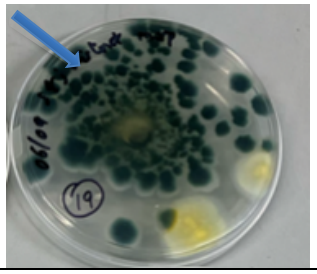
Sample 15	Recycled packaging 2	FSL S13–250	*Aspergillus*	Medium sized white mold with green center colony	
Sample 16	Recycled packaging 2	-	*Penicillium*	Medium sized white mold with green center colony	
Sample 17	Non-recycled packaging 1	FSL S13–251	*Penicillium*	Penicillium like green sporous mold colonies	
Sample 18	Non-recycled packaging 1	FSL S13–252	*Penicillium*	Penicillium like green sporous mold colonies	
Sample 19	Non-recycled packaging 2	FSL S13–253	*Penicillium*	Penicillium like dark green sporous mold colonies	

Table 1: *Distinct morphologies of the 19 samples from the different types of packaging materials tested along with the genus name identified.*

3.2 Gel electrophoresis

The gel electrophoresis image displayed in figure 2 showcases bright amplified DNA gel bands for all 19 samples interspersed with observed smearing in interstitial regions. The first well on the left contained pGEM ladder bands which served as a reference for estimating the size of the DNA fragments of the samples. Through a comparative analysis of the migration distances between the ladder and sample gel bands, the approximate size of the DNA fragments was determined to be 600 base pairs (bp). The presence of smearing in gel electrophoresis may arise from inadequate PCR product purification or the presence of contaminants such as proteins or undesired organic compounds like ethanol, phenols [17].

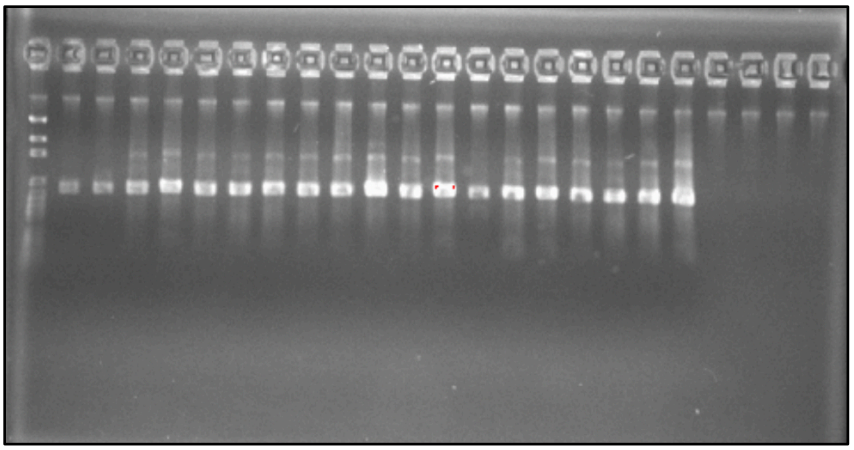


Figure 2: *An EtBr-stained agarose gel showing DNA fragments produced by PCR amplification of ITS region. In the above image, from left to right the wells are loaded with pGEM ladder followed by the 19 samples*

Upon careful examination of the image, the presence of double bands is seen, potentially resulting from the presence of multiple copies of ITS region in *Penicillium* species because the ITS region located between the small subunit (SSU) and large subunit (LSU) DNA genes can exhibit sequence variation and multiple copies in certain fungal species. At the same time, further investigations and analyses would be necessary to confirm the exact causes of the double bands observed. One such method can be gel extraction of the double bands. Nevertheless, results obtained from Sanger

sequencing exhibited that the sequencing reaction was successful and was able to be read without any disturbances or noises. Thus, the presence of demultiplication of double bands can be deemed negligible.

3.3 Statistical data analysis

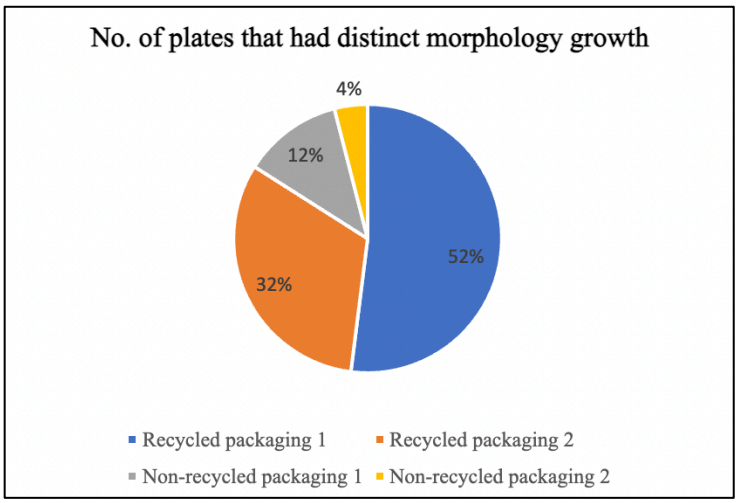


Figure 3: *The above pie chart shows the relative proportion of fungal distinct morphological growth in the different types of packaging materials tested*

Based on the visual analysis of figure 3, it can be observed that recycled packaging 1,2 exhibited distinct morphological growth with percentages of 52%,32% respectively, out of the total number of processed containers (n=48). Conversely, non-recycled packaging demonstrates a lower percentage of distinct morphological growth.

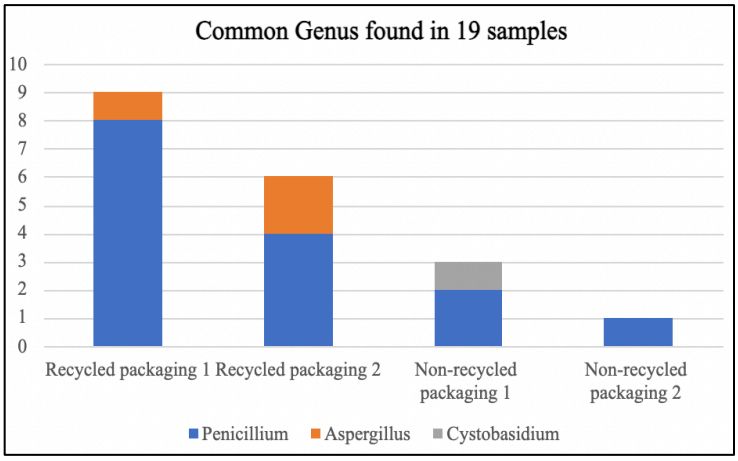


Figure 4: *The graph shows the occurrence of the most common fungal genera found in the packaging materials tested*

The results obtained in this study showed that the 19 yogurt containers tested exhibited broadly three fungal species, namely *Penicillium* (78.9%), *Aspergillus* (15.7%), *Cystobasidium* (5.4%).

The impact of recycled packaging on fungal growth can be attributed to the following factors. Firstly, recycled packaging materials contain a higher concentration of organic compounds which serve as nutrient sources for fungal growth. Secondly, these materials tend to exhibit enhanced moisture retention in comparison to non-recycled paper materials thereby creating a favorable environment for fungal growth particularly in instances where they are stored in damp or humid conditions. Additionally, the overall processing of the recycled materials used in food contact surfaces and environmental conditions surrounding storage of the packaging such as temperature, humidity, light exposure can exert influence on the growth of fungi.

As seen in Figure 1, recycled packaging 1 and 2 exhibited a greater surface area of packaging material at the bottom of the container, which may serve as an additional factor contributing to the increased likelihood of fungal adhesion from the production lines.

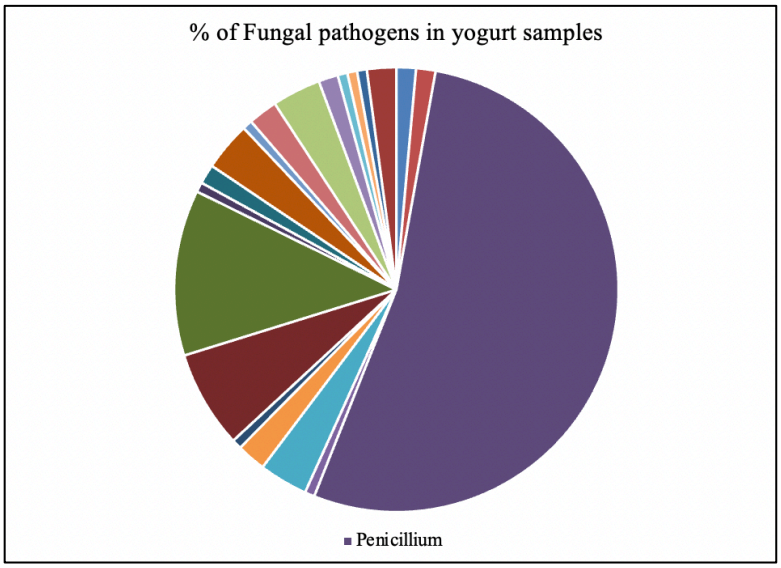


Figure 5*: The above chart illustrates the diversity of fungal spoilage biota and percentage of Penicillium (53%) in yogurt samples*

A comparative analysis was conducted between the fungal pathogen results obtained from yogurt containers and the database of yogurt samples tested from the same cultured dairy production facility (source: Shi, in progress, unpublished). The analysis revealed a predominant presence of *Penicillium* in both the yogurt

samples and the containers; this suggests that the prevalence maybe attributed to the migration of fungal spores from the exterior surface of the container's labels. Investigating the potential causes of interior contamination within the yogurt packaging containers, its postulated that the stacking and storage of the containers within the production facility prior to use may serve as one contributing factor. Hence, it not only results in external contamination but also extends to the yogurt sample, thereby compromising its quality. However, further investigation into the factors influencing this phenomenon is needed for a comprehensive understanding of the microbial dynamics in the packaging and its potential impact on the product quality.

It is imperative to acknowledge that the aforementioned factors may contribute to the observed outcomes. Nevertheless, it is equally crucial to consider the conditions prevailing at the dairy plant, the intricacies of the production process, handling procedures by the employers and the potential presence of airborne fungal spores.

3.4 Interventions

To mitigate fungal contamination and growth in dairy processing plants, several interventions can be implemented. Firstly, screening tests should be conducted for packaging materials and those failing to meet the necessary requirements should be excluded from use. Secondly, yogurt samples can be analysed for the presence of sorbate, a widely employed food preservative and antifungal agent. Sorbate acts by disrupting the cellular metabolism and membrane function of fungi thereby inhibiting their growth and reproduction [18]. Additionally, discontinuing the use of recycled packaging materials and packaging containing cardboard can be considered. Alternative packages incorporating antifungal or antimicrobial agents like benzoic acid or sorbic acid can be utilized as

effective measures against fungal proliferation and contamination [19]. These interventions aim to ensure the production of high-quality and safe dairy products by effectively controlling fungal related risks in dairy processing plants.

4. LIMITATIONS OF THE STUDY

Some of the limitations encountered in the conducted study are as follows. Firstly, the use of ITS PCR might have resulted in the appearance of double bands during gel electrophoresis which could be attributed to the inherent limitations associated with ITS as a genetic marker as ITS is a multi-copy amplicon. To gain further insights, microscopic analysis of fugal samples can be performed to complement the molecular approach. Secondly, to enhance the robustness of the findings, increasing the number of sample analyses is recommended. Expanding the sample size can strengthen statistical power and improve the generalizability of the results. Furthermore, obtaining additional data from the processing facility regarding the production processes and conditions at the plant would be beneficial. Augmenting the dataset with detailed information from the plant can provide a more comprehensive understanding of the factors influencing fungal contamination in the dairy production environment. Addressing these limitations can contribute to the overall validity and comprehensiveness of the study's conclusions.

5. CONCLUSION

Overall, the study revealed that outer labels of the yogurt containers made from recycled packaging materials exhibited a higher percentage of fungal contamination compared to non-recycled containers. This observation could be attributed to the likelihood that recycled packaging materials may contain a higher proportion of organic matter and moisture, creating a favorable environment for fungal growth. Additionally, the increased surface area of recycled packaging materials could contribute to a higher chance of fungal colonization. To mitigate these concerns, the industry could consider utilizing different types of packaging materials while also considering sustainability aspects. Furthermore, it is important to acknowledge that factors beyond packaging materials, such as the prevailing conditions at the processing plant and the handling practices by the employees, may also influence fungal contamination levels. Understanding and addressing these multiple factors are crucial for maintaining product quality and safety in the yogurt production process.

REFERENCES

1. *4JI-04PA Cultured Dairy Products*. (n.d.). Retrieved August 1, 2023, from http://www2.ca.uky.edu/agcomm/pubs/4ji/4ji04pa/4ji04pa.pdf
2. Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. *Journal of Dairy Science*, *100*(12), 9987–10013. https://doi.org/10.3168/jds.2017-12981
3. Barukčić, I., Ščetar, M., Lisak Jakopović, K., Kurek, M., Božanić, R., & Galić, K. (2021). Overview of packaging materials for Dairy packaging. *Hrvatski Časopis Za Prehrambenu Tehnologiju, Biotehnologiju I Nutricionizam*, *16*(3-4), 85–93. https://doi.org/10.31895/hcptbn.16.3-4.5
4. Buehler, A. J., Martin, N. H., Boor, K. J., & Wiedmann, M. (2018). Evaluation of biopreservatives in Greek yogurt to inhibit yeast and mold spoilage and development of a yogurt spoilage predictive model. *Journal of Dairy Science*, *101*(12), 10759–10774. https://doi.org/10.3168/jds.2018-15082
5. DeNovix. (2019, March 9). *TN 130 Purity Ratios Explained*. DeNovix. https://www.denovix.com/tn-130-purity-ratios-explained/
6. Deshwal, G. K., Panjagari, N. R., & Alam, T. (2019). An overview of paper and paper based food packaging materials: health safety and environmental concerns. *Journal of Food Science and Technology*, *56*(10), 4391–4403. https://doi.org/10.1007/s13197-019-03950-z
7. Garnier, L., Valence, F., & Mounier, J. (2017). Diversity and Control of Spoilage Fungi in Dairy Products: An Update. *Microorganisms*, *5*(3), 42. https://doi.org/10.3390/microorganisms5030042

8. Garnier, L., Valence, F., Pawtowski, A., Auhustsinava-Galerne, L., Frotté, N., Baroncelli, R., Deniel, F., Coton, E., & Mounier, J. (2017). Diversity of spoilage fungi associated with various French dairy products. *International Journal of Food Microbiology*, *241*, 191–197. https://doi.org/10.1016/j.ijfoodmicro.2016.10.026
9. Garnier, L., Valence, F., Pawtowski, A., Auhustsinava-Galerne, L., Frotté, N., Baroncelli, R., Deniel, F., Coton, E., & Mounier, J. (2017). Diversity of spoilage fungi associated with various French dairy products. *International Journal of Food Microbiology*, *241*, 191–197. https://doi.org/10.1016/j.ijfoodmicro.2016.10.026
10. Gurr, M. I. (1984). The Nutritional Role of Cultured Dairy Products. *Canadian Institute of Food Science and Technology Journal*, *17*(2), 57–64. https://doi.org/10.1016/s0315-5463(84)72357-1
11. Hladíková, Z., Kejlová, K., Sosnovcová, J., Jírová, D., Vavrouš, A., Janoušek, A., Syčová, M., & Špelina, V. (2016). Microbial contamination of paper-based food contact materials with different contents of recycled fiber. *Czech Journal of Food Sciences*, *33*(No. 4), 308–312. https://doi.org/10.17221/645/2014-cjfs
12. Huang, T., Qian, Y., Wei, J., & Zhou, C. (2019). Polymeric Antimicrobial Food Packaging and Its Applications. *Polymers*, *11*(3), 560. https://doi.org/10.3390/polym11030560
13. Ledenbach, L. H., & Marshall, R. T. (2009). Microbiological Spoilage of Dairy Products. *Compendium of the Microbiological Spoilage of Foods and Beverages*, 41–67. https://doi.org/10.1007/978-1-4419-0826-1_2
14. Martin, K. J., & Rygiewicz, P. T. (2005, May 18). *Fungal-specific PCR primers developed for analysis of the its region of environmental DNA extracts - BMC microbiology*. BioMed Central. https://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-5-28

15. Raheem, D. (2013). Application of plastics and paper as food packaging materials ? An overview. *Emirates Journal of Food and Agriculture*, *25*(3), 177. https://doi.org/10.9755/ejfa.v25i3.11509
16. Recent trends in packaging of dairy and food products - dairy knowledge. (n.d.-a). https://www.dairyknowledge.in/sites/default/files/ch5_1.pdf
17. Rejeesh, C. R., & Anto, T. (2022). Packaging of milk and dairy products: Approaches to sustainable packaging. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2022.07.467
18. Resistance and adaptation to Food Antimicrobials, sanitizers, and other ... (n.d.). https://www.researchgate.net/profile/P-Davidson-2/publication/237373178_Resistance_and_Adaptation_to_Food_Antimicrobials_Sanitizers_and_Other_Process_Controls/links/5416db750cf2fa878ad42fc7/Resistance-and-Adaptation-to-Food-Antimicrobials-Sanitizers-and-Other-Process-Controls.pdf?origin=publication_detail
19. Sanger, F., & Coulson, A. R. (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology*, *94*(3), 441–448. https://doi.org/10.1016/0022-2836(75)90213-2
20. Ščetar, M., Galić, K., Božanić, R., Lisak Jakopović, K., Kurek, M., & Barukčić, I. (2019). Packaging perspective of milk and dairy products. *Mljekarstvo*, *69*(1), 3–20. https://doi.org/10.15567/mljekarstvo.2019.0101
21. Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Bolchacova, E., Voigt, K., Crous, P. W., Miller, A. N., Wingfield, M. J., Aime, M. C., An, K.-D. ., Bai, F.-Y. ., Barreto, R. W., Begerow, D., Bergeron, M.-J. ., Blackwell,

M., & Boekhout, T. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, *109*(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

22. Snyder, A. B., Churey, J. J., & Worobo, R. W. (2016). Characterization and control of Mucor circinelloides spoilage in yogurt. *International Journal of Food Microbiology*, *228*, 14–21. https://doi.org/10.1016/j.ijfoodmicro.2016.04.008
23. Suberu, H & Mohammed, Neimat & Aminu, Mubarak & Naka Keta, Jibrin. (2019). Evaluation of Fungi Species from Commercial Yoghurts in. 6. 72-77.
24. Vasileios Triantafyllou, K. Akrida-Demertzi, & Demertzis, P. G. (2002). Migration studies from recycled paper packaging materials: development of an analytical method for rapid testing. *Analytica Chimica Acta*, *467*(1-2), 253–260. https://doi.org/10.1016/s0003-2670(02)00189-7
25. Wu, Y.-M., Hu, C.-Y., Wang, L., Cheng, J., Wang, Z.-W., & Zhu, Y. (n.d.). *Migration of Contaminants from Packaging Materials into Dairy Products**. Retrieved August 1, 2023, from https://file.scirp.org/pdf/21-1.51.pdf
26. Zamani Mazdeh, F., Sasanfar, S., Chalipour, A., Pirhadi, E., Yahyapour, G., Mohammadi, A., Rostami, A., Amini, M., & Hajimahmoodi, M. (2017). Simultaneous Determination of Preservatives in Dairy Products by HPLC and Chemometric Analysis. *International Journal of Analytical Chemistry*, *2017*, 1–8. https://doi.org/10.1155/2017/3084359