12-2024

Metabolic Capabilities of *Latilactobacillus curvatus* and the Potential Use as an Adjunct Culture for Food Safety and Quality Control in Yogurt

R. Chase Wahlstrom
Utah State University

METABOLIC CAPABILITIES OF *LATILACTOBACILLUS CURVATUS* AND THE POTENTIAL USE AS AN ADJUNCT CULTURE FOR FOOD SAFETY AND QUALITY CONTROL IN YOGURT

by

R. Chase Wahlstrom

A thesis proposal submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

Taylor Oberg, Ph.D. Major Professor	Prateek Sharma, Ph.D. Committee Member
Robert Ward, Ph.D. Committee Member	Richard Cutler, Ph.D. Vice Provost of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2024

ABSTRACT

Metabolic Capabilities of *Latilactobacillus curvatus* and the Potential Use as an Adjunct Culture for Food Safety and Quality Control in Yogurt

by

R. Chase Wahlstrom, Master of Science

Utah State University, 2024

Major Professor: Dr. Taylor S. Oberg
Department: Nutrition, Dietetics and Food Sciences

Given the ever-increasing demand for clean-label products, understanding and optimizing biopreservation using lactic acid bacteria is paramount in the food and dairy industries. *Latilactobacillus curvatus* is a promising candidate as a bioprotective adjunct culture in dairy fermentations. Genomic analysis of a dairy isolate, WSU-1, identified the presence of the propane-diol utilization pathway, which can produce 3-hydroxypropionate with reuterin as an intermediate or propionate, depending on the substrate. Initial GC-MS analysis of fermentate from a controlled fermentation showed the production of 3-hydroxypropionate and propionate. A colorimetric assay for reuterin determination indicated a concentration of 1 mM after 24 hours.

An in vitro method was developed to test the antifungal capabilities of WSU-1 under three conditions: standard MRS (de Man, Rogosa, and Sharpe) agar, carbohydrate-restricted (CR) MRS with 200 mM glycerol, and CR-MRS with 200 mM 1,2-propanediol, using a modified overlay method in 24-well plates. A 5-point visual grading

scale qualitatively demonstrated varying antifungal activity against all eight yeast and nine mold strains.

This capability was further tested in situ in a yogurt system. Three 50-gallon yogurt vats were produced in triplicate, containing either the addition of WSU-1 or WSU-1 with 200 mM glycerol. A control vat was made without the adjunct or glycerol. Yogurt was packaged into 8-ounce cups and inoculated in triplicate with a single mold or yeast strain at an inoculation rate of 10^3 CFU/g. Uninoculated cups were kept as controls. Samples were held at 8 °C for four weeks.

Standard plate counts analyzed Yeast growth weekly, showing no statistically significant inhibition in any yeast strain (Tukey HSD post hoc analysis with adjusted p-values < 0.05). Mold growth was visually analyzed using a 5-point visual classification scale, with subsequent analysis using a Kruskal-Wallis test with Dunn post-hoc analysis and Bonferroni corrections. Results showed limited inhibition in treatment yogurts compared to controls across replicates and time points.

These results indicate that while WSU-1 exhibited antifungal activity in a controlled in vitro study, these effects were not substantiated to the same extent in the yogurt system, highlighting the need for further validation in real-life food systems.

(178 pages)

PUBLIC ABSTRACT

Metabolic Capabilities of *Latilactobacillus curvatus* and the Potential Use as an Adjunct Culture for Food Safety and Quality Control in Yogurt

R. Chase Wahlstrom

In the quest for healthier and more natural food products, using beneficial lactic acid bacteria to preserve food, known as biopreservation, is becoming increasingly important. This research focused on a strain of beneficial bacteria called *Latilactobacillus curvatus* and its ability to inhibit fungal growth both in lab settings and in a yogurt system. This has the potential to enhance the safety and quality of dairy products.

We studied a specific strain of this bacteria, named WSU-1, found in dairy. Through detailed analysis, we discovered that this strain could produce substances that may help protect food from harmful microbes. In laboratory tests, we found that WSU-1 can produce compounds like 3-hydroxypropionate and reuterin, known for their antimicrobial properties.

We conducted experiments in the lab and in a yogurt production setting to test the bacteria's effectiveness in actual food conditions. In the lab, WSU-1 successfully inhibited the growth of various harmful yeast and mold strains. However, when we tested it in yogurt, the results were mixed. While WSU-1 showed some ability to prevent mold and yeast growth in controlled conditions, this effect was not as strong in the yogurt tests.

Our findings suggest that while WSU-1 has potential as a natural preservative, more research is needed to understand how it can be effectively used in food products.

This work is a step toward developing safer, more natural ways to keep our food fresh and free from harmful microbes.

DEDICATION

To my fantastic wife, Nikki, for your constant support; you are amazing.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my parents for their unending support throughout this research and the entirety of my education. I am immensely grateful to my wonderful wife, Nikki, for her moral support and invaluable advice while navigating grad school; you are the best. I also wish to thank the entire BUILD Dairy organization for their support throughout this project, as well as during my undergraduate research and education. I can confidently say that I would not be here today without their support. Lastly, I would like to thank my committee, especially my advisor, Taylor Oberg, for all your assistance and mentorship throughout this project.

CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

1,3-PDO = 1,3-propanediol

3-HP = 3-hydroxypropionate

3-HPA = 3-hydroxypropionaldehyde

BCP = Bromocresol purple

BPW = Buffered peptone water

CR-MRS = Carbohydrate-restricted MRS

GRAS = Generally recognized as safe

LAB = Lactic acid bacteria

ME = Malt Extract

MRS = De Man–Rogosa–Sharpe

NSLAB = Non-starter lactic acid bacteria

PBS = Phosphate-buffered saline

RAST = Rapid Annotation using Subsystem Technology

SLAB = Starter lactic acid bacteria

Tm = Primer melting temperature

INTRODUCTION

Bio-preservation is currently one of the most compelling topics in the food and dairy industry. In response to consumer demand for cleaner labels—featuring fewer ingredients and more natural components—food industries are compelled to explore natural methods of food preservation that uphold food quality and shelf-life standards. Bio-preservation harnesses the potential of organisms such as lactic acid bacteria (LAB), leveraging their unique metabolic capabilities to suppress spoilage organisms, thereby reducing reliance on artificial preservatives.

Among these organisms, *Latilactobacillus curvatus* strain WSU-1 emerges as a promising candidate for bioprotective applications, particularly in dairy products like yogurt. The distinctive metabolic and physiological traits of *Lat. curvatus* WSU-1 enable it to inhibit various microbes through the production of antimicrobial compounds, such as reuterin, alongside other competitive advantages. Unraveling the full scope of its metabolic capabilities and assessing its efficacy as a protective adjunct culture against mold and yeast spoilage holds significant implications for the food and dairy industries.

By delving into the mechanisms by which *Lat. curvatus* WSU-1 preserves yogurt, we aim to expand our understanding of biopreservation and contribute to the development of safer, more natural food preservation techniques that align with consumer preferences and industry standards.

HYPOTHESIS AND OBJECTIVES

Hypotheses:

1. *Lat. curvatus* strain WSU-1 can produce the antimicrobial compound reuterin in bulk fermentation and inhibit an array of dairy spoilage fungi, including yeasts and molds, in an in vitro system.
2. *Lat. curvatus* can produce reuterin in situ in a yogurt fermentation and inhibit an array of dairy spoilage yeasts and molds in a yogurt system.

Objectives:

1. The metabolic capabilities of *Lat. curvatus* will be analyzed in model bulk fermentations and tested in vitro for antifungal properties.
2. *Lat. curvatus* will be tested in situ in a yogurt system, and its antifungal capabilities will be examined over a typical shelf life against an array of common dairy spoilage fungi.
3. Yogurt produced with and without *Lat. curvatus* adjunct will be subject to consumer testing to determine if there is a significant difference between the yogurts.

LITERATURE REVIEW

Bio-preservatives and their importance

One of the leading causes of product loss and one of the biggest health concerns in the food industry is contamination, be it from mold, yeast, or bacteria. Dairy products have been found to be very susceptible to fungal spoilage (Suriyarachchi and Fleet, 1981; Kure et al., 2004; Ledenbach and Marshall, 2009). Thus, effectively identifying sources of contamination and developing inhibitory products against these organisms has been a prominent topic in the dairy industry for many years.

Post-pasteurization contamination has been identified as the primary concern of mold contamination, either through the air or improperly cleaned processing and packing equipment because these organisms have not been found to survive pasteurization (Beletsiotis et al., 2011). Regardless of the source, inhibiting the growth of these organisms below the required threshold is essential to prevent significant waste in the dairy industry.

Yogurt is a fermented dairy product with a low pH and high nutritional content. Because of its low pH, several acid-tolerant molds and yeasts have been identified as the primary sources of contamination. Belonging to the phyla Ascomycota and Zygomycota and specifically, from the genera *Aspergillus spp. Penicillium spp., Fusarium spp.,* and *Alternaria spp.* among molds and *Candida spp., Mucor spp.,* and *Rhizopus spp.* Among the yeasts (Beletsiotis et al., 2011).

The spoilage effects of yeast in yogurt have been covered extensively in the literature (Suriyarachchi and Fleet, 1981; Fleet and Mian, 1987; Fleet, 1990). Although accounting for many spoilage issues, yeasts do not present a substantial health risk, as noted by Fleet. However, there are several potentially dangerous bacteria, such as E. coli and Listeria, that could be present in dairy products (Ryser et al., 1985; Hicks and Lund, 1991; Arocha et al., 1992). In addition, mycotoxins, resulting from mold growth, are a concern when it comes to fungal contamination because of their danger to public health as well as the dairy economy (Filtenborg et al., 1996; Dalié et al., 2010; Becker-Algeri et al., 2016).

Previous attempts to mitigate spoilage by unwanted microbes have led to the development and use of many different artificial preservatives, such as benzoates and sorbates, and antimicrobials like natamycin (Fleet and Mian, 1987; Brul and Coote, 1999; Paul Ross et al., 2002; Mroueh et al., 2008). However, shorter ingredient labels and a reduction in the use of synthetic chemicals are becoming essential to satisfy consumer demand for clean-label products across the food industry (Mills et al., 2011).

This poses the challenge of maintaining control of spoilage and pathogenic organisms without compromising quality and maintaining a clean label. Bio-preservation through antimicrobial production in lactic acid bacteria (LAB) has emerged as a front-runner for clean-label food preservation in the dairy industry (Crowley et al., 2013a; Makki et al., 2020).

LAB as bio-preservative cultures

The use of LAB as a food preservation method spans centuries and is generally recognized as safe (GRAS) by the USDA and FDA. This group of bacteria produces a highly diverse array of fermentation products such as flavor compounds, organic acids, proteases, low molecular weight antimicrobial compounds, and bacteriocins (Klaenhammer, 1988; Muriana and Luchansky, 1993). However, the production of antimicrobial compounds is not the only mechanism for inhibition in LAB, and often, these compounds are found below MIC when analyzed alone.

The diverse physiological capabilities and biochemistry permit LAB to outgrow and outcompete many other organisms, and this has recently been found to be one of the most essential factors permitting LAB to outcompete and inhibit other contaminate organisms. One of these mechanisms is manganese scavenging (Siedler et al., 2019, 2020; Shi and Maktabdar, 2022), where the presence of an mntH gene has displayed a distinct phylogenetic pattern within the *Lactobacillus* genus (Hohle and O'Brian, 2009; Siedler et al., 2020; van Gijtenbeek et al., 2021). Both antimicrobial compound production and competitive exclusion culminate in making LAB bio-preservatives an additional tool in food preservation hurdle technology (Rouse et al.; Singh and Shalini, 2016).

LAB efficacy as a preservative has been shown against many organisms, including pathogenic bacteria (Castellano et al., 2008; Mobarak et al., 2015) and mold and yeast spoilage (Gerez et al., 2013; Leyva Salas et al., 2017; Salas et al., 2018). Additionally, the use of LAB as a bioprotective agent has been demonstrated in many

fields throughout the food industry, including dairy (Sedaghat et al., 2016), meats (Castellano et al., 2008, 2010), fruits (Crowley et al., 2013b), cereals (Russo et al., 2017) among others.

The efficiency of different commercial protective cultures in dairy products, such as cottage cheese, queso fresco, Greek yogurt, and others, against common yeasts and molds has been studied. It was found that commercial bio-preservative cultures vary substantially in their ability to inhibit molds and yeasts in different dairy matrices (Buehler et al., 2018; Makki et al., 2020, 2021). Studies analyzing various LAB in a yogurt system have shown high variation in fungal susceptibility between LAB species (Delavenne et al., 2013). In addition, there have been studies investigating the inhibitory effects of protective cultures against pathogenic bacteria typically found in dairy, such as *E. coli* 0157:H7 and *Listeria monocytogenes* (Al-Nabulsi et al., 2021; van Gijtenbeek et al., 2021).

Latilactobacillus curvatus potential as a bio-preservative

Latilactobacillus curvatus is a facultative heterofermentative lactic acid bacterium that has garnered a lot of interest as both a candidate probiotic as well as a bioprotective agent in various food products (Chen et al., 2020; Heidari et al., 2022) and has been isolated from a diverse array of food products. Most commonly associated with aged meats and meat packing (Hammes et al., 1990; Hammes and Hertel, 1998; Castellano et al., 2010), *Lat. curvatus* has also been isolated out of dairy (Kask et al., 2003), sourdough bread (Michel et al., 2016), and honey (Bulgasem et al., 2018), as well as many

fermented vegetables such as pickles, sauerkraut, and kimchi (Vogelxy et al., 1993; Jung et al., 2011; Nakano et al., 2016).

Recently, *Lat. curvatus* has been identified as a member of the NSLAB (non-starter lactic acid bacteria) population found in many aged hard and semi-hard cheeses and is the most common NSLAB found in North American cheeses (Broadbent et al., 2003; Oberg et al., 2022). In previous research, several strains of *Lat. curvatus* were isolated from aged cheddar cheese, and next-generation whole genome sequencing was performed (Broadbent et al., 2014). After analyzing the genomes, several interesting potential metabolic pathways were identified.

One interesting pathway has the potential to produce propionic acid from lactate, or 1-2 propanediol, a potential antimicrobial, and another pathway has the potential to metabolize glycerol and produce reuterin (Terán et al., 2018), a potent antimicrobial that has been shown to be effective against common pathogenic organisms found in dairy products (Talarico and Dobrogosz, 1989; El-Ziney and Debevere, 1998).

Lat. curvatus **antimicrobial capabilities**

Reuterin

Reuterin is defined as a dynamic system composed of 3-hydroxypropionaldehyde (3-HPA), its hydrate form, its dimer, and, as of 2016, acrolein (Engels et al., 2016), which is a result of spontaneous dehydration of 3-HPA in aqueous solutions (Vollenweider and Lacroix, 2004; Engels et al., 2016). The biological formation of reuterin as well as 3-hydroxypropionic acid has been well documented in bacterial genera

Clostridia, *Klebsiella*, *Citrobacter*, as well as *Lactobacilli*, an important member of the lactic acid bacteria (Talarico et al., 1988; Dishisha et al., 2014).

The synthesis of reuterin has been identified as an intermediate product of the PDU pathway, a glycerol fermentation pathway in which glycerol is converted into 3-hydroxypropionic acid (3-HP) and 1,3-propandiol (1,3PD) catalyzed by a B12-dependent glycerol dehydratase, with 3-HPA serving as an intermediate (Fig. 1) (Dishisha et al., 2014; Gopi et al., 2015).

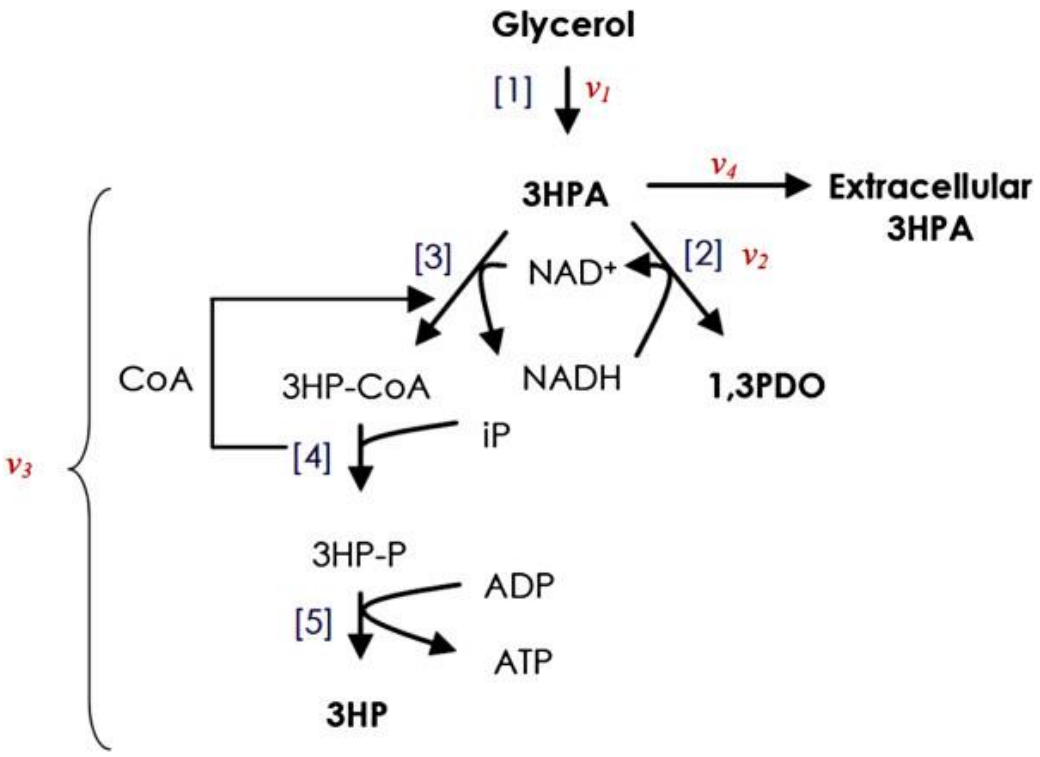


Figure 1. Fermentation mechanism of glycerol to its two end products 3-HP and 1,3PD by means of the intermediate 3-HPA catalyzed by a B-12 dependent dehydrogenase (Dishisha et al., 2014).

The antimicrobial mechanism of reuterin induces oxidative stress through modification of thiol groups in proteins and small molecules, depleting glutathione and modifying functional enzymes (Schaefer et al., 2010; Vollenweider et al., 2010; Engels et al., 2016). Reuterin is effective at inhibiting a broad range of Gram-positive and Gram-negative bacteria, bacterial spores, fungi, and protozoa, including food-borne pathogens, such as *E. coli* 0157:H7 studied in White brined cheeses (Ávila et al., 2014; Al-Nabulsi et al., 2021) as well as *L. monocytogenes* and *S. aureus* in milk and cottage cheese (El-Ziney and Debevere, 1998; Arqués et al., 2004). However, it was noted that Gram-positive bacteria were more resistant (Ortiz-Rivera et al., 2017).

Research has been conducted to determine the minimum inhibitory activity (MIC) and minimum fungicidal activity (MFC) of reuterin against a panel of abundant common fungi related to food contamination and spoilage. The study also tested the antifungal activity of purified reuterin in yogurt, where concentrations above 1.38 mM inhibited the visual growth of fungi but also resulted in a yellowish color to the yogurt (Vimont et al., 2019).

Additional literature has found that the fermentation of glycerol to reuterin can cause shifts in the L* a* b* color spectrum (Gómez-Torres et al., 2014); they observed that cheese made with *Lim. reuteri* in the presence of 100mM glycerol caused positive change on the a* scale, resulting in a pink-reddish color. Additionally, (Ortiz-Rivera et al., 2017) noted that a fermented milk product without reuterin displayed higher L* values than a fermented milk product with reuterin. Still, fermented milk products with reuterin tended to show higher a* and b* values than their reuterin-free counterparts, which signifies a shift from light greenish tones to yellowish-orange tones. However,

they concluded that the presence of reuterin did not cause relevant changes in the quality parameters of the fermented milk product, including pH, acidity, soluble solids, color, and rheological aspects (Ortiz-Rivera et al., 2017).

Propionic acid

Propionic acid is a ubiquitous fatty acid present in many processed foods and animal feedstocks. It is a natural intermediate and metabolite in many biological processes. Designated as generally regarded as safe (GRAS) by the US Food and Drug Administration, propionic acid has shown little toxicity in humans (Gad, 2014). In addition, it has been shown to have a strong inhibitory effect on many molds and yeasts typically found as spoilage organisms in lactic acid fermented foods by inducing fungal cell death through mitochondria-mediated apoptosis (Moon, 1983; Yun and Lee, 2016).

A mechanism to produce propionate from 1-2PD has been found in other bacteria such as *Lim. reuteri* encoded within the *pdu* operon, as shown in (Figure 2) (Rolf et al., 1998). Like the formation of reuterin, the metabolism of 1-2PD to propanol and propionate is catalyzed by a cobalamin-dependent diol dehydratase (EC 4.2.1.28). 1,2-PD utilization occurs, with disproportionation to propionate and propanol. Cobalamin is also synthesized (Sriramulu et al., 2008).

Resting cells of *Lim. reuteri* induced in the presence of 1,2PD have shown significant capabilities to convert aqueous glycerol to 1,3PD, 3HPA, and a compound proposed to be 3-hydroxypropionic acid (Amin et al., 2013). Helping to illustrate the potential of the pathway to produce propionate but also be a key in the formation of reuterin.

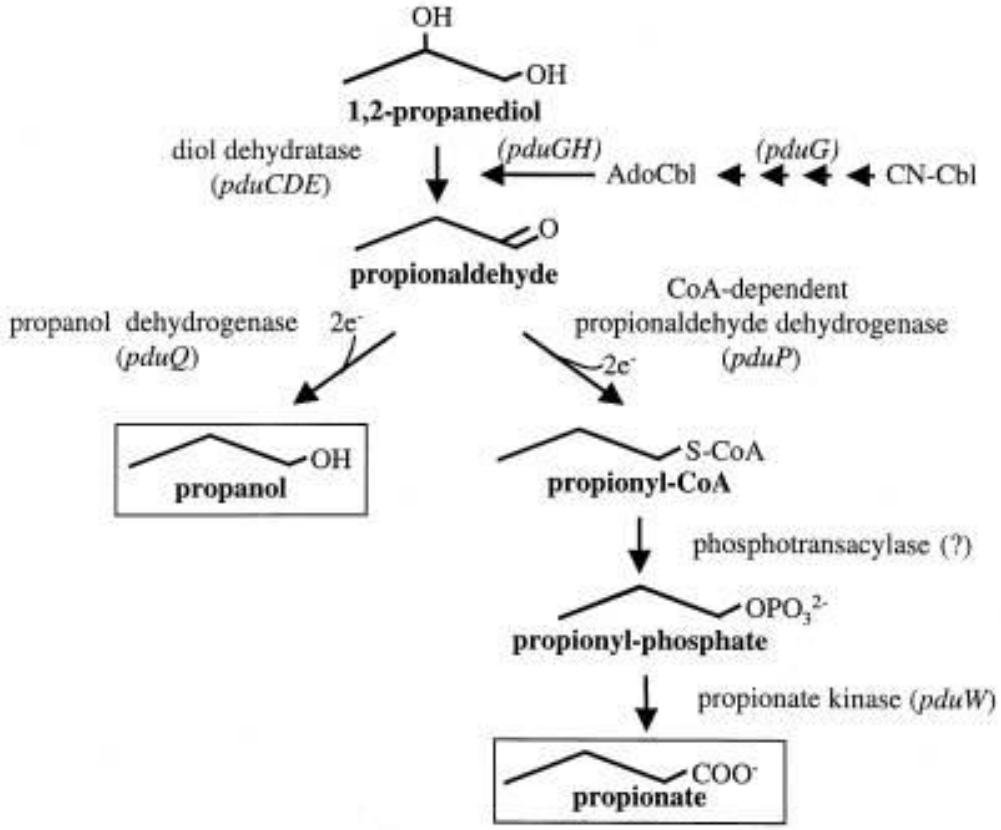


Figure 2. Mechanism for the metabolism of 1-2PD to propionate through PDU operon (Rolf et al., 1998).

Summary

The literature demonstrates the critical need for effective bio-preservatives in the dairy industry to combat contamination and spoilage caused by molds, yeasts, and bacteria. Traditional preservatives are becoming less favorable due to consumer demand for clean-label products, leading to increased interest in natural preservation methods such as the use of lactic acid bacteria (LAB).

LAB, including *Latilactobacillus curvatus*, have shown promising antifungal and antimicrobial properties through mechanisms such as competitive exclusion, manganese

scavenging, and the production of bioactive compounds like reuterin and propionic acid. While in vitro studies have highlighted the potential of LAB in controlling spoilage organisms, in-situ applications, particularly in complex food matrices like yogurt, present additional challenges and variables.

This study's comprehensive exploration of *Lat. curvatus* WSU-1 aims to bridge the gap between in vitro efficacy and practical in-situ application. By understanding the genomic, biochemical, and antifungal properties of *Lat. curvatus*, this research seeks to evaluate its potential as a bio-preservative in dairy products. The findings will contribute to the broader knowledge of bio-preservation and support the development of effective natural preservation strategies in the food industry.

METHODS

Objective 1: In vitro analysis of *Lat. curvatus*

Culture preparation and enumeration

This study utilized nine mold and eight yeast strains for both the in vitro and in situ analyses against *Latilactobacillus curvatus* strain WSU-1. All strains are listed in Table 1 and will be referred to by their label designation. All fungal strains were obtained from the Food Safety Laboratory isolate collection at Cornell University. These isolates are the same strains previously analyzed against commercial protective cultures in cottage cheese and queso fresco (Makki et al., 2020, 2021). Previously isolated samples of *Lat. curvatus* strain WSU-1 were revived from freezer samples from the Oberg lab at Utah State University.

To ensure a consistent inoculum rate of *Lat. curvatus* in all trials of this study, *Lat. curvatus* was grown overnight and diluted to an optical density value of 1 measured at 600nm (OD_{600}). This culture was serially diluted and plated on MRS agar in triplicate, incubated anaerobically for 48 hours, and counted. This was repeated in triplicate. All subsequent analyses in this study using *Lat. curvatus* were done using a culture at OD_{600} 1 to ensure consistent inoculum concentrations.

To differentiate *Lat. curvatus* from the starter lactic acid bacteria (SLAB) used in yogurt production, the vancomycin resistance of *Lat. curvatus* was tested by plating an OD_{600} 1 culture on MRS + 50ug/mL vancomycin plates and standard MRS plates.

Following anaerobic incubation at 37°C for 48 hours, plates were counted and analyzed to see if there was any difference. This test was also conducted with the starter cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* to determine if *Lat. curvatus* could be quantified in a co-culture while inhibiting the predominant SLAB found in yogurt production.

Enumeration and standardization of yeast strains used for inoculation in all phases of this study were prepared by diluting overnight cultures grown in Malt Extract (ME) Broth (Becton Dickinson, Franklin Lakes, NJ) to OD_{600} 1; the cultures were then serially diluted using 1:10 dilutions in Buffered Peptone Water (BPW) (Becton Dickinson, Franklin Lakes, NJ), plated on ME agar, and incubated at 25°C for 48 hours. Plates were counted, and the concentration was determined based on the dilution factor. The results are the average of triplicate plating. Additionally, counts were compared to those using a Neubauer-improved counting chamber.

The preparation of mold spore inoculum for all trials in this project was adapted from the protocol outlined in (Makki et al., 2021). The nine mold strains were grown on MEA for two weeks. Plates were then flooded with 25 mL of Phosphate Buffered Solution containing 0.1% Tween 80, and the surface was gently scraped using a sterile plate spreader to release spores from the mycelium. The solution was filtered through 4 layers of cheesecloth to remove residual mycelium from the spore solution. The resulting solution was enumerated using a Neubauer-improved counting chamber. The resulting solution was divided into 1mL aliquots for each solution made to limit them to a single freeze-thaw cycle.

Table 1. Mold and Yeast Reference

Label[1]	Strain[2]	Type	ID[3]	Isolate Source
Y1	*Pichia fermentans*	Yeast	B90001	Raw Milk
Y3	*Clavispora lusitaniae*	Yeast	B90007	Raw Milk
Y4	*Debaryomyces hansenii*	Yeast	B90013	Cheese
Y5	*Debaryomyces prosopidis*	Yeast	B90028	Cheese
Y6	*Candida zeylanoides*	Yeast	B90031	Cheese
Y8	*Rhodotorula mucilaginosa*	Yeast	E20331	Dairy Processing Environment
Y9	*Meyerozyma guilliermondii*	Yeast	E20377	Yogurt
Y10	*Torulaspora delbrueckii*	Yeast	E20442	Yogurt
M1	*Penicillium commune*	Mold	B90026	Cheese
M3	*Penicillium citrinum*	Mold	E20297	Yogurt
M4	*Penicillium decumbens*	Mold	E20320	Dairy Processing Environment
M5	*Aspergillus cibarius*	Mold	E20323	Dairy Processing Environment
M6	*Penicillium roqueforti*	Mold	E20329	Dairy Processing Environment
M7	*Penicillium chrysogenum*	Mold	E20332	Dairy Processing Environment
M8	*Phoma dimorpha*	Mold	E20369	Yogurt
M9	*Mucor racemosus*	Mold	E20368	Yogurt
M10	*Trichoderma amazonicum*	Mold	E20387	Yogurt

1. Abbreviated labels used in both in vitro and in situ studies.
2. All strains received from Food Safety Laboratory isolate collection at Cornell by Dr. Samuel Alcaine (Cornell University, Ithaca, NY).
3. ID refers to identification in the Food Microbe tracker database (www.food microbetracker.com

DNA sequencing and culture identification

Five isolates of *Lat. curvatus* strain WSU-1 were revived from freezer stocks. Each isolate was grown in MRS broth media and incubated at 37°C for 48 hours, then streaked for isolation on MRS agar plates. The plates were grown in an anaerobic chamber with BD GasPak (Becton Dickinson, Franklin Lakes, NJ) for 48 hours at 37°C. Individual colonies for each strain were then inoculated into MRS broth and grown for 48 hours at 37°C, which were then used for 16S rRNA DNA extraction.

DNA extraction was performed using the MasterPure Gram Positive DNA Purification Kit (Biosearch Technologies, Hoddesdon, United Kingdom), with the addition of mutanolysin (Sigma-Aldrich, St. Louis, MO). DNA purity and concentration were determined by spectroscopy using a NanoDrop One (ThermoScientific, Waltham, MA) and fluorometry using a Quibit 4 fluorometer (ThermoScientific).

Amplification of DNA through PCR was conducted using Invitrogen Platinum Hot Start PCR 2X Master Mix (ThermoScientific). Each strain was amplified using three different primer sets (Eurofins, Luxemburg), as detailed in Table 2. The PCR was run for 40 cycles in a thermocycler. Confirmation and quantification of amplification were conducted using a DNA 7500 bioanalyzer (Agilent, Santa Clara, CA). Following confirmation of amplification, purity based on amplicon size, and quantification of DNA, excess oligos and primers were removed using a SpinPrep PCR cleanup kit (Millipore Corp., Burlington, MA).

Purified PCR products for all strains were sent to the USU Center for Integrated Biosystems for DNA sequencing using an ABI PRISM 3730 DNA Analyzer. Resulting

sequences were analyzed and trimmed using 4Peaks software (4Peaks, RRID: SCR_000015), and identity was determined using NCBI BLAST (Altschul et al., 1990).

Yeast & Mold

Identity Confirmation of all fungal strains was confirmed through 18S rRNA sequencing. Mold strains were grown on ME agar plates and incubated for two weeks at 25°C. Yeast strains were inoculated into ME broth, incubated at 25°C for 48 hours, then streaked for isolation on ME agar, incubated for 48 hours at 25°C, and reinoculated into ME broth for another 48 hours. The Fungi/Yeast Genomic DNA Isolation Kit (Norgen, Thorold, ON, Canada) extracted DNA for both yeast and mold.

DNA amplification by PCR was conducted using Invitrogen Platinum Hot Start PCR 2X Master Mix, with 18S rRNA primers (ITS1-F and ITS4-R), as detailed in Table 2. The PCR reaction was carried out in a thermocycler with an annealing temperature (Tm) of 52.7°C and run for 35 cycles. Identity was confirmed as previously outlined for *Lat. curvatus*.

Table 2. Table of the primer sequences used in 16S and 18S rRNA sequencing.

Seq. Name	Seq 5' to 3'	Tm
27F	AGAGTTTGATCMTGGCTCAG	59.4
1492R	ACGGYTACCTTGTTACGACTT	59.6
16SUNIF	TTTGATCCTGGCTCAGGAC	60.2
16SUNIR	TGGTTGGATACCGTCACTAC	60.4
CurvatusF	ACTCTCATTGAATTAGGACGT	56.7
CurvatusR	CCCGTGTTGGTACTATTTAAT	56.7
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	57.1
ITS4-R	TCCTCCGCTTATTGATATGC	58.4

Genomic analysis

Previously, *Lat. curvatus* strain WSU-1 underwent whole genome sequencing and was uploaded to the Rapid Annotation using Subsystem Technology (RAST) prokaryotic genome annotation service (Aziz et al., 2008). The *Lat. curvatus* WSU-1 genome was analyzed using RAST to identify potential antimicrobial-producing genes.

BCP carbohydrate analysis

In addition to 16S rRNA sequencing, a carbohydrate metabolism profile of *Lat. curvatus* WSU-1 was conducted to confirm identity compared to previous API carbohydrate fermentation panel results on known WSU-1 isolates (unpublished data). Based on this data, the strains should be lactose and citrate negative and positive for

galactose, glucose, and ribose, with no gas production. The isolate with the lowest galactose utilization was selected for future trials.

The test was run on five different WSU-1 isolates using bromocresol purple (BCP) broth (Sigma Aldrich) containing one of 5 carbon sources: 1% lactose, 1% galactose, 1% glucose, 1% ribose, and 0.2% citric acid, Durham tubes were added to each tube to detect gas production. Tubes were analyzed based on color change from purple to yellow and cell growth with a 5-point visual classification scale:

(-) no growth and no color change,

(+-) light purple color,

(+) very light yellow/grey color,

(++) yellow color,

(+++) strong yellow color and pellet present.

Gas production, if present, will be indicated with (*).

Lat. curvatus metabolic analysis

Glycerol fermentation analysis

Batch fermentations were conducted using a Sartorius bioreactor to determine the functionality of the *pdu* operon in *Lat. curvatus* WSU-1 and quantify its products. The fermentations involved growing *Lat. curvatus* WSU-1 in carbohydrate-restricted MRS (CR-MRS) media supplemented with coenzyme B12 (Sigma-Aldrich) at a concentration of 2 mg/L, with either 200 mM glycerol or no glycerol. *Lat. curvatus* was inoculated at a rate of 1% from an overnight culture standardized by spectrophotometry to an OD_{600} of 1. Additionally, a negative control of sterile CR-MRS was also included for all subsequent assays. The fermentations were run for 24 ± 2 hours.

To analyze the metabolic products of *Lat. curvatus* WSU-1 fermentation of glycerol under various conditions, a cell-free supernatant had to be created for all fermentation conditions tested. Following fermentation, 50 mL of the culture was centrifuged at 10,000 x g for 10 minutes. The resulting supernatant was then transferred and sterilized by filtering it through a 0.20 m filter. The cell-free supernatant was stored at -20 °C until use in subsequent assays. This process was repeated three times.

Variable carbohydrate fermentation

Adapting the protocol from (Lüthi-Peng et al., 2002a), *Lat. curvatus* fermentation of 200 mM glycerol in the presence of either 20 mM glucose, galactose, or ribose and 2 mg/L B12 was conducted in 100 mL medicine bottles. 0.2 μm filter sterilized carbohydrate stock solutions of 20% were made and added to CR-MRS post-autoclave as indicated. An overnight culture of *Lat curvatus* was standardized to an OD_{600} of 1, pelleted at 1500 x g for 10 minutes, rinsed with BPW to remove residual carbohydrates, resuspended in CR-MRS, and inoculated at 1%. Bottles were sealed with a rubber septum, and the headspace was purged with nitrogen gas to ensure anaerobic conditions. Bottles were incubated at 37 °C for 48 hours, with samples being collected aseptically using a sterile needle through the septum cleaned with 70% isopropyl alcohol. Cell-free supernatant was prepared as previously specified. Reuterin concentration was determined by colorimetric method at 24 and 48 hours for all samples.

Two-step fermentation

To determine the quantity of reuterin produced by glycerol fermentation alone, a modified version of the two-step fermentation outlined in (Doleyres Beck S Vollenweider C Lacroix, 2005) was used. An overnight culture of *Lat. curvatus* standardized to an OD_{600} of 1 was inoculated into 1 liter of MRS containing 20 mM glycerol and 0.2 mg/L B12 and incubated for 18 hours in a bioreactor. After incubation, the culture was pelleted at 1,500 x g for 10 minutes, washed with PBS buffer, pelleted again, resuspended in PBS, and inoculated into DI water containing 200 mM glycerol, 2 mg/mL B12. This was incubated at 37 °C for 18 hours. Samples were taken at 2hr, 4hr, and 18hr, with the supernatant prepared as previously outlined and tested for reuterin production using the colorimetric method described below.

Detection and quantification of reuterin and PDU pathway

Previously prepared cell-free supernatants were analyzed for reuterin production as well as metabolic end products of the PDU pathway. Gas chromatography-mass spectrometry (GC-MS) was employed to detect the presence of 1,3-propanediol (1,3-PD) and 3-hydroxypropionaldehyde (3-HP) to verify the functionality of the pathway. The intermediate, reuterin, was quantified using a modified colorimetric method initially proposed by (Circle et al., 1945) and adapted by (Lüthi-Peng et al., 2002b; Doleyres Beck S Vollenweider C Lacroix, 2005).

To a 1.5 mL cuvette, 330μL of sample or standard was mixed with 75μL of 0.1 M tryptophan suspended in 0.05 N HCl, 150 μl EtOH, and 945μL of concentrated fuming HCl. The cuvettes were covered and incubated for 50 minutes at 40 °C. Immediately following incubation, samples were read at 560nm. Results were compared to a standard

curve constructed with concentrations ranging from 1.5 to 15 µg/mL of acrolein (2-propenal), setting the limit of quantification (LOQ) at 1.5 µg/mL.

The standard curve was generated using a purified acrolein standard (Restek, Bellefonte, PA), prepared, and analyzed in the same manner as the samples. This standardization ensured accuracy and consistency in quantifying reuterin across all samples.

Antifungal Challenge of Lat. curvatus

In addition to genomic and biochemical analysis of *Lat. curvatus* metabolic capabilities, the primary interest in this study was to understand the antifungal effects of *Lat. curvatus* WSU-1. To achieve this, two different assays were developed: one tested the antifungal effects of the cell-free supernatant of *Lat. curvatus* fermentations, and the other tested its antifungal capabilities when grown in co-culture with various mold and yeast species.

Yeast inhibition 96 well plate

In this trial, all eight yeast strains were tested against four different concentrations (0.0%, 0.1%, 1.0%, and 10.0%) of WSU-1 supernatant containing reuterin and supernatant fermented without glycerol (thus not containing reuterin). This trial was conducted in a 96-well plate with a total volume of 300 µL in each well. Each well contained 267 µL of malt extract (ME) broth, 30 µL of the appropriate supernatant dilution, and 3 µL of yeast culture standardized to an OD_{600} of 1, resulting in a 1.0% inoculation. The plates were incubated for 48 hours at 25°C, and OD_{600} readings were taken at 6, 24, and 48 hours.

24 well overlay method

The inhibitory effects of *Lat. curvatus* WSU-1 were tested against eight yeast and nine mold strains utilizing a modified overlay method in 24-well plates adapted from the literature (Delavenne et al., 2013; Cheong et al., 2014; Inglin et al., 2015). This assay is a development of a high-throughput method for analyzing the in vitro antifungal efficacy of candidate biopreservative bacteria under various growth conditions.

Each mold and yeast strain was tested individually employing a soft agar overlay onto agar containing *Lat. curvatus* WSU-1 grown under three different conditions. Rows separated each growth condition in the 24-well plate, where each column separated the 17 different fungi or controls. The three conditions were MRS agar, CR-MRS agar + 200 mM glycerol + 2 mg/L B12, and CR-MRS agar + 200 mM 1-2 propanediol + 2 mg/L B12.

Plates were prepared aseptically in a biosafety cabinet by filling each well with 600 µL of the specified media, allowing them to cool and solidify. Each well was spotted with 10 µL of overnight standardized 1 OD_{600} *Lat. curvatus* WSU-1 culture. The first row in each plate contained standard MRS and was spotted with sterile MRS broth, serving as a positive control for fungal growth. Spots were allowed to dry, and plates were covered with a lid treated with 0.05% Triton 100X in 20% ethanol to limit condensation on the plate lid and prevent cross-contamination between wells (Brewster, 2003). Plates were grown in an anaerobic chamber and incubated at 37°C for 48 hr.

Following incubation, a fungal overlay was conducted using ME soft agar (0.7% agar), which was boiled and distributed into 1.5 mL microcentrifuge tubes. Tubes were autoclaved and tempered to 40 °C on a heat block. One at a time, each tube was

inoculated with either 50 µL of a 6 log_{10} spores/mL solution for mold or 50 µL 1 OD_{600} overnight yeast. Tubes were vortexed to ensure homogenization of the fungi, and 200 µL of the solution was overlaid onto each of the wells in the column for final concentrations of 4 log_{10} yeast or spores/mL. Plates were incubated at 25°C for 96 hours. Following incubation, plates were photographed under controlled identical lighting and distance, then qualitatively assessed for the level of inhibition using a 5-point visual classification scale. Results were reported as follows: (-) No Inhibition, (+*)* Slight fading/color change, (+) Fading of growth/slight ring, (++*) Ring of inhibition present, (++) Complete visual inhibition/no growth.

Objective 2: in-situ analysis of *Lat. curvatus* WSU-1 in yogurt a system

Experimental Design

The experiment employed a factorial split-plot design to investigate the effects of treatments on the outgrowth of fungi in yogurt batches over time.

Factors

Main factor: Treatments - Control, Treatment 1, Treatment 2 (3 levels)

Sub-factor: Fungi species - 17 different species (factors nested within the primary treatment factor)

Temporal factor: Time points – 5-time points (factors nested within the primary treatment factor and sub-factor)

Design structure

Each treatment level (Control, Treatment 1, Treatment 2) represents a whole plot. The 17 different fungi species were randomly assigned to sub-plots within each plot. Each sub-plot corresponded to a cup of yogurt inoculated with a single fungal species. Measurements of outgrowth were taken at five predetermined time points. Replication: The entire experiment was replicated three times (three biological replicates), resulting in nine yogurt batches.

Yogurt Production

Yogurt was produced using newly acquired yogurt equipment at the Gary H. Richardson Dairy Products Laboratory (DPL) at Utah State University. For each of the three trials, three 50-gallon batches of yogurt were produced using whole-fat milk sourced from the George B. Caine Dairy in Logan, Utah. The milk was standardized with 4.5% non-fat dry milk and 0.8% stabilizer and made using a commercial starter culture blend (YFL-702, Chr. Hansen, Hørsholm, Denmark). Each batch was made with one of three treatments: a control yogurt with only starter cultures (C), yogurt made with the adjunct *Lat. curvatus* WSU-1 (5 $\log_{10}$ CFU/mL) (W), and yogurt made with the adjunct plus 200 mM glycerol (WG).

The mixture was HTST pasteurized and homogenized before being pumped into the fermentation vessel. Once in the vessel, the mixture was heated to 185°F, held for 30 minutes, cooled to 109°F, and inoculated with the starter cultures and *Lat. curvatus* WSU-1 if indicated. The contents were mixed and allowed to ferment until a pH of 4.6 was reached, at which point glycerol was added (approximately 4 hours post-inoculation) if indicated, as proposed by (Ortiz-Rivera et al., 2017). The yogurt was mixed, run through a smoothing valve, and packaged into 200 g cups. All yogurt batches and

subsequent mold and yeast inhibition tests were performed in triplicate. Each make sheet can be found in Appendix A.

For each yogurt treatment, cups were inoculated in triplicate with one of the pre-standardized molds or yeasts at a rate of approximately 2 $\log_{10}$ spores/yeast per gram or kept as a negative control. Samples were stored between 8-10 °C for four weeks to simulate standard yogurt shelf life under moderate abuse conditions. Mold and yeast growth was analyzed every week.

Preparation of WSU-1 Inoculum

For each trial, *Lat. curvatus* WSU-1 was grown in bulk fermentation using a bioreactor. It was cultured in a simulated dairy medium for 20 hours at 37 °C with pH control set at 5.5 using 14% ammonium hydroxide (NH4OH). The fermentation vessel was inoculated at a rate of 1% with an overnight WSU-1 culture, diluted to an OD_{600} value of 1. Following overnight fermentation, the concentration reached approximately 8 $\log_{10}$ CFU/mL. To achieve a total inoculation rate of 5 $\log_{10}$ CFU/mL, 50 gallons (approximately 189 liters) of yogurt was inoculated with 189 mL of the culture.

Yogurt Sampling and Analysis

WSU-1 Concentration over time

Lat. curvatus WSU-1 concentration in control yogurt (not inoculated with fungi) was analyzed weekly by plate count. For each of the three yogurt treatments, 11 g was weighed into a 99 mL bottle of Butterfield's Buffer (3M Flip-Top dilution bottle, St Paul, MN). The bottle was shaken to homogenize the sample, and 10-fold dilutions were performed in 9 mL sterile blanks of BPW. Then, 10 µL samples of the appropriate dilution were plated on MRS agar containing 50 µg/mL vancomycin (ThermoScientific)

to inhibit the growth of the starter cultures. Control yogurt without *Lat. curvatus* was plated as a negative control to ensure no other NSLAB were present in the yogurt at concentrations that would obscure results. Plates were incubated anaerobically at 37 °C for 48 hours and counted.

ANOVA was conducted to test the differences in *Lat. curvatus* WSU-1 concentrations in treatment yogurts over time. Plate counts were $\log_{10}$ transformed, and ANOVA was run at each time point with all replicates, with a significance level set at $\alpha = 0.05$.

Yeast Analysis

Yeast samples were enumerated at the time of inoculation, day 5, and weekly for four weeks, totaling five time points. The analysis involved performing standard plate counts for the three cups of inoculated yogurt from each of the three treatments for all eight yeasts. Briefly, yogurt cups were mixed aseptically with a sterile spoon to obtain a representative sample and avoid contamination. As with *Lat. curvatus* WSU-1 sampling, 11 g was weighed into a 99 mL bottle of Butterfield's Buffer, the bottle was shaken to homogenize the sample, 10-fold dilutions were performed in 9 mL tubes of sterile BPW, and 100 µL was plated onto ME agar, incubated at 25 °C for 48 hours, and counted. Uninoculated control yogurt was also plated to control for contaminant yeast.

ANOVA was conducted to analyze the main effects of treatment over time. Yeast plate count results were $\log_{10}$ transformed, and a separate ANOVA was run for each yeast strain at each time point. The significance level was set at $\alpha = 0.05$. If significant differences were found, Tukey's HSD post-hoc test was used to determine significant

differences between treatment groups. For these studies, a biological difference was defined as having both a significant difference ($P < 0.05$) and a difference of at least 1 log CFU/g.

Mold Analysis

Mold-inoculated yogurt cups were visually analyzed to detect and characterize mold growth. Every week for five weeks, samples were photographed in a sterile biosafety cabinet under controlled lighting conditions at a consistent distance. After obtaining all the pictures from all three replicate trials, the photos were grouped by mold species and week. Blinding of treatment was performed by a separate researcher who randomized each group of photos. Pictures from each group were rated using an adapted 5-level categorization compared to an uninoculated control from the same time point, as proposed by (Makki et al., 2021). All pictures were re-randomized and reanalyzed for replicate observations. Results were analyzed using a Kruskal-Wallis test with Dunn post hoc comparison and Bonferroni corrections.

Table 3. Visual classification scale for all nine mold strains used in the study. A visual representation of this table can be found in the appendix B (Figure A1 10)

Mold	Scale	Description
M1&M3	**1**	Same as control
	2	Matte spot present, no elevated filamentous outgrowth present
	3	Spot of uncolored filamentous outgrowth present
	4	Fully colored filamentous growth spot
	5	Full colored filamentous outgrowth covering majority of surface, with distinct color change in the remainder of yogurt
M4	**1**	Same as control
	2	Slight yellowing of yogurt surface, still glossy
	3	Slight yellowing, with notable surface gelation and possible slight cracking
	4	Yellowing with gelation and possible cracking
	5	Pronounced dark yellowing with pronounced gelling and surface change
M5	**1**	Same as control
	2	Transitioning color change, with possible light syneresis
	3	Small matte spot with yellowing around
	4	Pronounced uncolored filamentous outgrowth from spot
	5	Full colored outgrowth of spot covering majority of surface
M6	**1**	Same as control
	2	Transitioning to matte appearance of middle spot
	3	Small lightly colored spot
	4	Light colored spot, and matte appearance covering majority of surface
	5	Complete colored outgrowth covering complete surface
M7&M8	**1**	Same as control
	2	Slight color change to surface with slight syneresis
	3	Slight color change and syneresis with small spot present
	4	Larger pronounced spot with uncolored filamentous growth
	5	Large colored filamentous spot
M9	**1**	Same as control
	2	Transitioning surface color and texture
	3	Yellowed surface mostly transitioned to matte surface
	4	Fully matte yellowed surface with slight surface texture change/wrinkling
	5	Yellow and brown surface, surface wrinkling and filamentous growth
M10	**1**	Same as control
	2	Slight change in surface color and texture, very small cracks visible
	3	More pronounced color change with surface transitioning to matte
	4	More pronounced surface cracking
	5	Fully matte colored surface with filamentous growth around edges

Ancillary Yogurt Analyses

At Weeks 0, 1, 2, 3, and 4, 150-mL yogurt samples were taken and frozen at -20 °C in 50 mL conical tubes to be later tested for changes in carbohydrates, reuterin concentration, and B12 concentration. Samples were taken for all replicates and tested simultaneously following the final yogurt make.

Sugar Analysis

Changes in residual lactose and galactose between Weeks 1 and 4 were assessed on all three replicates of all three treatments. The analysis was performed using a Carrez clarification kit coupled with a lactose/galactose detection kit, both obtained from Megazyme (Megazyme, Bray, Ireland).

To clarify the yogurt samples for lactose determination, 1 g of yogurt was added to a 100 mL volumetric flask, and 60 mL of water was added. The flask was placed in a 50 °C water bath for 15 minutes, stirring occasionally. Following incubation, 600 µL of Carrez solution 1 was added, and the solution was vortexed for 1 minute. Then, 600 µL of Carrez solution 2 was added and remixed. Finally, 10 mL of 100 mM NaOH was added, and the solution was brought to a total volume of 100 mL with nano-pure water. The solution was filtered through a 5 µm filter to remove precipitate, discarding the first few mL of filtrate.

The resulting filtered solution was then used for lactose/galactose determination following the Megazyme procedure A (Standard Assay Procedure). The resulting solutions were measured spectrophotometrically at OD_{340}, and concentrations were determined based on the calculations provided by Megazyme.

Results were analyzed using ANOVA comparing the sugar concentrations of all treatments at the two determined time points.

Reuterin Quantification

Reuterin quantification was performed on yogurt samples as previously specified, with a few alterations. Yogurt was treated following a modified protocol for lactose determination using Carrez clarification to reduce the sample's dilution (Yang et al., 2021). To prepare the sample, 5 g of yogurt was mixed with 5 mL of 50% ethanol and vortexed well. Then, 120 µL of Carrez solution 1 was added and vortexed for 1 minute, followed by 120 µL of Carrez solution 2 and another 1-minute vortex. The sample was centrifuged at 1,900 RCF and filtered through a 5 µm filter. Following this, samples were run as previously specified in the in vitro analysis and compared to a 1.5-15 µg/mL 2-propenal standard curve created in Carrez-treated and filtered control yogurt.

B12 Quantification

Samples from all three replicates of all three treatments were sent externally for coenzyme B12 quantification (Merieux NutriSciences, Silliker Inc., Crete, IL). The AOAC method 960.46 was followed for the quantification of B12.

Objective 3: Sensory Evaluation

Difference Testing, Triangle tests

All three yogurts were prepared approximately two weeks before the sensory tests. Two triangle tests were presented individually to 64 panelists, each testing one of the two treatments against the control. All samples were presented in a random and balanced manner and assigned a unique 3-digit number, as shown in the worksheet (Appendix C) generated using the SIMs 2000 software (Sensory Computer Systems LLC, NJ). Panelists filled out the score sheet on a computer in the testing booth with instructions on performing the test (Appendix C). Tests were administered using SIMs 2000 software.

Results were tabulated, and the number of correct answers was compared to a Triangle Test of Similarity: Critical Number of Correct Answers table using a pre-determined significance value of 0.05 to determine significance. The software's statistical package performed this comparison automatically. The null hypothesis of no difference was rejected if the number of correctly identified different samples exceeded the critical number given in the table.

The first test compared yogurt made with *Lat. curvatus* adjunct (treatment A) against control yogurt. Trays contained either two control samples and one treatment A yogurt or two treatment A yogurts and one control. The second test followed the same pattern but with yogurt made with *Lat. curvatus* adjunct and 200 mM glycerol. The trays were prepared following the worksheet, aligning the samples in order from left to right, including water and unsalted crackers to cleanse the palate. The test was given to a total of 64 panelists.

Colorimetry

Yogurt from this study was analyzed using a Hunter colorimeter, looking at the L* a* and b* CIELAB color scale to determine any detectable differences in color between treatments. Yogurt from all three treatments was measured in a consistent location under consistent lighting. Approximately 80g of yogurt was weighed into a petri dish so that it was flush against the lid to remove all air bubbles and read through the lid. Interference caused by the lid of the petri dish was accounted for and subtracted from the readings by blanking the colorimeter through the Petri dish lid. Readings were taken at one week and four weeks.

Color differences were quantified using the Delta E (ΔE) value, a standard metric for measuring the distance between two colors in a three-dimensional color space. The calculation of ΔE values involves the following steps:

Color Measurement: The color of each yogurt sample was measured using a colorimeter. The colorimeter provides readings in the CIELAB color space, which is a color-opponent space with dimensions L* (lightness), a* (green to red), and b* (blue to yellow).

Reference and Sample Values: For each treatment, we identified a reference color from the control yogurt (L1*, a1*, b1*) and compared it to the color of one of two treatments independently (L2*, a2*, b2*). This was performed twice, once for each treatment, and yielded two ΔE values.

Delta E Calculation: The ΔE value was calculated using the following formula, which represents the Euclidean distance between two points in the CIELAB color space:

$$\Delta E=\sqrt{(L2*-L1*)^2+(a2*-a1*)^2+(b2*-b1*)^2}$$

This formula accounts for the differences in lightness (ΔL*), red/green value (Δa*), and yellow/blue value (Δb*).

Interpretation of Delta E Values: The resulting ΔE values indicate the perceptible difference between the colors of the reference and treated samples. A higher ΔE value corresponds to a more noticeable color difference, while a lower ΔE value suggests that the colors are more similar (Karma, 2020). Generally, ΔE values can be interpreted as follows:

$\Delta E < 1$: Not perceptible by the human eye.

$1 \leq \Delta E < 2$: Perceptible through close observation.

$2 \leq \Delta E < 10$: Perceptible at a glance.

$\Delta E \geq 10$: Large color difference.

Using ΔE values, we could objectively quantify the color differences between various yogurt samples subjected to the treatments, thereby assessing the impact of each treatment on the visual quality of the yogurt.

Statistical Analysis

All statistical analyses, apart from the triangle test, were performed using R-studio (Version 2023.12.1+402). The significance level for all analyses was set at alpha = 0.05. The triangle test was analyzed using the SIMS2000 software to calculate significance based on a triangle table.

RESULTS AND DISCUSSION

Culture Preparation, Identification, and Standardization

DNA sequencing

The identity of all *Latilactobacillus curvatus* WSU-1 isolates and fungal strains used in this project was confirmed by 18S or 16S rRNA sequencing. Table 4 presents the results of the rRNA sequencing with the percent identity match provided by NCBI BLAST. All species had >95% sequence identity except for *Aspergillus cibarius*, which had 86.36%: these results and the analysis of growth morphologies allowed for the confident confirmation of the strains used. Complete sequences of each culture can be found in Appendix D.

Table 4. Fungal and bacterial DNA confirmation results for all strains used in the study. All strains, except for *Latilactobacillus curvatus*, were sequenced with 18S rRNA. NCBI BLAST results are shown with percent identity.

Label	Presumed Strain	18S/16S rRNA BLAST results	ID %
Y1	*Pichia fermentans*	*Pichia fermentans*	98.97
Y3	*Clavispora lusitaniae*	*Clavispora lusitaniae*	98.96
Y4	*Debaryomyces hansenii*	*Debaryomyces hansenii*	99.37
Y5	*Debaryomyces prosopidis*	*Debaryomyces* species	98.82
Y6	*Candida zeylanoides*	*Candida zeylanoides*	96.72
Y8	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa*	100
Y9	*Meyerozyma guilliermondii*	*Meyerozyma guilliermondii*	99.50
Y10	*Torulaspora delbrueckii*	*Torulaspora delbrueckii*	99.87
M1	*Penicillium commune*	*Penicillium commune*	100
M3	*Penicillium citrinum*	*Penicillium citrinum*	100
M4	*Penicillium decumbens*	*Penicillium decumbens*	99.31
M5	*Aspergillus cibarius*	*Aspergillus cibarius*	86.36
M6	*Penicillium roqueforti*	*Penicillium roqueforti*	99.65
M7	*Penicillium chrysogenum*	*Penicillium chrysogenum*	95.64
M8	*Phoma dimorpha*	*Phoma species*	98.89
M9	*Mucor racemosus*	*Mucor racemosus*	99.51
M10	*Trichoderma amazonicum*	*Trichoderma amazonicum*	99.33
5-14	*Latilactobacillus curvatus*	*Latilactobacillus curvatus*	100
6-11	*Latilactobacillus curvatus*	*Latilactobacillus curvatus*	99.8
9-22	*Latilactobacillus curvatus*	*Latilactobacillus curvatus*	100
PacSeq1	*Latilactobacillus curvatus*	*Latilactobacillus curvatus*	100

Carbohydrate fermentation panel

In addition to 16S rRNA sequencing, the BCP carbohydrate fermentation panel run on all five *Lat. curvatus* WSU-1 isolates matched carbohydrate fermentation results from previous research, further validating that these were indeed *Lat. curvatus* WSU-1 strains (unpublished data). Of particular interest in this panel was the confirmation that all

isolates were lactose-negative, distinguishing WSU-1 from other *Lat. curvatus* strains. Additionally, none of the isolates produced gas across all carbohydrates tested (Table 5).

The five isolates of strain WSU-1 exhibited variations in the rate and efficacy of carbohydrate fermentation. All strains effectively fermented glucose and ribose within 72 hours, with some notably fermenting within 18 hours, as indicated by the change in color (Table 5). The strain designated as 5-14 was selected for use in subsequent studies due to its perceived slower fermentation of ribose and glucose and decreased efficiency in fermenting galactose. This was particularly relevant for the yogurt trial, aiming to optimize glycerol fermentation in yogurt while limiting the fermentation of other carbohydrates, primarily galactose, which is present due to the metabolic profile of the starter *Streptococcus thermophilus*.

Table 5. BCP results for *Lat. curvatus* WSU-1 isolates grown with five different carbohydrates and observed at two time points (18 and 72 hours). Five different WSU-1 isolates were tested, as shown on the y-axis. Results are presented on a 5-point scale; (-) no growth and no color change, (+-) Light purple color, (+) very light yellow/grey color, (++) Yellow color, (+++) Strong yellow color and pellet present)

18hr	**1% Lactose**	**1% Glucose**	**1% Galactose**	**1% Ribose**	**0.2% Citric Acid**	**Control**
9_22	-	++	+	+++	-	-
5_14	-	+-	+-	+	-	-
Pac Seq 1	-	+	+-	++	-	-
Pac Seq 2	-	+	+-	+++	-	-
6_11	-	+	+-	++	-	-
72hr	**1% Lactose**	**1% Glucose**	**1% Galactose**	**1% Ribose**	**0.2% Citric Acid**	**Control**
9_22	-	+++	++	+++	-	-
5_14	-	+++	+-	+++	-	-
Pac Seq 1	-	+++	+	+++	-	-
Pac Seq 2	-	+++	+	+++	-	-
6_11	-	+++	+-	+++	-	-

Genomic Analysis Results

In analyzing the *Lat. curvatus* WSU-1 genome, several systems and genes were targeted, focusing primarily on the presence or absence of *pdu* operon genes, manganese transport genes, and B12 synthesis genes. Thus, this was not a complete comprehensive evaluation of the entire genome.

Manganese Transport and Regulation Genes

Table 6 lists the genes identified in the *Lat. curvatus* WSU-1 genome related to manganese transport and regulation. Multiple genes encoding manganese transport proteins (MntH/MntABC) and Mn-dependent transcriptional regulators (MntR) were

found. These genes suggest a robust system for manganese sequestration, which has been shown to be a very effective antifungal mechanism in some LAB. Although the genes are present, their functionality and expression were not tested, and the levels of manganese present were not measured. However, the potential implications of their presence are further analyzed later. Primarily of interest, however, were the MntH proteins, which are driven by a proton gradient. These proteins are importers that work mainly at low pH, like that found in yogurt, due to the high proton concentration outside the cell. MntABC proteins are ATP-driven and function primarily at neutral pH, which is of less interest in this study.

Table 6. Genes identified in the *Latilactobacillus curvatus* WSU-1 genome related to manganese and cobalamin import.

Gene ID	Function
LBCU_0388	Manganese transport protein MntH
LBCU_0389	Manganese transport protein MntH
LBCU_0435	Manganese transport protein MntH
LBCU_0558	Mn-dependent transcriptional regulator MntR
LBCU_1379	Manganese transport protein MntH
LBCU_1893	Manganese transport protein MntH
LBCU_1894	Mn-dependent transcriptional regulator MntR
LBCU_1040	Manganese ABC transporter, ATP-binding protein SitB
LBCU_1041	Manganese ABC transporter, inner membrane permease protein SitD
LBCU_1042	Manganese ABC transporter, periplasmic-binding protein SitA
LBCU_0744	Substrate-specific component CbrT of predicted cobalamin ECF transporter
LBCU_0745	Duplicated ATPase component CbrU of energizing module of predicted cobalamin ECF transporter
LBCU_0746	Transmembrane component CbrV of energizing module of predicted cobalamin ECF transporter

Propanediol Utilization Genes

The complete complex of essential *pdu* genes related to propanediol utilization (PDU) were identified, as shown in Table 7. As previously discussed, these genes are crucial in the catabolism of glycerol into the intermediate reuterin and the end products 3-HP and 1,3-PD. Although all the required genes in the *pdu* operon were found, this does not confirm the functionality of the pathway, which was further analyzed in the following metabolic analysis section.

Table 7. Genes identified in the *Latilactobacillus curvatus* WSU-1 genome related to the *pdu* operon.

Gene ID	Pdu Protein	Function
LBCU_0751	PduV	Propanediol utilization protein PduV
LBCU_0752	PduU	Propanediol utilization polyhedral body protein PduU
LBCU_0753	PduW	Acetate/propionate family kinase (EC 2.7.2.1)
LBCU_0754	PduQ	Putative iron-containing NADPH-dependent propanol dehydrogenase
LBCU_0755	PduP	CoA-acylating propionaldehyde dehydrogenase
LBCU_0756	PduO	Cob(I)alamin adenosyltransferase PduO (EC 2.5.1.17)
LBCU_0757	PduO	Cob(I)alamin adenosyltransferase PduO (EC 2.5.1.17)
LBCU_0758	PduP	Ethanolamine utilization protein EutN/carboxysome structural protein Ccml
LBCU_0760	PduL	Phosphate Proanoyltransferase PduL
LBCU_0762	PduK	Propanediol utilization polyhedral body protein PduK
LBCU_0763	PduG	Propanediol dehydratase reactivation factor small subunit
LBCU_0764	PduH	Propanediol dehydratase reactivation factor large subunit
LBCU_0765	PduE	Propanediol dehydratase small subunit (EC 4.2.1.28)
LBCU_0766	PduD	Propanediol dehydratase medium subunit (EC 4.2.1.28)
LBCU_0767	PduC	Propanediol dehydratase large subunit (EC 4.2.1.28)
LBCU_0768	PduB	Propanediol utilization polyhedral body protein PduB
LBCU_0769	PduJ	Propanediol utilization polyhedral body protein PduJ
LBCU_0770	PduS	Oxidoreductase
LBCU_0771	PduU	Propanediol utilization polyhedral body protein PduU
LBCU_1910	PduO	Cob(I)alamin adenosyltransferase PduO (EC 2.5.1.17)

B12 Synthesis Genes

Because of the importance of B12 in the initial conversion of glycerol to reuterin, a comparative genomic analysis was conducted to determine the presence of B12 synthesis genes in *Lat. curvatus* WSU-1 compared to *Limosilactobacillus reuteri,* a strain with confirmed B12 synthesis capabilities. As shown in Table 8, B12 synthesis genes were absent in *Lat. curvatus* WSU-1, whereas they were found in *Lim. reuteri*. The absence of these genes in *Lat. curvatus* WSU-1 could substantially impact its efficacy in glycerol fermentation, which will be discussed further in later sections.

Table 8. Function-based genome comparison of *Latilactobacillus curvatus* WSU-1 and *Limosilactobacillus reuteri,* analyzing the presence of cobalamin synthesis genes.

		Genes Present?	
Subsystem	**Role**	***Lat. curvatus***	***Lim. reuteri***
Cobalamin synthesis	Adenosylcobinamide kinase (EC 2.7.1.156)	no	yes
Cobalamin synthesis	Adenosylcobinamide-phosphate guanylyltransferase (EC 2.7.7.62)	no	yes
Cobalamin synthesis	Adenosylcobinamide-phosphate synthase (EC 6.3.1.10)	no	yes
Cobalamin synthesis	Cobalamin synthase (EC 2.7.8.26)	no	yes
Cobalamin synthesis	Cobalt-precorrin-6x reductase (EC 1.3.1.54)	no	yes
Cobalamin synthesis	Cobyric acid synthase (EC 6.3.5.10)	no	yes
Cobalamin synthesis	L-threonine 3-O-phosphate decarboxylase (EC 4.1.1.81)	no	yes
Cobalamin synthesis	Nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase (EC 2.4.2.21)	no	yes
Cobalamin synthesis	Sirohydrochlorin cobaltochelatase CbiK (EC 4.99.1.3)	no	yes

Culture Standardization

Latilactobacillus curvatus WSU-1

Consistent values of 8 $\log_{10}$ CFU/g were obtained when standardizing *Lat. curvatus* WSU-1. Plating WSU-1 on MRS agar plates supplemented with 50 μg/mL vancomycin had no significant effect on its growth (p-value > 0.05, paired t-test) (Table 9). However, the yogurt starters *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were significantly inhibited up to the highest concentration tested at 9 $\log_{10}$ CFU/g (p-value <0.0001) (Table 8). Thus, this method effectively selected WSU-1 in co-culture with *S. thermophilus* and *L. bulgaricus* starters and was later used to enumerate WSU-1 in yogurt.

Table 9. *Latilactobacillus curvatus* WSU-1 growth on standard MRS compared to MRS containing 50 μg/mL vancomycin compared to yogurt starters *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; Results shown as counts in log10 CFU/mL.

Media Type	**WSU-1**	***S. thermophilus, L. bulgaricus* blend[a]**
MRS	8.8	9
MRS + 50ug/mLVancomycin	8.7	< 2[b]

Results are shown as the average of 3 replicates.
[a] Plating of each replicate occurred from the same bag to show the difference in the plating method.
[b] Starter plated on vancomycin plate were significantly inhibited p-value<0.0001

Yeast

Comparing standard plate counts and enumeration with a hemocytometer, standard viable plate counts consistently showed lower numbers than the hemocytometer

for most yeast strains (Table 10). This suggests that plate counts more accurately represent yeast viability than the hemocytometer, especially with extended growth periods. This pattern was consistent over three replicates. Consequently, values from plate counts were used for inoculating samples in both in vitro analyses and the in situ yogurt trial. The in situ yogurt trial also used the plate count method to quantify yeast growth over time.

Spore aliquots for all nine mold strains were successfully prepared and enumerated (Table 11). These results are an average of 5 replicates. These solutions were subdivided into 1 mL aliquots and used for all in vitro and in-situ analyses.

Table 10. Standardization of yeast cultures at OD_{600} = 1 on Potato dextrose agar as well as with a hemocytometer. All results are an average of five replicates and expressed as $\log_{10}$ CFU/mL.

Yeast	ID	Plate Count	Hemocytometer count
P. fermentans	Y1	6.8	7.4
C. lusitaniae	Y3	7.0	7.2
D. hansenii	Y4	6.4	7.2
D. prosopidis	Y5	6.3	7.1
C. zeylanoides	Y6	6.6	7.0
R. mucilaginosa	Y8	6.6	7.0
M. guillermondii	Y9	6.8	7.4
T. delbrueckii	Y10	6.9	7.0

Table 11. Standardization of Mold spore solutions enumerated using a hemocytometer. Results are an average of five replicates and expressed as $\log_{10}$ spores/mL.

Mold	ID	Hemocytometer Spore count
P. commune	M1	7.7
P. citrinum	M3	8.0
P. decumbens	M4	7.3
A. cibarius	M5	6.1
P. roqueforti	M6	7.8
P. chrysogenum	M7	8.5
Ph. dimorpha	M8	6.6
M. racemosus	M9	7.2
T. amazonicum	M10	8.3

Metabolic Analysis of *Lat. curvatus*

GC-MS analysis of bulk fermentation

GC-MS analysis of bulk fermentation confirmed the functionality of the *pdu* operon in *Lat. curvatus* WSU-1. Only the cultures containing glycerol and B12 produced substantial amounts of the end products 3-HP and 1,3-PD (Figures 3 and 4). This indicates that the *pdu* operon is functional; however, the lack of end products in the glycerol-only supernatant further supports previous findings that *Lat. curvatus* WSU-1 cannot synthesize B12 and must instead utilize exogenous B12 already present in the system.

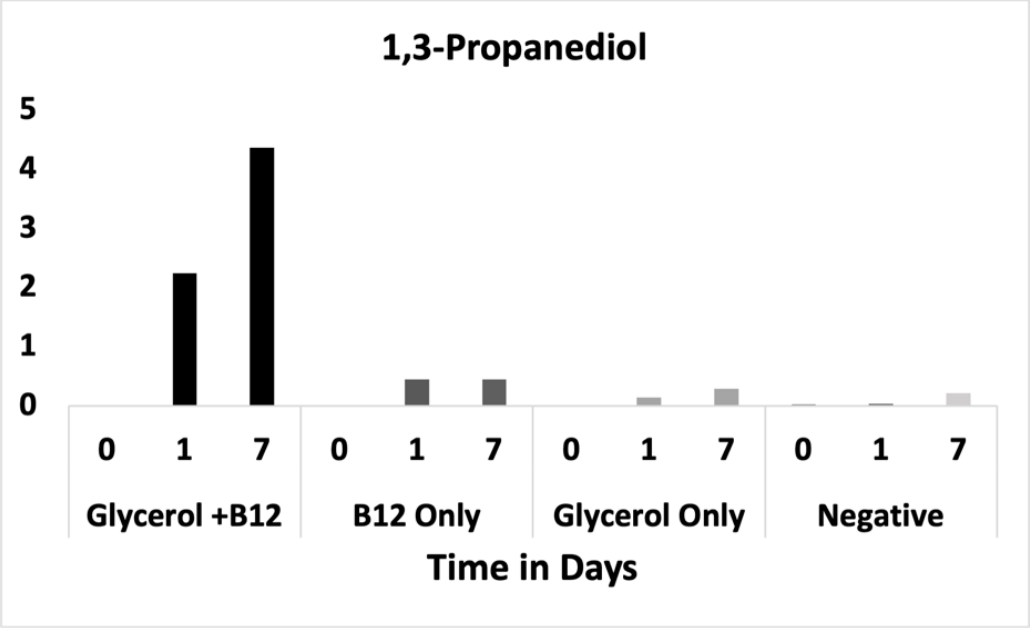


Figure 3. 1,3-Propanediol GCMS results. Cell-free supernatant of *Lat. curvatus* WSU-1 grown under four different conditions: CR-MRS supplemented with either 200 mM glycerol + 0.2 mg/L B12, 0.2 mg/L B12 only, 200 mM glycerol only, or nothing as a negative control.

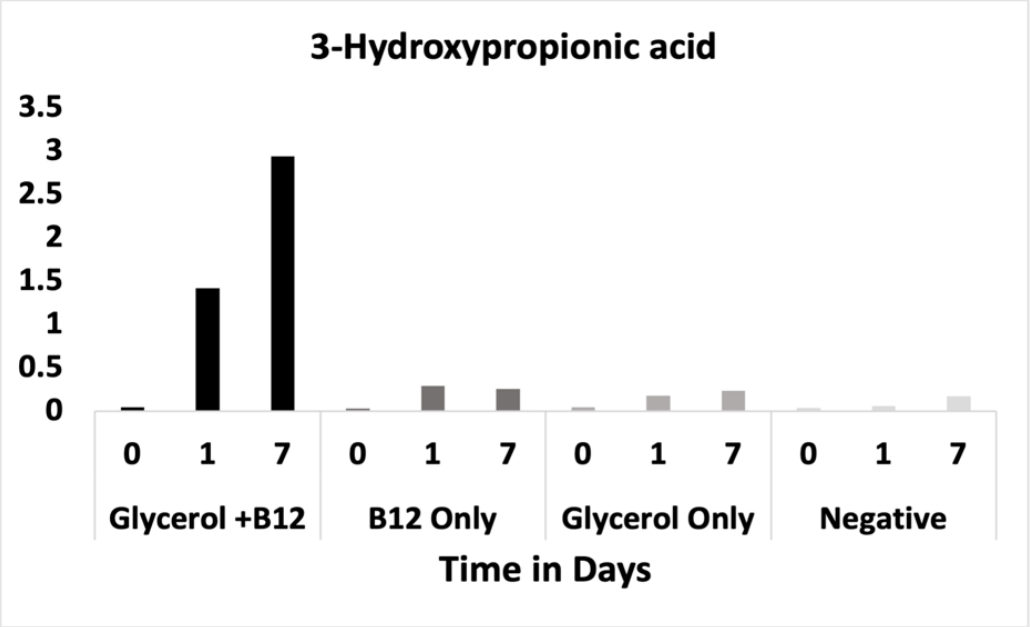


Figure 4. 3-Hydroxypropionic acid GCMS results. Cell-free supernatant of *Lat. curvatus* WSU-1 grown under four different conditions: CR-MRS supplemented with either 200 mM glycerol + 0.2 mg/L B12, 0.2 mg/L B12 only, 200 mM glycerol only, or nothing as a negative control.

Reuterin analysis in bulk fermentations

The quantification of reuterin under various fermentation conditions and time points is presented in Table 12. These results indicate significant variations in reuterin production depending on the substrate used and the duration of fermentation. The quantification was performed using a standard curve of acrolein, shown in Figure 5, which had an R-squared value of 0.9995, indicating a high level of accuracy in the measurement.

The highest concentration of reuterin was observed in the Glycerol + B12 condition at 24 hours, with a concentration of 45.8 μg/mL (0.816 mM). However, this concentration decreased substantially by the 48-hour mark to 23.8 μg/mL (0.42 mM). This notable reduction suggests that reuterin is further metabolized to the end products 3-HP and 1,3-PD. In a culture with no competing organisms, the export of reuterin extracellularly is not as necessary, leading to its conversion to these end products. This finding correlates with the GC-MS data, which showed the highest concentration of end products after 48 hours of fermentation (Figures 3-4).

The "No Glycerol" and "Glycerol + B12 Two-step" conditions produced negligible amounts of reuterin, with concentrations below the LOQ of 1.5 μg/mL. Interestingly, the tested conditions showed that the two-step fermentation process did not enhance reuterin synthesis. Other studies using this protocol on the bacterium *Lim. reuteri* showed pronounced conversion of glycerol in the two-step fermentation, where glycerol was the sole substrate (Lüthi-Peng et al., 2002b; Doleyres Beck S Vollenweider

C Lacroix, 2005). This indicates that *Lat. curvatus* WSU-1 may not be as efficient at metabolizing glycerol when present as the sole substrate.

Fermentations with the addition of ribose or galactose at 10% of the concentration of glycerol produced varying levels of reuterin. Ribose-containing fermentations yielded lower levels of reuterin, with concentrations of 7.1 μg/mL (0.126 mM) at 24 hours and 7.0 μg/mL (0.125 mM) at 48 hours. The minimal change between the 24-hour and 48-hour time points suggests that reuterin production from ribose quickly reaches a plateau, with little additional production or degradation over the extended fermentation period. In contrast, fermentations with galactose resulted in reuterin concentrations of 9.9 μg/mL (0.177 mM) at 24 hours and 11.4 μg/mL (0.203 mM) at 48 hours. The slight increase in reuterin production over time indicates a continued or delayed synthesis of reuterin in the presence of galactose.

Overall, glycerol, in combination with vitamin B12, proved to be the most effective substrate for reuterin synthesis. However, even under optimal conditions, the production rate was substantially lower than that reported in previous research with *Lim. reuteri (Doleyres Beck et al., 2005; Ortiz-Rivera et al., 2017).* Additionally, glycerol fermentations supplemented with ribose or galactose produced substantially lower levels of reuterin. This suggests that the presence of other sugars may interfere with reuterin production, contradicting previous studies that found an increase in production in *Lim. reuteri* (Lüthi-Peng et al., 2002). These results indicate that, although functional, the *pdu* operon in *Lat. curvatus* may still be incomplete or less effective compared to bacteria with functional B12 synthesis genes.

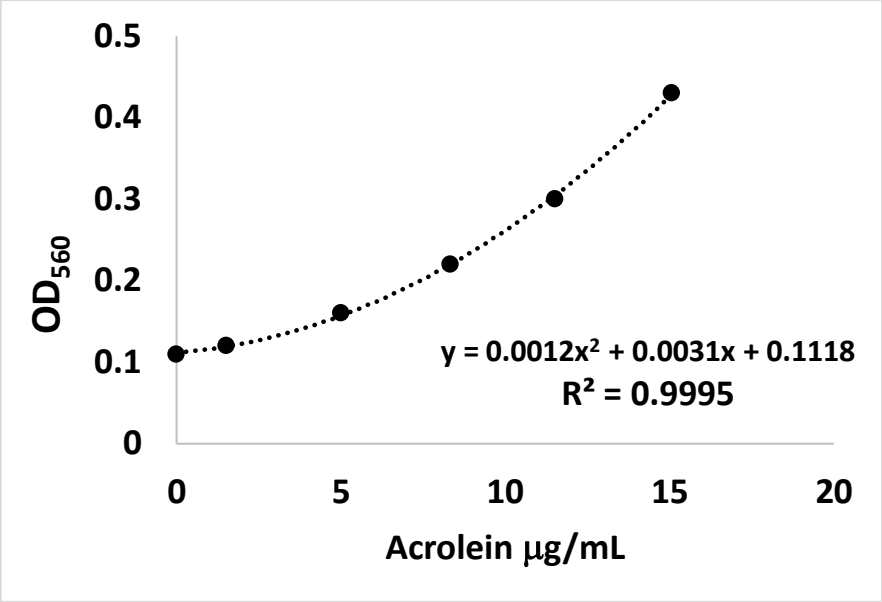


Figure 5. Standard curve of acrolein (1.5-15µg/mL) for the quantification of reuterin measured at OD_{560}.

Table 12. Quantification of reuterin in bulk fermentations: Results were quantified by comparing the OD560 values of the solution to the acrolein standard curve, accounting for dilution as needed. Substrate concentrations are as follows: glycerol (200mM), ribose (20mM), Galactose (20mM), and B12 (0.2µg/mL) mM concentrations calculated based on the molecular weight of acrolein.

Sample	µg/mL	mM
Glycerol + B12 24hr	45.8	0.816
Glycerol + B12 48hr	23.8	0.42
No Glycerol	<1.5	0
Glycerol + B12 Two step	<1.5	0
Glycerol + B12 + Ribose 24hr	7.1	0.126
Glycerol + B12 + Ribose 48hr	7	0.125
Glycerol + B12 + Galactose 24hr	9.9	0.177
Glycerol + B12 + Galactose 48hr	11.4	0.203

In vitro Antifungal Analyses

96-well plate assay

From the 96-well plate analysis of the eight yeast strains, we examined the impact of metabolites produced by *Lat. curvatus* WSU-1 fermentation under two conditions on yeast growth. Our findings revealed that the extent of inhibition varied among yeast strains, treatments, and treatment concentrations. Despite these variations, several noticeable trends were identified, providing insights into the metabolic interactions between WSU-1 and the yeast strains.

Across the strains, glycerol supernatant generally affected growth in a concentration-dependent manner, often inhibiting growth compared to the negative control. However, the degree of inhibition and the specific concentrations that yielded the most significant effects varied among the strains (Figures 6-13).

- Strains Y3, Y4, and Y5**:** Demonstrated apparent inhibitory effects from glycerol supernatant at one or more concentrations when compared to the negative control. In contrast, other strains exhibited minimal to no inhibition, particularly when considering the final growth measurement at 140 hours.

The growth patterns under the no glycerol supernatant treatment varied as well. Still, it was observed that the no glycerol supernatant often resulted in similar or greater inhibition compared to the glycerol-containing supernatant. Initially, it was hypothesized that glycerol supernatant would exhibit higher levels of inhibition due to the presence of

reuterin. This unexpected finding suggests that WSU-1 may produce higher levels of other unknown inhibitory compounds in the absence of glycerol.

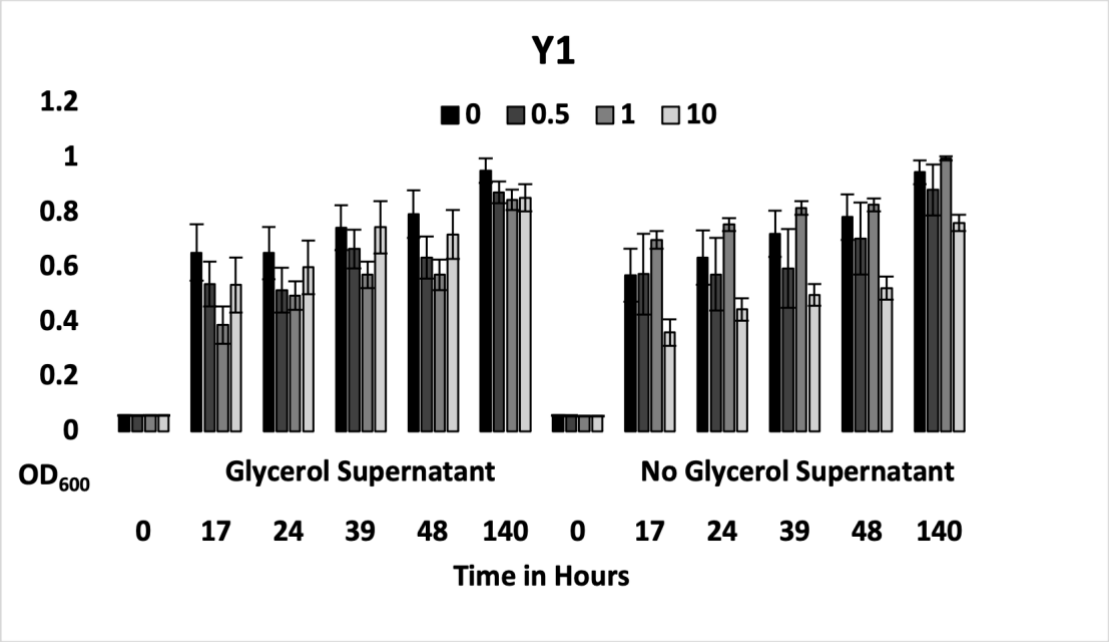


Figure 6. Growth of *Pichia fermentans* (Y1) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

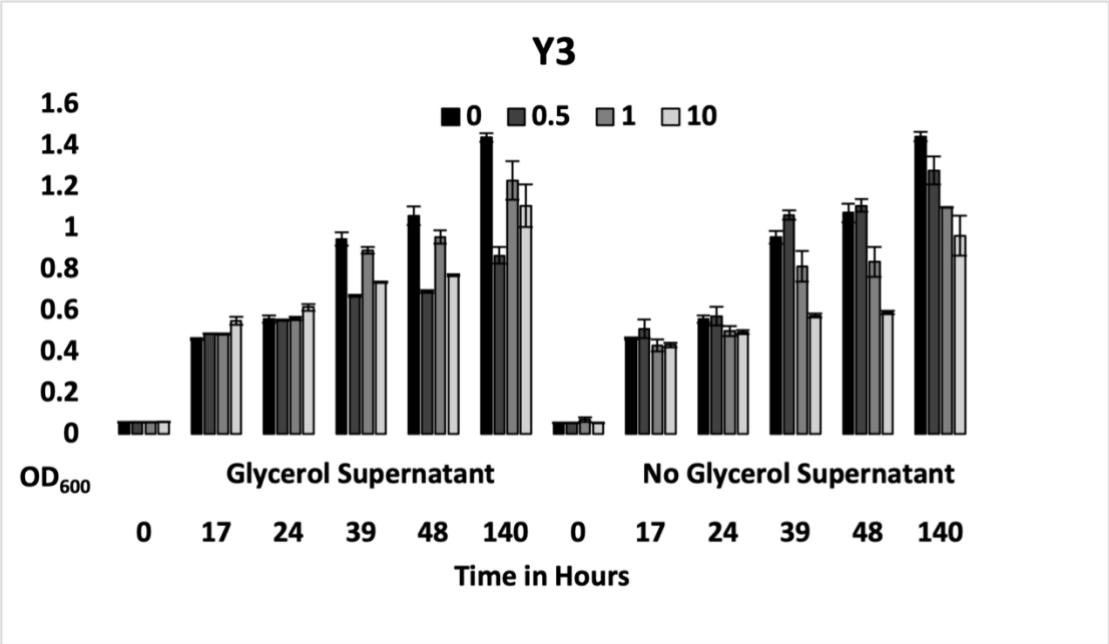


Figure 7. Growth of *Clavispora lusitaniae* (Y3) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

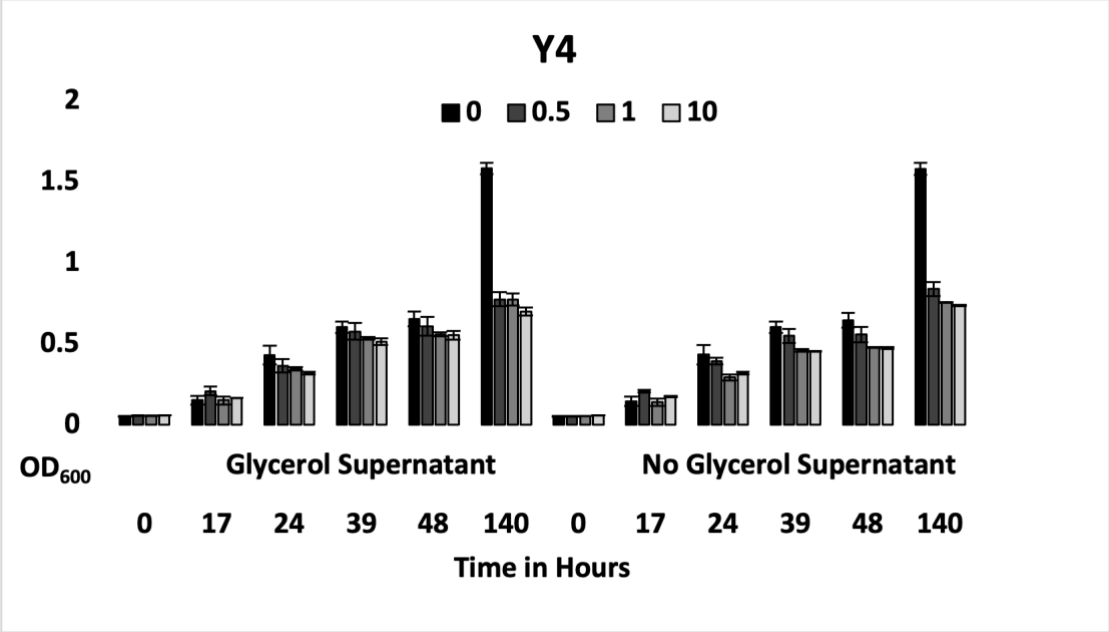


Figure 8. Growth of *Debaryomyces hansenii* (Y4) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

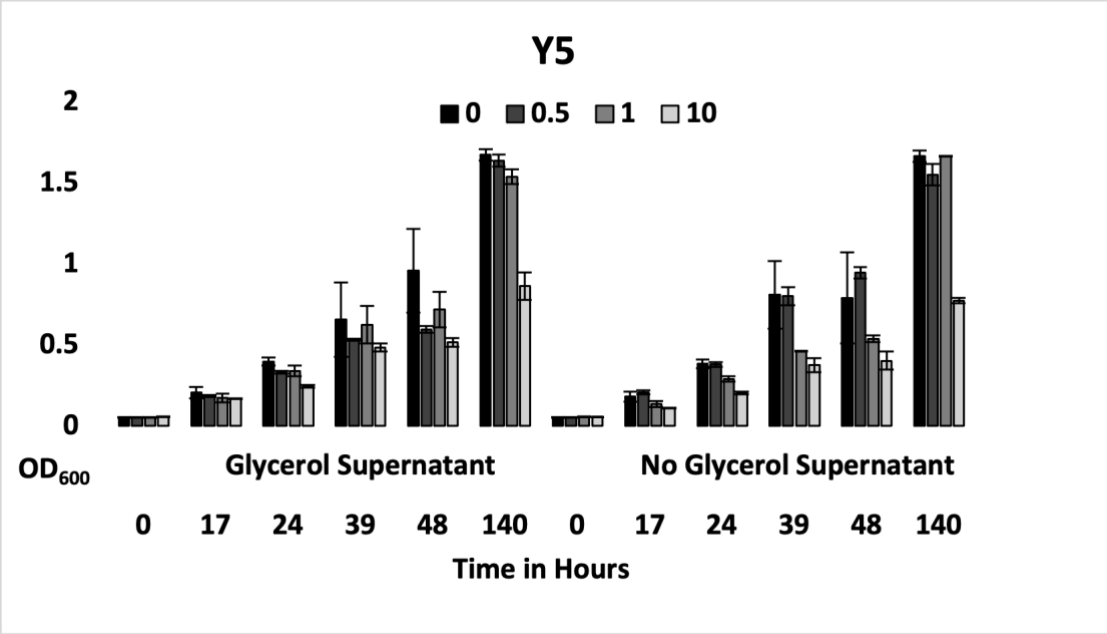


Figure 9. Growth of *Debaryomyces prosipidis* (Y5) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

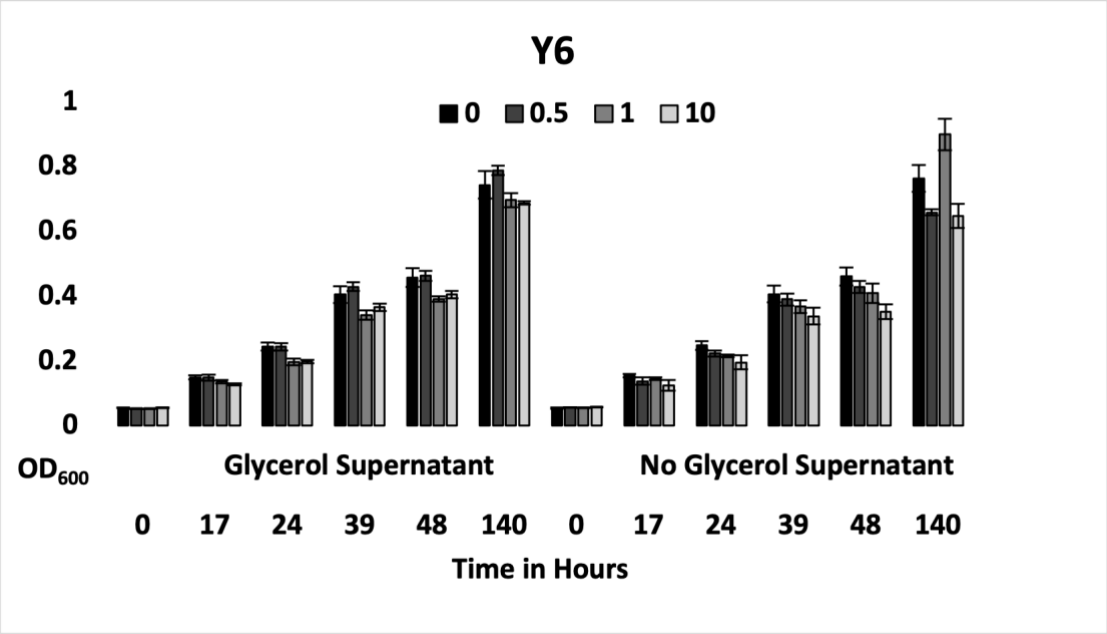


Figure 10. Growth of *Candida zeylanoides* (Y6) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

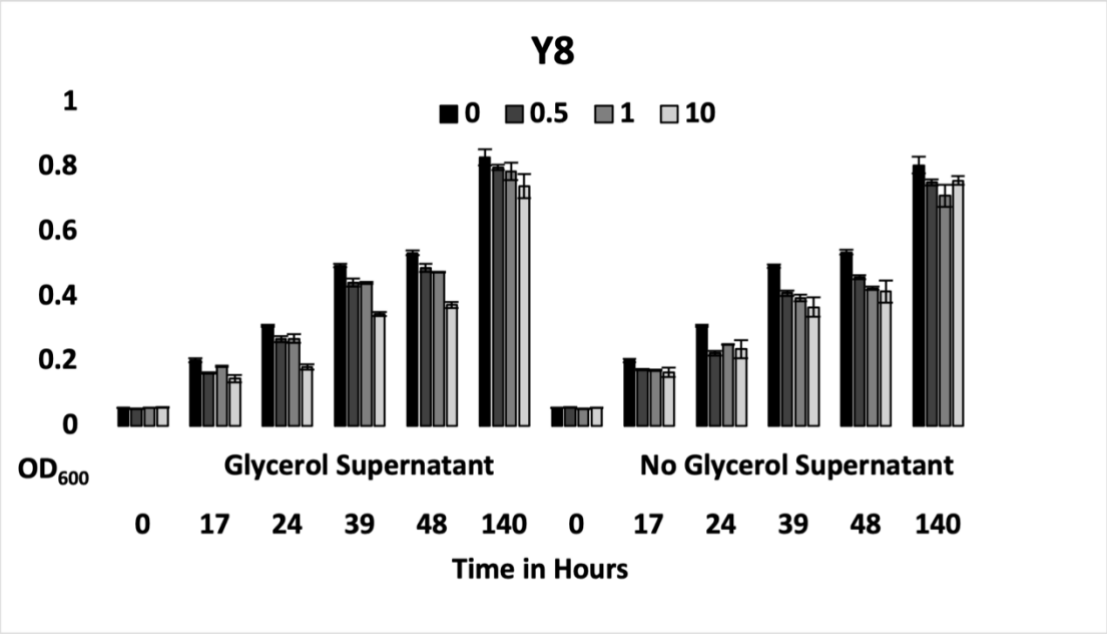


Figure 11. Growth of *Rhodotorula mucilaginosa* (Y8) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

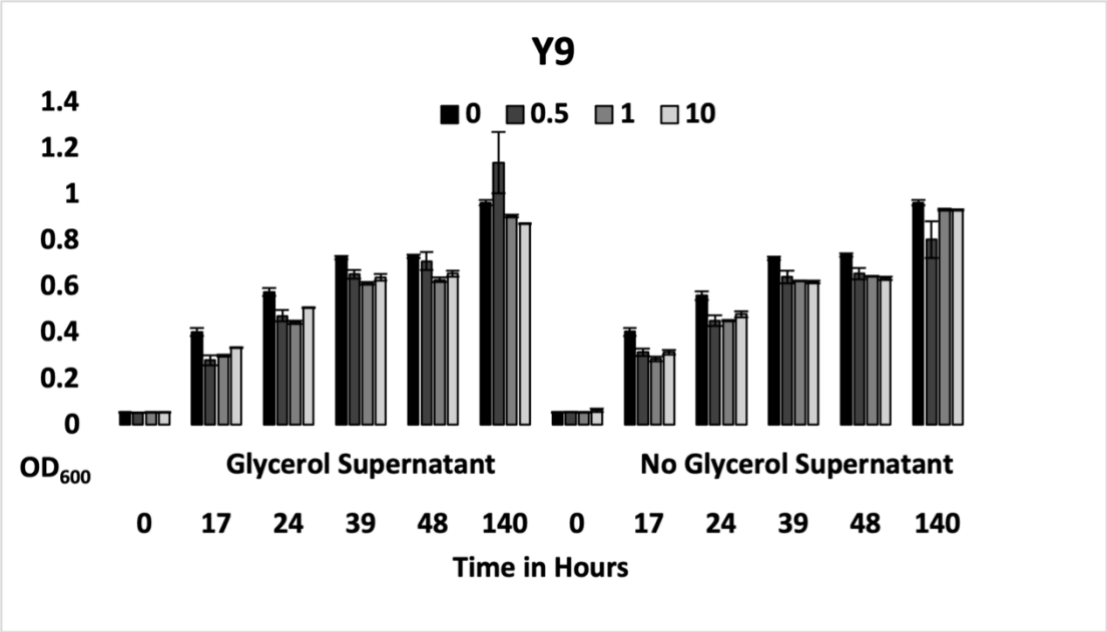


Figure 12. Growth of *Meyerozyma guilliermondii* (Y9) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

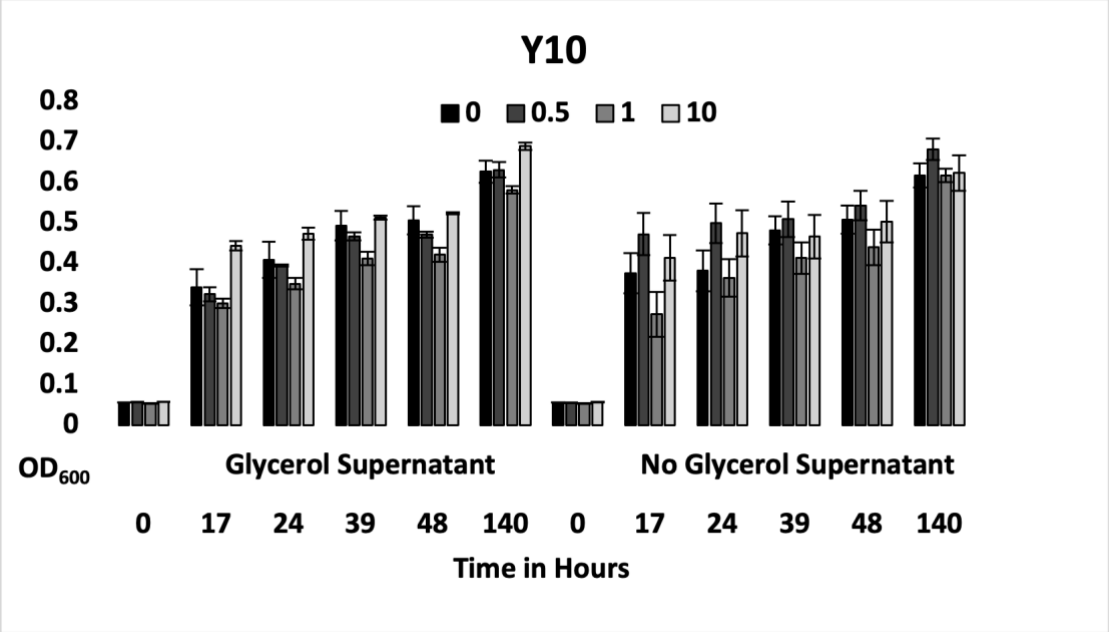


Figure 13. Growth of *Torulaspora delbrueckii* (Y10) in a 96-well plate using malt extract broth, subjected to four concentrations (0%, 0.5%, 1.0%, and 10%) of WSU-1 cell-free supernatant grown in CR-MRS. The supernatant was prepared either with glycerol (200 mM) and B12 (2 mg/L) or without any added substrate. Absorbance values at OD_{600} were measured at time points 0, 17, 24, 39, 48, and 140 hours. Data points represent the mean of three replicates, with error bars indicating the standard error of the mean (SEM).

24-well plate assay

The antifungal efficacy of *Latilactobacillus curvatus* WSU-1 was evaluated using a modified overlay method in 24-well plates, facilitating a high-throughput assessment of its bio-preservative potential under various growth conditions. This method tested the effects of WSU-1 grown in different media against eight yeast and nine mold strains.

Mold Inhibition Trials

Figure 14 and Table 13 detail the results from the mold inhibition trials conducted under three conditions:

1. MRS + WSU-1: This condition showed the highest level of inhibition among the three treatments, completely inhibiting 6 of the 9 molds, substantially inhibiting *Penicillium commune*, with only *Penicillium roqueforti* and *Penicillium decumbens* showing minimal to no inhibition.
2. CR-MRS + WSU-1 + Glycerol: Displayed a general decrease in inhibition compared to the standard MRS + WSU-1, though it still completely inhibited 3 strains. The remaining strains showed limited to no inhibition.
3. CR-MRS + WSU-1 + 1-2PD: Similar to the glycerol condition, this medium exhibited reduced inhibition compared to MRS + WSU-1.

Yeast Inhibition Trials

Figure 15 and Table 14 provide insights into the yeast inhibition trials. WSU-1's efficacy against yeast under the same three conditions revealed more varied results than with the molds, showing generally lower levels of inhibition:

1. MRS + WSU-1: This treatment inhibited the broadest range of yeast strains, although it did not completely inhibit any yeast strain.
2. CR-MRS + WSU-1 + Glycerol: While this treatment inhibited fewer yeast strains, the extent of inhibition was more profound, with three strains being completely inhibited and one exhibiting a pronounced ring of inhibition.
3. CR-MRS + WSU-1 + 1-2PD: Showed the lowest inhibitory effects, with most strains experiencing little to no inhibition. Slight rings of inhibition were noted in *Pichia fermentans* and *Torulaspora delbrueckii*.

These findings indicate that the antifungal capabilities of *Lat. curvatus* WSU-1 can be influenced by the composition of the growth medium. The standard MRS medium supported the highest level of mold inhibition. In contrast, yeast inhibition was more varied, with standard MRS affecting the broadest range of strains, although glycerol enhanced MRS proved most potent against the few strains it did affect.

The level of inhibition of the mold with standard MRS was relatively unexpected and was contrary to what was initially hypothesized. One potential reason why *Lat. curvatus* WSU-1 was more effective on standard MRS could be because it appeared to be able to reach a higher biomass. This larger biomass could prove more effective in producing alternative antifungal compounds or, with the higher biomass, it could be more likely to sequester a higher amount of manganese, rendering it unavailable for fungal growth.

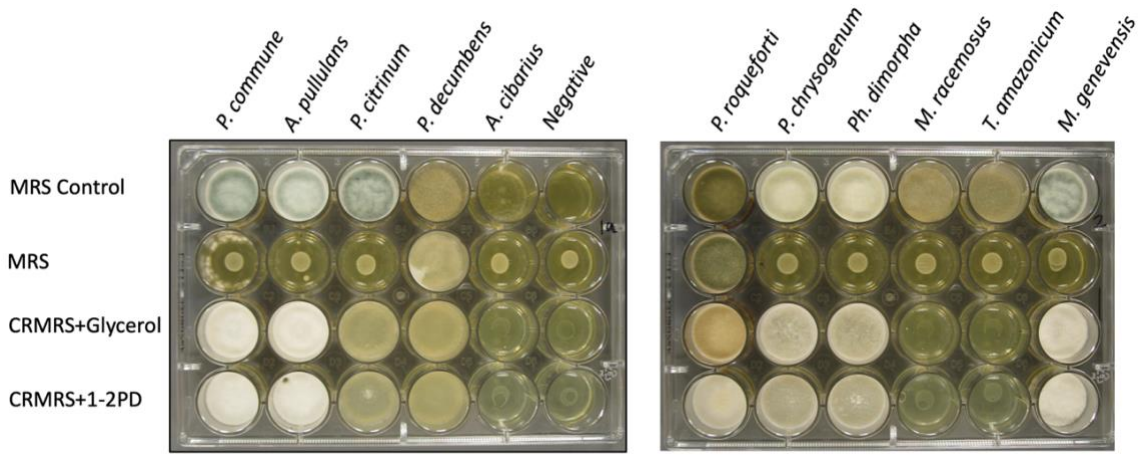


Figure 14. Inhibition trial of nine mold species in a 24-well plate, using a modified soft agar overlay method against *Latilactobacillus curvatus* WSU-1 grown under three different conditions: standard MRS, CR-MRS with 200 mM glycerol, or CR-MRS with 200 mM 1,2-propanediol. The top row represents a negative control of MRS with no WSU-1 culture. All mold strains were inoculated in malt extract soft agar at a concentration of 5 $\log_{10}$ spores/mL, and plates were incubated for 96 hours at 25°C. The image shows one of three replicates, all with comparable growth results.

Table 13. Results of 24-well plate mold inhibition trial. All results shown were run in triplicate. Results reported as follows: (-) No Inhibition, (+*) Slight fading/color change, (+) Fading of growth/slight ring, (++*) Ring of inhibition present, (++) complete visual inhibition/no growth.

Mold 96 hr.	MRS Control	MRS + WSU-1	CRMRS + WSU-1 + Glycerol	CRMRS + WSU-1 + 1-2PD
P. commune	-	++*	+*	+*
P. citrinum	-	++	+*	+*
P. decumbens	-	+*	+*	+*
A. cibarius	-	++	++	++
P. roqueforti	-	-	+*	+*
P. chrysogenum	-	++	-	-
Ph. dimorpha	-	++	-	-
M. racemosus	-	++	++	++
T. amazonicum	-	++	++	++
Negative control	-	-	-	-

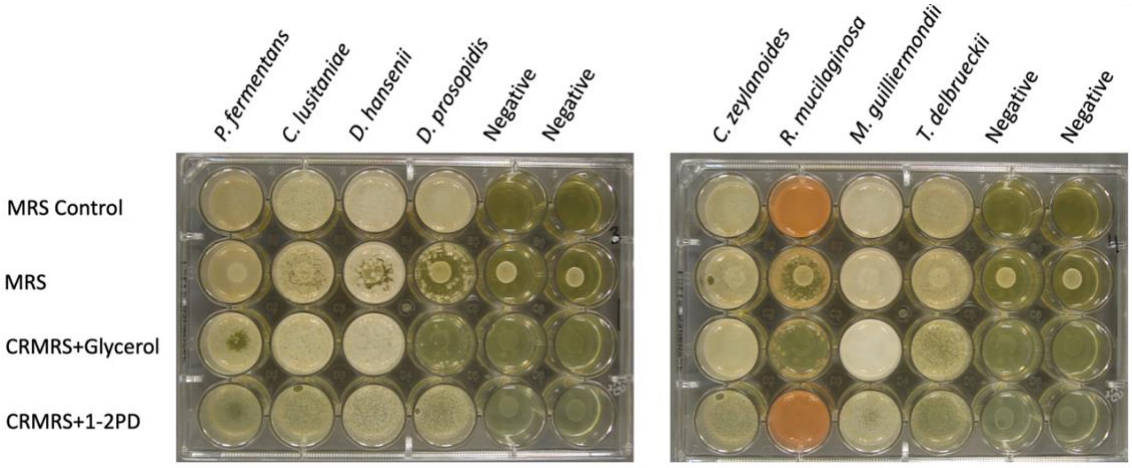


Figure 15. Inhibition trial of 8 yeast species in a 24-well plate, using a modified soft agar overlay method against *Latilactobacillus curvatus* WSU-1 grown under three different conditions: standard MRS, CR-MRS with 200 mM glycerol, or CR-MRS with 200 mM 1,2-propanediol. The top row represents a negative control of MRS with no WSU-1 culture. All yeast strains were inoculated in malt extract soft agar at a concentration of 5 $\log_{10}$ CFU/mL, and plates were incubated for 96 hours at 25°C. The image shows one of three replicates, all with comparable growth results.

Table 14. Results of 24-well plate yeast inhibition trial. All results shown were run in triplicate. Results reported as follows: (-) No Inhibition, (+*) Slight fading/color change, (+) Fading of growth/slight ring, (++*) Ring of inhibition present, (++) complete visual inhibition/no growth.

Yeast 96 hr.	MRS Control	MRS + WSU-1	CRMRS + WSU-1 + Glycerol	CRMRS + WSU-1 + 1-2PD
P. fermentans	-	-	++*	+
C. lusitaniae	-	+*	-	+*
D. hansenii	-	+	-	+*
D. prosopidis	-	++*	++	+*
C. zeylanoides	-	+*	-	+*
R. mucilaginosa	-	++*	++	-
M. guillermondii	-	+*	-	+
T. delbrueckii	-	+*	+	++*
Negative Control	++	++	++	++

The assessment of *Latilactobacillus curvatus* WSU-1's characteristics and antifungal efficacy provided significant insights relevant to its application as a bio-preservative. The metabolic analysis revealed WSU-1's unique carbohydrate fermentation profile, particularly its ability to ferment glucose and ribose efficiently while being lactose-negative and non-gas producing. These metabolic traits are particularly advantageous in settings where the fermentation of specific sugars needs to be controlled or minimized, such as in yogurt production.

The antifungal activity assays further illustrated the bio-preservative capabilities of WSU-1, showing variable but promising results across different media conditions. The modified overlay method confirmed that the antifungal properties of WSU-1 could be modulated by the growth medium, with the standard MRS medium supporting the highest level of mold inhibition. This suggests that the antifungal metabolites generated by WSU-1 are influenced by the culture’s metabolic state, which can be tailored by altering growth substrates.

However, the results also indicated that while WSU-1 can effectively inhibit several molds, its impact on yeasts was less consistent, with some strains showing resistance to the antifungal effects. This variability underscores the complexity of microbial interactions.

Building on these findings, the next objective focuses on applying WSU-1 in yogurt production to evaluate its practical efficacy and impact on product quality, particularly in the presence of competing microorganisms.

In-situ Yogurt Trial

WSU-1 concentration in yogurt over time

As depicted in Figure 16, the concentration of *Lat. curvatus* WSU-1 in yogurt supplemented with glycerol was consistently higher than that in the control group without glycerol across weeks 2, 3, and 4. This statistically significant trend suggests that glycerol supports and actively enhances the proliferation of WSU-1.

The consistently higher counts of WSU-1 in the glycerol-enriched yogurt indicate that WSU-1 likely utilizes glycerol as a substrate, corroborating findings from previous in vitro studies. However, the precise quantity of glycerol fermented by WSU-1 during these trials remains undetermined. Examination of the intermediate reuterin in glycerol fermentation is discussed in the ancillary yogurt test section.

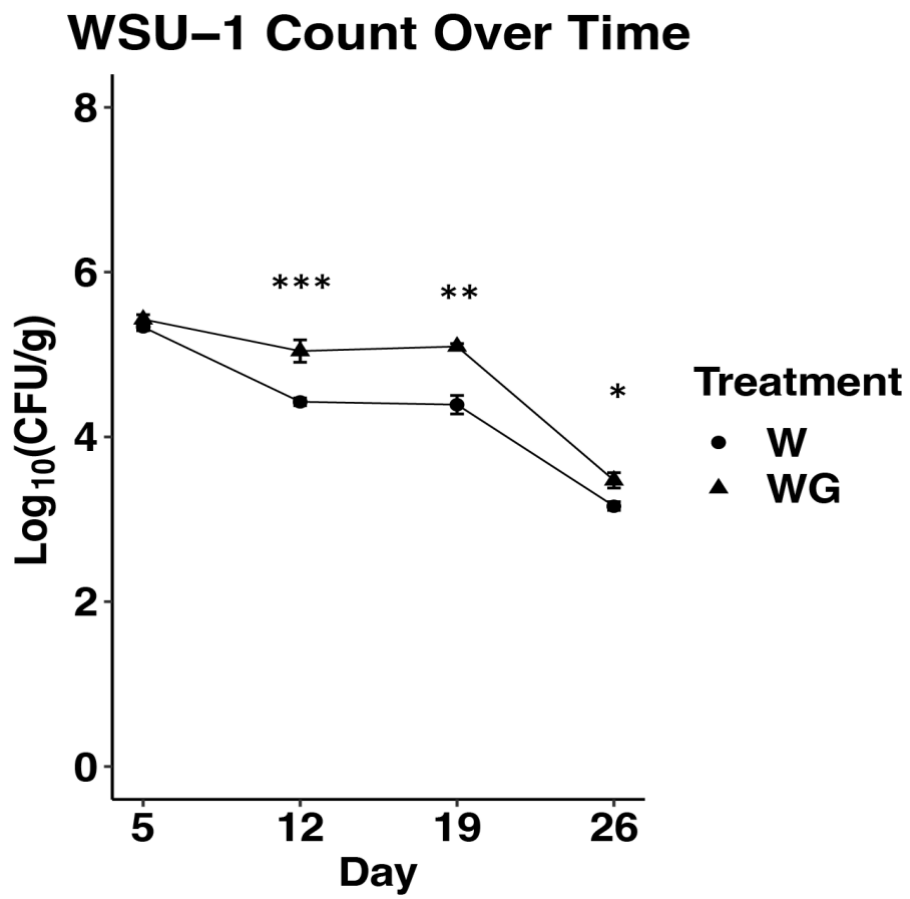


Figure 16. Plate counts of *Latilactobacillus curvatus* strain WSU-1 in yogurt inoculated with 5 $\log_{10}$ CFU/g WSU-1 **(W)** and yogurt inoculated with 5 $\log_{10}$ CFU/g WSU-1 supplemented with 200 mM glycerol **(WG)**. Plate counts were conducted at four time points (5, 12, 19, and 26 days) on MRS agar supplemented with 50 µg/mL vancomycin, and the plates were incubated anaerobically at 37°C for 48 hours. Results represent the mean of nine total replicates, comprising three technical replicates for each of the three biological replicates at each time point. Error bars indicate the standard error of the mean (SEM). Significance markers: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Yeast yogurt trial

The yeast growth over time in three different yogurt treatments was evaluated and is depicted in Figure 17. Some significant differences were found in yeast growth between treatments; however, there was no discernible trend. Some strains showed lower counts in the treatment compared to the control, while others showed the control with lower counts than the treatments. Although significant differences were detected, they

were less than a log difference, and all counts were above 7 log10 CFU/g, well above the detectable limit of 5 log10 CFU/g, which has been determined as the limit at which consumers can detect flavor and textural differences (Makki et al., 2021). This suggests that there was no substantial inhibition of any yeast strains in either treatment.

Significant differences in these results likely reflect a well-designed experiment with very low variation between replicates rather than a pronounced inhibition of yeast growth. Therefore, the inhibitory results observed in the in vitro analysis could not be replicated.

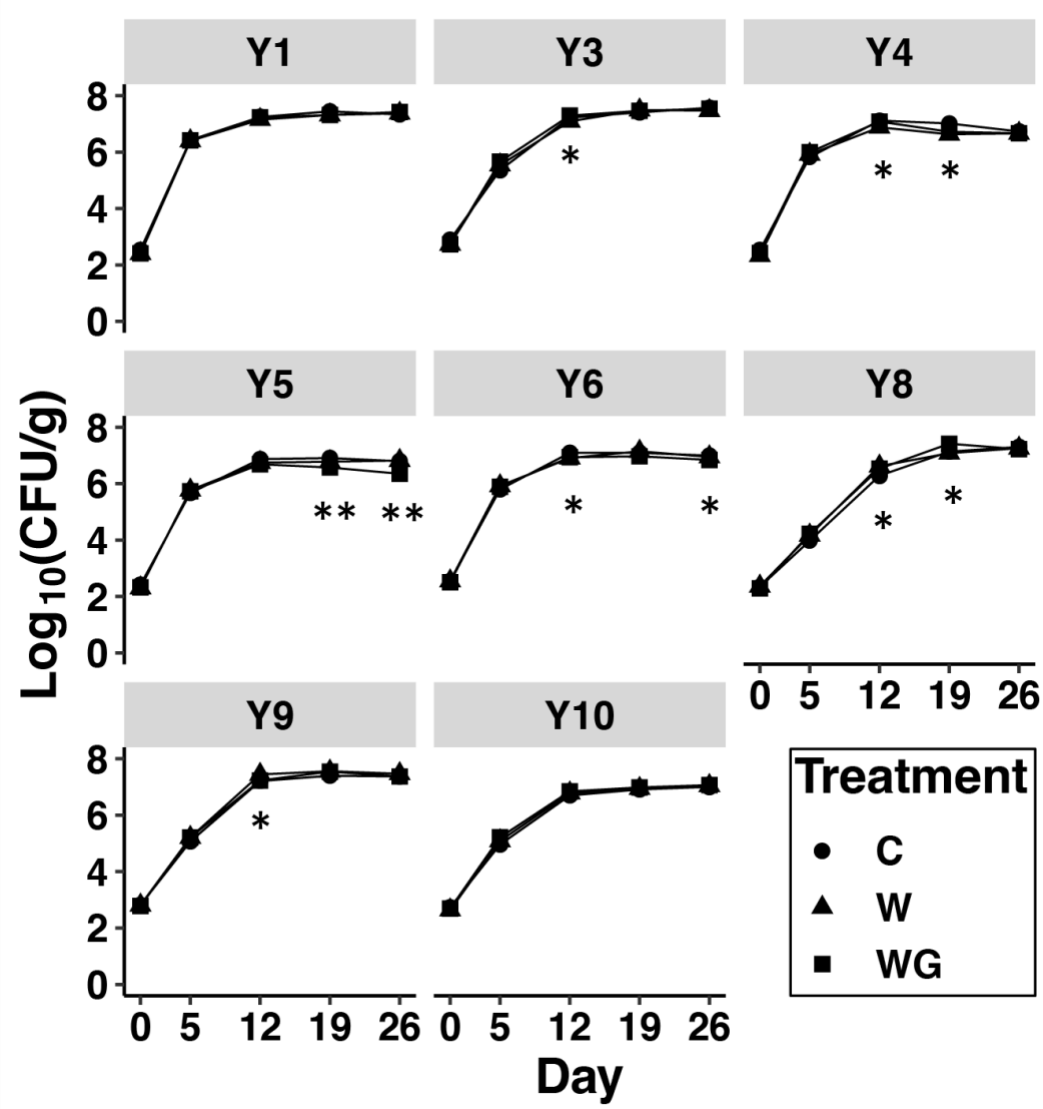


Figure 17. Comparison of yeast enumerations in three yogurt treatments over time. The line graphs depict the logarithmic count of yeast (CFU/g) over time for treatments: **C** (Control), **W** (inoculated with 5 $\log_{10}$ CFU/g WSU-1), and **WG** (inoculated with 5 $\log_{10}$ CFU/g WSU-1 supplemented with 200 mM glycerol). The eight yeast strains tested are **Y1** (*Pichia fermentans)*, **Y3** (*Clavispora zeylanoides*), **Y4** (*Debaryomyces hansenii*), **Y5** (*Debaryomyces prosipidis)*, **Y6** (*Candida zeylanoides*), **Y8** (*Rhodotorula mucilaginosa*), **Y9** (*Meyerozyma guilliermondii*), **Y10** (*Torulaspora delbrueckii).* Measurements were taken at multiple time points (Day 0, Day 5, Day 12, Day 19, Day 26). Values represent the mean of three replicates, with error bars depicting the standard error of the mean (SEM). Significance markers result from pairwise comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Mold yogurt trial

Figure 18 depicts the level of mold outgrowth across the three yogurt treatments. These results are based on the average visual classification rating of mold outgrowth across nine replicates of each yogurt treatment. The Kruskal-Wallis test detected significant differences in mold growth. A compilation of all mold images can be found in Appendix B.

Like the yeast results, although significant differences were present in a few strains, they were not indicative of substantial inhibition, as all strains still showed pronounced visible outgrowth. Overall, the treatments appeared to work better on some mold species compared to yeast. For instance, M3 (*Penicillium citrinum*) and M10 (*Trichoderma amazonicum*) exhibited the largest amount of inhibition across the treatments, with treatments holding mold growth close to the detectable limit. However, like with the yeast, none of the mold strains were completely inhibited below detectable limits, and notable significant differences were only present in a select few.

Therefore, it is concluded that the inhibitory results observed in the in vitro analyses for both mold and yeast could not be substantiated or replicated in the in situ yogurt trial.

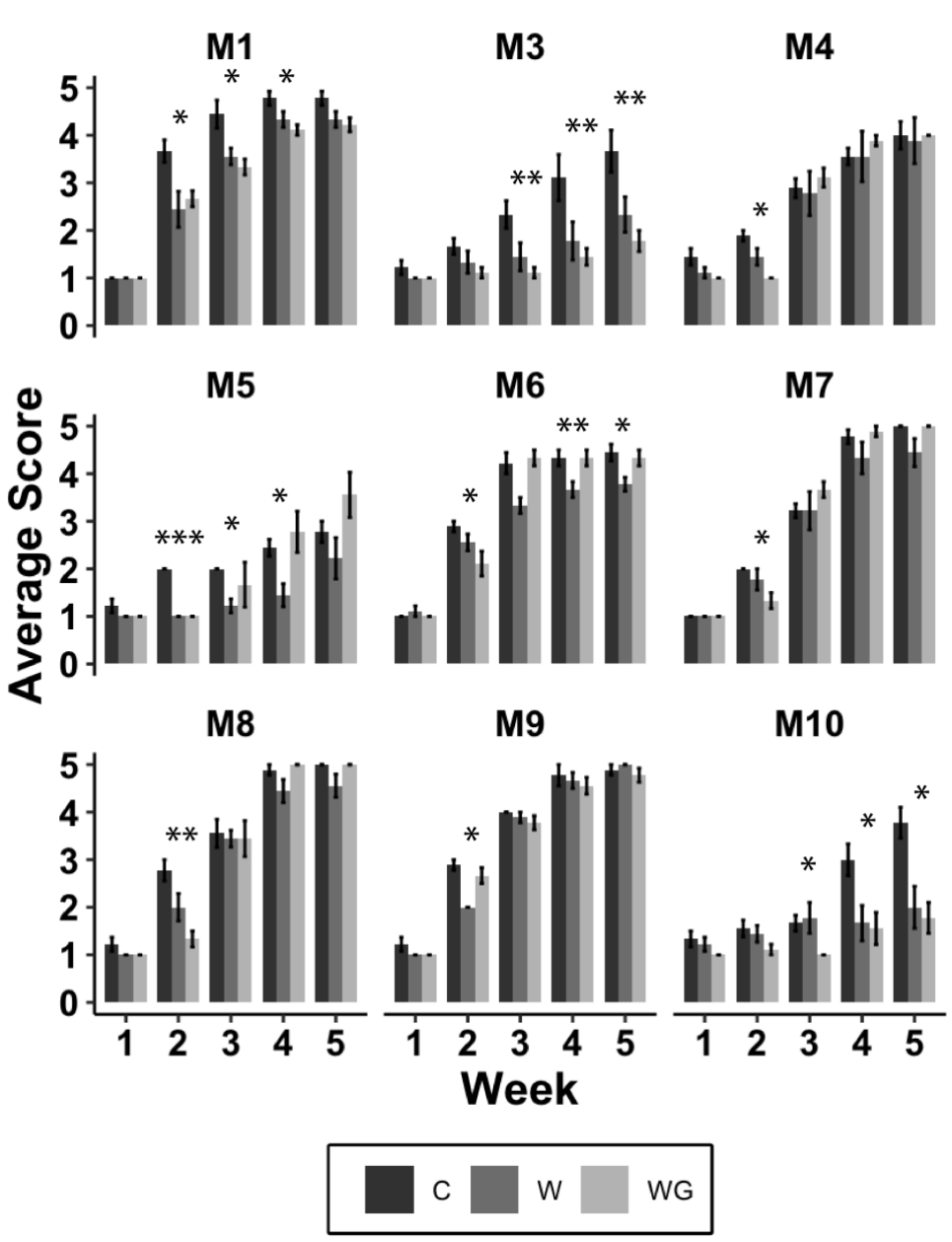


Figure 18. Statistical analysis of the visual classification of mold outgrowth in three yogurt treatments. The bar graphs show the average classification scores for treatments: **C** (Control), **W** (inoculated with 5 $\log_{10}$ CFU/g WSU-1), and **WG** (inoculated with 5 $\log_{10}$ CFU/g WSU-1 supplemented with 200 mM glycerol), over a period of five weeks. The nine mold species tested are **M1** (*Penicillium commune*) **M3** (*Penicillium citrinum*) **M4** (*Penicillium decumbens*) **M5** (*Aspergillus cibarius*) **M6** (*Penicillium roqueforti*) **M7** (*Penicillium chrysogenum*) **M8** (*Phoma dimorpha*) **M9** (*Mucor racemosus*) **M10** (*Trichoderma amazonicum*). Values represent the mean of three replicates, with error bars depicting the standard error of the mean (SEM). Significance markers as shown as a result of the Kruskal-wallis test with Dunn post hoc analysis: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Ancillary Analyses

Reuterin

The concentration of reuterin in all replicates of both treatments was below the LOQ of 1.5 µg/mL (Table 15) when quantified using a standard curve with an R-squared of 0.9969 (Figure 19). As previously discussed, these findings contrast with the higher reuterin production observed in bulk fermentations with glycerol and B12. As discussed below, several potential factors could contribute to this discrepancy, such as the carbohydrate and B12 concentration in the yogurt.

Sugar

No significant difference in lactose and galactose concentrations was found between treatments or the time points analyzed (Figures 20 and 21). Although there appeared to be an increase in galactose concentration, it was not significant. This was interesting because there was a drop in pH from 0 to 4 weeks, which would suggest sugar fermentation (Table 15). The presence of galactose in yogurt could help explain the lack of reuterin production, as the presence of galactose reduced reuterin concentration in the in vitro analysis. However, this is unlikely as there was no significant drop in either carbohydrate tested.

B12

B12 synthesis is a process known only to prokaryotes, particularly lactic acid bacteria, such as *Limosilactobacillus reuteri*. It is a highly complex process controlled by several different gene clusters and is a field of active study. *Lat. curvatus*, as previously shown, lacks the ability to synthesize coenzyme B12, which could account for the lack of reuterin production in yogurt and the diminished production in vitro.

B12 concentrations in the yogurt were substantially lower than those used in the in vitro studies, ranging between 0.18-0.2 µg/100g compared to 200 µg/100g used in the in vitro studies. This lower concentration could be a leading factor in the lack of reuterin production in yogurt compared to in vitro analyses, thus decreasing its anti-fungal capabilities.

Table 15. Ancillary tests of yogurt treatments over time. The table presents the measurements of lactose (g/L), galactose (g/L), B12 (µg/100g), reuterin (µg/mL), and pH for different yogurt treatments at two time points (Time 0 and Week 4). The treatments include Control, WSU-1, and WSU-1 supplemented with 200mM glycerol. Values for B12 are provided at Time 0 for each treatment, and reuterin levels are indicated as being below the limit of quantification (<1.5 µg/mL) across all samples.

Yogurt Treatment	Lactose g/L	Galactose g/L	B12 ug/100g	Reuterin g/mL	pH
Control Time 0	51.71	7.65	0.19	<1.5	4.61
Control Week 4	51.31	8.30	-	<1.5	4.38
WSU-1 Time 0	52.70	7.65	0.2	<1.5	4.61
WSU-1 Week 4	52.12	8.69	-	<1.5	4.38
WSU-1 +Glycerol Time 0	51.60	7.32	0.18	<1.5	4.62
WSU-1 + Glycerol Week 4	50.22	7.71	-	<1.5	4.40

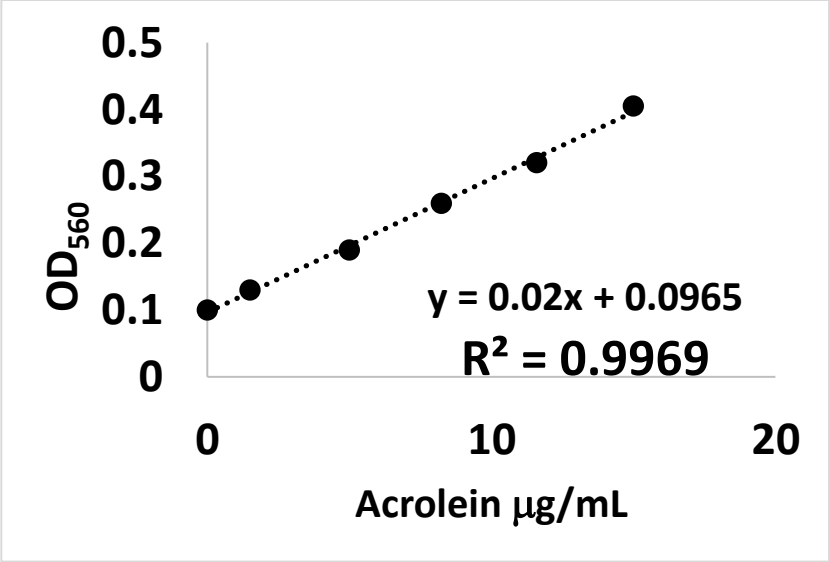


Figure 19. Acrolein standard curve (1.5-15 μg/mL) for the quantification of reuterin in yogurt using a colorimetric assay. The standard curve was constructed by measuring the absorbance at 560 nm (OD560) of acrolein at different concentrations.

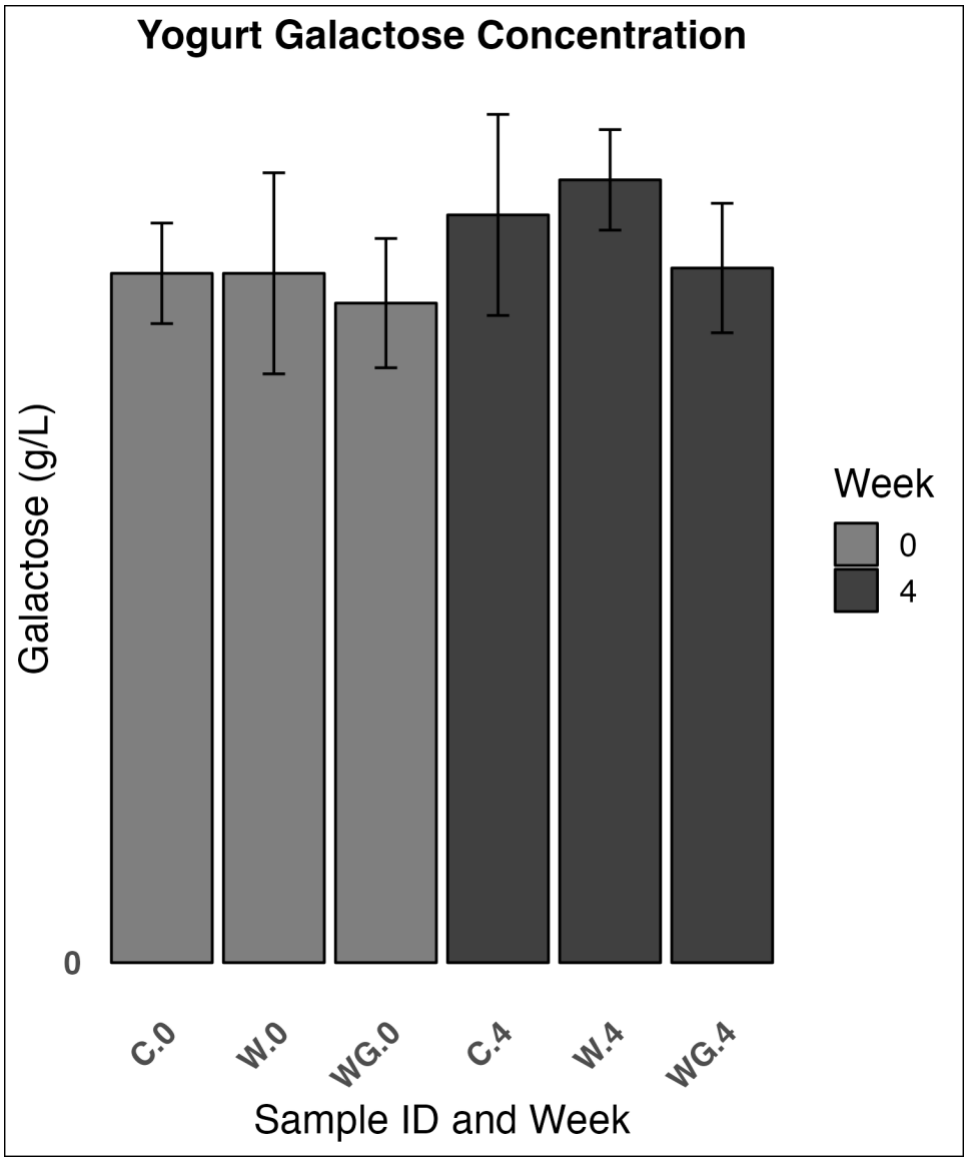


Figure 20. Galactose concentration in yogurt treatments over time. Galactose concentration (g/L) was quantified for each treatment: C (Control), W (inoculated with 5 log10 CFU/g WSU-1), and WG (inoculated with 5 log10 CFU/g WSU-1 supplemented with 200 mM glycerol) at time 0 and after 4 weeks. Results are presented as the mean of three replicates for each treatment, with error bars representing the standard error of the mean (SEM). No significant difference was found between treatments or time points as tested by ANOVA.

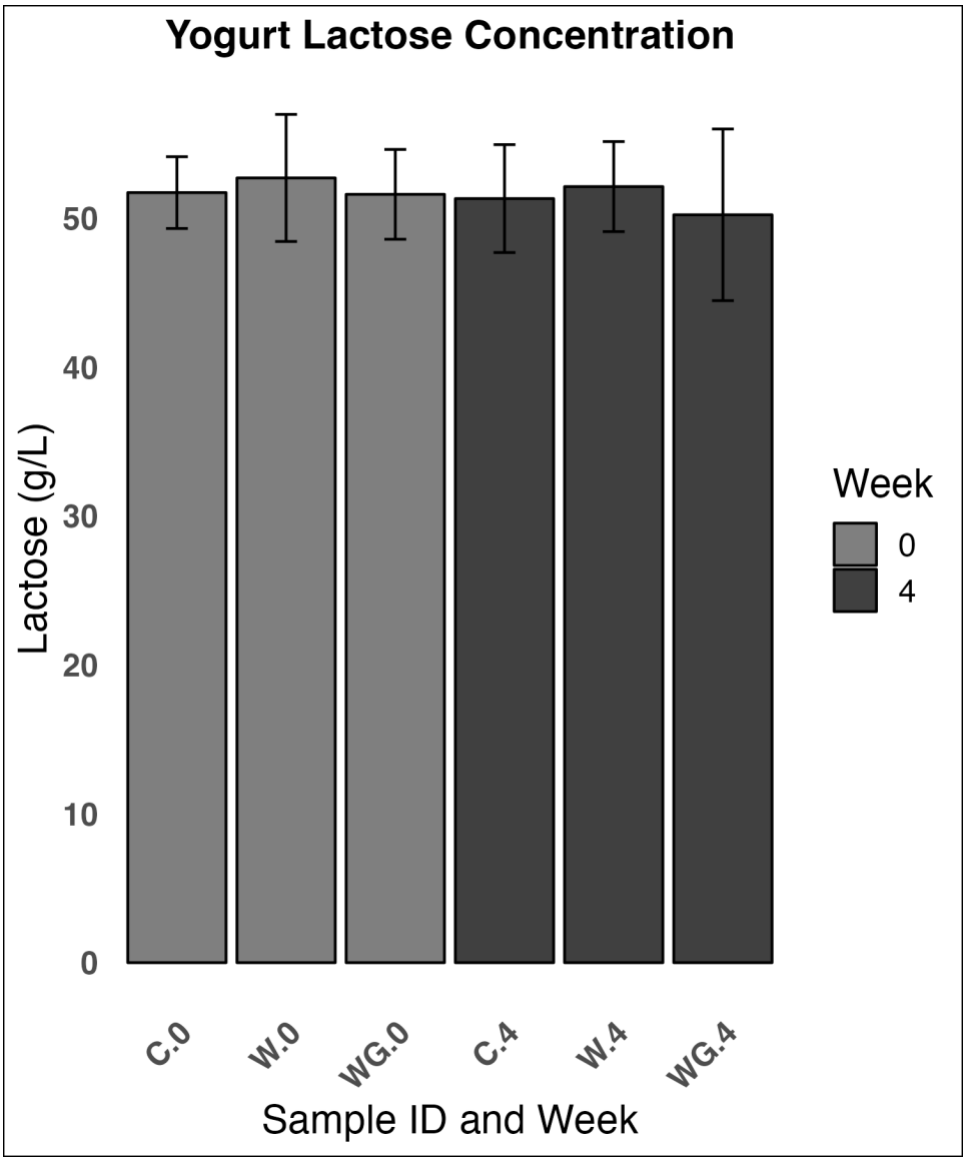


Figure 21. Lactose concentration in yogurt treatments over time. Lactose concentration (g/L) was quantified for each treatment: C (Control), W (inoculated with 5 log10 CFU/g WSU-1), and WG (inoculated with 5 log10 CFU/g WSU-1 supplemented with 200 mM glycerol) at time 0 and after 4 weeks. Results are presented as the mean of three replicates for each treatment, with error bars representing the standard error of the mean (SEM). No significant difference was found between treatments or time points as tested by ANOVA.

Sensory Evaluation

Difference Tests

Both triangle tests concluded that the WSU-1 and WSU-1 + glycerol yogurts were significantly different from the control yogurt, with p-values of 0.0087 and <0.0001, respectively, as shown in Table 16. Comments from panelists, found in Appendix C, commonly cited variations in sweetness, acidity, and texture as reasons for the perceived differences.

Table 16. Triangle test results of the three yogurt treatments. Control yogurt was compared to yogurt inoculated with 5 log10 CFU/g WSU-1, and control yogurt to yogurt inoculated with 5 log10 CFU/g WSU-1 supplemented with 200 mM glycerol. Results were collected and statistical analyses were conducted using SIMS2000 software. Results are considered significant if $P < 0.05$.

Test	WSU-1 Vs. Control	WSU-1 Glycerol Vs. Control
Total Number of Responses:	64	64
Correct:	31	46
Incorrect:	33	18
Probability of a Correct Guess:	33%	33%
P-Value when testing for a difference:	0.0087	< 0.0001
P-Value when testing for similarity:	0.4503	0.9999

Colorimetry

LAB color scale measurements for each yogurt treatment at weeks 1 and 4 showed no noticeable differences. Table 17 presents the L*, a*, and b* values for each

yogurt at each time point, showing only slight variations. Delta E values for the control yogurt compared to WSU-1 yogurt and the WSU-1 + glycerol yogurts were calculated and are shown in Table 18. Each of the 4 Delta E values, ranging from 0.102-0.189, is far below the threshold value of 1, which is the point at which the human eye can begin to detect minute color differences. Therefore, it can be concluded that neither treatment had any substantial impact on the color of the yogurt.

Table 17. LAB color values for yogurt treatments taken at 1 and 4 weeks post production. The table shows the L*, a*, and b* values for three treatments (**C** (Control), **W** (inoculated with 5 log10 CFU/g WSU-1), and **WG** (inoculated with 5 log10 CFU/g WSU-1 supplemented with 200 mM glycerol)) at Week 1 and Week 4. Results represent the mean of nine total replicates, comprising three technical replicates for each of three biological replicates at each time point.

	L*		**a***		**b***	
	Week 1	**Week 4**	**Week 1**	**Week 4**	**Week 1**	**Week 4**
C	91.79	91.59	-2.82	-2.76	8.68	8.67
W	91.69	91.59	-2.84	-2.70	8.58	8.45
WG	91.55	91.33	-2.90	-2.84	8.66	8.71

Table 18. Delta E values comparing LAB color differences between three yogurt treatments (**C** (Control), **W** (inoculated with 5 log10 CFU/g WSU-1), and **WG** (inoculated with 5 log10 CFU/g WSU-1 supplemented with 200 mM glycerol)) at Week 1 and Week 4. treatments. The table presents the calculated delta E values for comparisons between W and C, and WG and C at Week 1 and Week 4.

Comparison	**DeltaE**
W vs C at Week 1	0.1023
W vs C at Week 4	0.1666
WG vs C at Week 1	0.1806
WG vs C at Week 4	0.1889

CONCLUSION

The antifungal capabilities of *Latilactobacillus curvatus* strain WSU-1 were analyzed comprehensively throughout this research. Through genomic, biochemical, and in vitro methods, antifungal mechanisms present in WSU-1 were identified, and their functionality was confirmed. However, the promising in vitro results were not substantiated when WSU-1 was grown and challenged in situ in yogurt. This discrepancy highlights the complexity of food systems compared to controlled in vitro systems and underscores the necessity of practical in-situ trials when analyzing potential LAB for use as bio-preservative agents.

Despite the limited success in replicating the in vitro inhibitory effects in yogurt, WSU-1's unique metabolic capabilities and ability to thrive in dairy environments suggest that it still holds potential for use in the dairy industry. Its ability to produce reuterin and other antimicrobial compounds, given the right conditions, could be leveraged in specific applications where these conditions can be optimized. Moreover, the research emphasizes the critical role of manganese scavenging and competitive exclusion, which remain a valuable antifungal strategy although not fully explored in the yogurt trials.

Further research could explore optimizing WSU-1's growth conditions and possibly enhancing its antifungal properties through genetic or metabolic manipulation. Additionally, WSU-1 might be more effective in combination with other LAB strains or preservatives, creating a synergistic effect that enhances its bio-preservative potential.

Previous research on *Lat. curvatus* and similar bacteria suggests that it could also be used as a bio-preservative protecting against spoilage and pathogenic bacteria, either

through mechanisms discussed here or potentially additional mechanisms, such as the production of bacteriocins. Additionally, it has been suggested that *Lat. curvatus* could show promise in human health as a potential probiotic (Chen et al., 2020). Pairing both bio-preservative functionality as well as potential probiotic benefits could be very beneficial to the dairy industry.

In conclusion, while WSU-1 showed limited effectiveness as a stand-alone bio-preservative in yogurt, its unique properties and potential applications warrant further investigation. This research contributes to the broader understanding of LAB as bio-preservatives and accentuates the importance of conducting thorough in-situ trials to complement in vitro findings.

WORKS CITED

Al-Nabulsi, A.A., T.M. Osaili, S.B. Oqdeh, A.N. Olaimat, Z.W. Jaradat, M. Ayyash, and R.A. Holley. 2021. Antagonistic effects of *Lactobacillus reuteri* against *Escherichia coli* O157:H7 in white-brined cheese under different storage conditions. J Dairy Sci 104:2719–2734. doi:10.3168/JDS.2020-19308.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. doi:10.1016/S0022-2836(05)80360-2.

Amin, H.M., A.M. Hashem, M.S. Ashour, and R. Hatti-Kaul. 2013. 1,2 Propanediol utilization by *Lactobacillus reuteri* DSM 20016, role in bioconversion of glycerol to 1,3 propanediol, 3-hydroxypropionaldehyde and 3-hydroxypropionic acid. Journal of Genetic Engineering and Biotechnology 11:53–59. doi:10.1016/J.JGEB.2012.12.002.

Arocha, M.M., M. McVey, S.D. Loder, J.H. Rupnow, and L. Bullerman. 1992. Behavior of Hemorrhagic *Escherichia coli* O157:H7 During the Manufacture of Cottage Cheese. J Food Prot 55:379–381. doi:10.4315/0362-028X-55.5.379.

Arqués, J.L., J. Fernández, P. Gaya, M. Nuñez, E. Rodríguez, and M. Medina. 2004. Antimicrobial activity of reuterin in combination with nisin against food-borne pathogens. Int J Food Microbiol 95:225–229. doi:10.1016/J.IJFOODMICRO.2004.03.009.

Ávila, M., N. Gómez-Torres, M. Hernández, and S. Garde. 2014. Inhibitory activity of reuterin, nisin, lysozyme and nitrite against vegetative cells and spores of dairy-related *Clostridium* species. Int J Food Microbiol 172:70–75. doi:10.1016/J.IJFOODMICRO.2013.12.002.

Aziz, R.K., D. Bartels, A. Best, M. DeJongh, T. Disz, R.A. Edwards, K. Formsma, S. Gerdes, E.M. Glass, M. Kubal, F. Meyer, G.J. Olsen, R. Olson, A.L. Osterman, R.A. Overbeek, L.K. McNeil, D. Paarmann, T. Paczian, B. Parrello, G.D. Pusch, C. Reich, R. Stevens, O. Vassieva, V. Vonstein, A. Wilke, and O. Zagnitko. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9. doi:10.1186/1471-2164-9-75.

Becker-Algeri, T.A., D. Castagnaro, K. de Bortoli, C. de Souza, D.A. Drunkler, and E. Badiale-Furlong. 2016. Mycotoxins in Bovine Milk and Dairy Products: A Review. J Food Sci 81:R544–R552. doi:10.1111/1750-3841.13204.

Beletsiotis, E., D. Ghikas, and K. Kalantzi. 2011. Incorporation of microbiological and molecular methods in HACCP monitoring scheme of molds and yeasts in a Greek

dairy plant: A case study. Procedia Food Sci 1:1051–1059. doi:10.1016/J.PROFOO.2011.09.157.

Brewster, J.D. 2003. A simple micro-growth assay for enumerating bacteria. J Microbiol Methods 53:77–86. doi:10.1016/S0167-7012(02)00226-9.

Broadbent, J.R., K. Houck, M.E. Johnson, and C.J. Oberg. 2003. Influence of Adjunct Use and Cheese Microenvironment on Nonstarter Bacteria in Reduced-Fat Cheddar-Type Cheese. J Dairy Sci 86:2773–2782. doi:10.3168/JDS.S0022-0302(03)73874-0.

Broadbent, J.R., C.J. Oberg, T.S. Oberg, M.D. Culumber, D.J. McMahon, and R.E. Ward. 2014. Genome Analysis of Dominant *Lactobacillus curvatus* Non-Starter Lactic Acid Bacteria Isolated from Cheddar Cheese. Page.

Brul, S., and P. Coote. 1999. Preservative agents in foods: Mode of action and microbial resistance mechanisms. Int J Food Microbiol 50:1–17. doi:10.1016/S0168-1605(99)00072-0.

Buehler, A.J., N.H. Martin, K.J. Boor, and M. Wiedmann. 2018. Evaluation of biopreservatives in Greek yogurt to inhibit yeast and mold spoilage and development of a yogurt spoilage predictive model. J Dairy Sci 101:10759–10774. doi:10.3168/JDS.2018-15082.

Bulgasem, B.Y., M.N. Lani, Z. Hassan, W.M. Wan Yusoff, and S.G. Fnaish. 2018. Antifungal Activity of Lactic Acid Bacteria Strains Isolated from Natural Honey against Pathogenic Candida Species. https://doi.org/10.5941/MYCO.2016.44.4.302 44:302–309. doi:10.5941/MYCO.2016.44.4.302.

Castellano, P., C. Belfiore, S. Fadda, and G. Vignolo. 2008. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. Meat Sci 79:483–499. doi:10.1016/J.MEATSCI.2007.10.009.

Castellano, P., C. González, F. Carduza, and G. Vignolo. 2010. Protective action of *Lactobacillus curvatus* CRL705 on vacuum-packaged raw beef. Effect on sensory and structural characteristics. Meat Sci 85:394–401. doi:10.1016/J.MEATSCI.2010.02.007.

Chen, Y., L. Yu, N. Qiao, Y. Xiao, F. Tian, J. Zhao, H. Zhang, W. Chen, and Q. Zhai. 2020. *Latilactobacillus curvatus*: A Candidate Probiotic with Excellent Fermentation Properties and Health Benefits. Foods 2020, Vol. 9, Page 1366 9:1366. doi:10.3390/FOODS9101366.

Cheong, E.Y.L., A. Sandhu, J. Jayabalan, T.T. Kieu Le, N.T. Nhiep, H.T. My Ho, J. Zwielehner, N. Bansal, and M.S. Turner. 2014. Isolation of lactic acid bacteria with antifungal activity against the common cheese spoilage mould *Penicillium commune*

and their potential as biopreservatives in cheese. Food Control 46:91–97. doi:10.1016/J.FOODCONT.2014.05.011.

Circle, S.J., L. Stone, and H. Walker. 1945. Acrolein Determination by Means of Tryptophane A Colorimetric Micromethod. Industrial & Engineering Chemistry Analytical Edition, 15:259–262.

Crowley, S., J. Mahony, and D. Van Sinderen. 2013a. Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. Trends Food Sci Technol 33:93–109. doi:10.1016/J.TIFS.2013.07.004.

Crowley, S., J. Mahony, and D. van Sinderen. 2013b. Broad-spectrum antifungal-producing lactic acid bacteria and their application in fruit models. Folia Microbiol (Praha) 58:291–299. doi:10.1007/S12223-012-0209-3/FIGURES/5.

Dalié, D.K.D., A.M. Deschamps, and F. Richard-Forget. 2010. Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. Food Control 21:370–380. doi:10.1016/J.FOODCONT.2009.07.011.

Delavenne, E., R. Ismail, A. Pawtowski, J. Mounier, G. Barbier, and G. Le Blay. 2013. Assessment of *lactobacilli* strains as yogurt bioprotective cultures. Food Control 30:206–213. doi:10.1016/J.FOODCONT.2012.06.043.

Dishisha, T., L.P. Pereyra, S.H. Pyo, R.A. Britton, and R. Hatti-Kaul. 2014. Flux analysis of the *Lactobacillus reuteri* propanediol-utilization pathway for production of 3-hydroxypropionaldehyde, 3-hydroxypropionic acid and 1,3-propanediol from glycerol. Microb Cell Fact 13:1–11. doi:10.1186/1475-2859-13-76/FIGURES/4.

Doleyres Beck S Vollenweider C Lacroix, Y.P. 2005. BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING Production of 3-hydroxypropionaldehyde using a two-step process with *Lactobacillus reuteri*. Appl Microbiol Biotechnol 68:467–474. doi:10.1007/s00253-005-1895-4.

El-Ziney, M.G., and J.M. Debevere. 1998. The Effect of Reuterin on *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Milk and Cottage Cheese. J Food Prot 61:1275–1280. doi:10.4315/0362-028X-61.10.1275.

Engels, C., C. Schwab, J. Zhang, M.J.A. Stevens, C. Bieri, M.O. Ebert, K. McNeill, S.J. Sturla, and C. Lacroix. 2016. Acrolein contributes strongly to antimicrobial and heterocyclic amine transformation activities of reuterin. Scientific Reports 2016 6:1 6:1–13. doi:10.1038/srep36246.

Filtenborg, O., J.C. Frisvad, and U. Thrane. 1996. Moulds in food spoilage. Int J Food Microbiol 33:85–102. doi:10.1016/0168-1605(96)01153-1.

Fleet, G.H. 1990. Yeasts in dairy products. Journal of Applied Bacteriology 68:199–211.

Fleet, G.H., and M.A. Mian. 1987. The occurrence and growth of yeasts in dairy products. Int J Food Microbiol 4:145–155. doi:10.1016/0168-1605(87)90021-3.

Gad, S.C. 2014. Propionic Acid. Encyclopedia of Toxicology: Third Edition 1105–1107. doi:10.1016/B978-0-12-386454-3.00914-3.

Gerez, C.L., M.J. Torres, G. Font de Valdez, and G. Rollán. 2013. Control of spoilage fungi by lactic acid bacteria. Biological Control 64:231–237. doi:10.1016/J.BIOCONTROL.2012.10.009.

van Gijtenbeek, L.A., Q. Singer, L.E. Steffensen, S. Neuens, H.S. Guldager, S. Bidstrup, T. Høgholm, M.G. Madsen, K. Glass, and S. Siedler. 2021. *Lacticaseibacillus rhamnosus* impedes growth of *listeria* spp. In cottage cheese through manganese limitation. Foods 10:1353. doi:10.3390/FOODS10061353/S1.

Gómez-Torres, N., M. Ávila, P. Gaya, and S. Garde. 2014. Prevention of late blowing defect by reuterin produced in cheese by a *Lactobacillus reuteri* adjunct. Food Microbiol 42:82–88. doi:10.1016/J.FM.2014.02.018.

Gopi, G.R., N. Ganesh, S. Pandiaraj, B. Sowmiya, R.G. Brajesh, and S. Ramalingam. 2015. A Study on Enhanced Expression of 3-Hydroxypropionic Acid Pathway Genes and Impact on Its Production in *Lactobacillus reuteri*. Food Technol Biotechnol 53:331–336. doi:10.17113/FTB.53.03.15.3976.

Hammes, W.P., A. Bantleon, and S. Min. 1990. Lactic acid bacteria in meat fermentation. FEMS Microbiol Rev 7:165–173. doi:10.1111/J.1574-6968.1990.TB04886.X.

Hammes, W.P., and C. Hertel. 1998. New developments in meat starter cultures. Meat Sci 49:S125–S138. doi:10.1016/S0309-1740(98)90043-2.

Heidari, Z., · Mohammad, F. Ghasemi, and · Leila Modiri. 2022. Antimicrobial activity of bacteriocin produced by a new *Latilactobacillus curvatus* sp.LAB-3H isolated from traditional yogurt 204:101. doi:10.1007/s00203-021-02641-8.

Hicks, S.J., and B.M. Lund. 1991. The survival of *Listeria monocytogenes* in cottage cheese. Journal of Applied Bacteriology 70:308–314. doi:10.1111/J.1365-2672.1991.TB02941.X.

Hohle, T.H., and M.R. O'Brian. 2009. The mntH gene encodes the major Mn(2+) transporter in *Bradyrhizobium japonicum* and is regulated by manganese via the Fur protein. Mol Microbiol 72:399–409. doi:10.1111/J.1365-2958.2009.06650.X.

Inglin, R.C., M.J.A. Stevens, L. Meile, C. Lacroix, and L. Meile. 2015. High-throughput screening assays for antibacterial and antifungal activities of *Lactobacillus* species. J Microbiol Methods 114:26–29. doi:10.1016/J.MIMET.2015.04.011.

Jung, J.Y., S.H. Lee, J.M. Kim, M.S. Park, J.W. Bae, Y. Hahn, E.L. Madsen, and C.O. Jeon. 2011. Metagenomic analysis of kimchi, a Traditional Korean fermented food. Appl Environ Microbiol 77:2264–2274. doi:10.1128/AEM.02157-10/SUPPL_FILE/AEM02157_10_SUPPL.DOC.

Karma, I.G.M. 2020. Determination and Measurement of Color Dissimilarity. International Journal of Engineering and Emerging Technology 5:67–71. doi:10.24843/IJEET.2020.v05.i01.p13.

Kask, S., K. Adamberg, A. Orłowski, F.K. Vogensen, P.L. Møller, Y. Ardö, and T. Paalme. 2003. Physiological properties of *Lactobacillus paracasei*, *L. danicus* and *L. curvatus* strains isolated from Estonian semi-hard cheese. Food Research International 36:1037–1046. doi:10.1016/J.FOODRES.2003.08.002.

Klaenhammer, T.R. 1988. Bacteriocins of lactic acid bacteria. Biochimie 70:337–349.

Kure, C.F., I. Skaar, and J. Brendehaug. 2004. Mould contamination in production of semi-hard cheese. Int J Food Microbiol 93:41–49. doi:10.1016/J.IJFOODMICRO.2003.10.005.

Ledenbach, L.H., and R.T. Marshall. 2009. Microbiological Spoilage of Dairy Products. Compendium of the Microbiological Spoilage of Foods and Beverages 41–67. doi:10.1007/978-1-4419-0826-1_2.

Leyva Salas, M., J. Mounier, F. Valence, M. Coton, A. Thierry, and E. Coton. 2017. Antifungal Microbial Agents for Food Biopreservation—A Review. Microorganisms 2017, Vol. 5, Page 37 5:37. doi:10.3390/MICROORGANISMS5030037.

Lüthi-Peng, Q., F.B. Dileme, and Z. Puhan. 2002a. Effect of glucose on glycerol bioconversion by *Lactobacillus reuteri*. Appl Microbiol Biotechnol 59:289–296. doi:10.1007/S00253-002-1002-Z/METRICS.

Lüthi-Peng, Q., S. Schärer, and Z. Puhan. 2002b. Production and stability of 3-hydroxypropionaldehyde in *Lactobacillus reuteri*. Appl Microbiol Biotechnol 60:73–80. doi:10.1007/S00253-002-1099-0/METRICS.

Makki, G.M., S.M. Kozak, K.G. Jencarelli, and S.D. Alcaine. 2020. Evaluation of the efficacy of commercial protective cultures against mold and yeast in queso fresco. J Dairy Sci 103:9946–9957. doi:10.3168/JDS.2020-18769.

Makki, G.M., S.M. Kozak, K.G. Jencarelli, and S.D. Alcaine. 2021. Evaluation of the efficacy of commercial protective cultures to inhibit mold and yeast in cottage cheese. J Dairy Sci 104:2709–2718. doi:10.3168/JDS.2020-19136.

Michel, E., C. Monfort, M. Deffrasnes, S. Guezenec, E. Lhomme, M. Barret, D. Sicard, X. Dousset, and B. Onno. 2016. Characterization of relative abundance of lactic acid bacteria species in French organic sourdough by cultural, qPCR and MiSeq high-throughput sequencing methods. Int J Food Microbiol 239:35–43. doi:10.1016/J.IJFOODMICRO.2016.07.034.

Mills, S., C. Stanton, C. Hill, and R.P. Ross. 2011. New Developments and Applications of Bacteriocins and Peptides in Foods. https://doi.org/10.1146/annurev-food-022510-133721 2:299–329. doi:10.1146/ANNUREV-FOOD-022510-133721.

Mobarak, E., R. Kasra-Kermanshahi, and E. Mobarak-Qamsari. 2015. Inhibition effect of Lactic acid bacteria against food born pathogen, *Listeria monocytogenes*. Review Article APPLIED FOOD BIOTECHNOLOGY 2:11–19. doi:10.22037/afb.v2i4.8894.

Moon, N.J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. Journal of Applied Bacteriology 55:453–460. doi:10.1111/J.1365-2672.1983.TB01685.X.

Mroueh, M., D. Issa, J. Khawand, B. Haraty, A. Malek, Z. Kassaify, and I. Toufeili. 2008. Levels of benzoic and sorbic acid preservatives in commercially produced yoghurt in Lebanon.

Muriana, P.M., and J.B. Luchansky. 1993. Biochemical Methods for Purification of Bacteriocins. Bacteriocins of Lactic Acid Bacteria 41–61. doi:10.1016/B978-0-12-355510-6.50011-7.

Nakano, K., A. Shiroma, H. Tamotsu, S. Ohki, M. Shimoji, N. Ashimine, M. Shinzato, M. Minami, T. Nakanishi, K. Teruya, K. Satou, C. Suzuki, H. Kimoto-Nira, M. Kobayashi, K. Mizumachi, R. Aoki, S. Miyata, K. Yamamoto, Y. Ohtake, T. Eguchi-Ogawa, N. Moriy, T. Hagi, M. Nomura, and T. Hirano. 2016. First Complete Genome Sequence of the Skin-Improving *Lactobacillus curvatus* Strain FBA2, Isolated from Fermented Vegetables, Determined by PacBio Single-Molecule Real-Time Technology. Genome Announc 4:884–900. doi:10.1128/GENOMEA.00884-16.

Oberg, T.S., D.J. McMahon, M.D. Culumber, O. McAuliffe, and C.J. Oberg. 2022. Invited review: Review of taxonomic changes in dairy-related *lactobacilli*. J Dairy Sci 105:2750–2770. doi:10.3168/JDS.2021-21138.

Ortiz-Rivera, Y., R. Sánchez-Vega, N. Gutiérrez-Méndez, J. León-Félix, C. Acosta-Muñiz, and D.R. Sepulveda. 2017. Production of reuterin in a fermented milk product by *Lactobacillus reuteri*: Inhibition of pathogens, spoilage microorganisms, and lactic acid bacteria. J Dairy Sci 100:4258–4268. doi:10.3168/JDS.2016-11534.

Paul Ross, R., S. Morgan, and C. Hill. 2002. Preservation and fermentation: past, present and future. Int J Food Microbiol 79:3–16. doi:10.1016/S0168-1605(02)00174-5.

Rolf, D., T.A. Bobik, and G. Gottschalk. 1998. Biochemistry of coenzyme B12-dependent glycerol and diol dehydratases and organization of the encoding genes. FEMS Microbiol Rev 22:553–566. doi:10.1111/J.1574-6976.1998.TB00387.X.

Rouse, S., D. Harnett, A. Vaughan, and D. Van Sinderen. Lactic acid bacteria with potential to eliminate fungal spoilage in foods. doi:10.1111/j.1365-2672.2007.03619.x.

Russo, P., M.P. Arena, D. Fiocco, V. Capozzi, D. Drider, and G. Spano. 2017. *Lactobacillus plantarum* with broad antifungal activity: A promising approach to increase safety and shelf-life of cereal-based products. Int J Food Microbiol 247:48–54. doi:10.1016/J.IJFOODMICRO.2016.04.027.

Ryser, E.T., E.H. Marth, and M.P. Doyle. 1985. Survival of *Listeria monocytogenes* During Manufacture and Storage of Cottage Cheese. J Food Prot 48:746–750. doi:10.4315/0362-028X-48.9.746.

Salas, M.L., A. Thierry, M. Lemaître, G. Garric, M. Harel-Oger, M. Chatel, S. Lê, J. Mounier, F. Valence, and E. Coton. 2018. Antifungal activity of lactic acid bacteria combinations in dairy mimicking models and their potential as bioprotective cultures in pilot scale applications. Front Microbiol 9:1787. doi:10.3389/FMICB.2018.01787/BIBTEX.

Schaefer, L., T.A. Auchtung, K.E. Hermans, D. Whitehead, B. Borhan, and R.A. Britton. 2010. The antimicrobial compound reuterin (3-hydroxypropionaldehyde) induces oxidative stress via interaction with thiol groups. Microbiology (N Y) 156:1589–1599. doi:10.1099/MIC.0.035642-0/CITE/REFWORKS.

Sedaghat, H., M.H. Eskandari, M. Moosavi-Nasab, and S.S. Shekarforoush. 2016. Application of non-starter lactic acid bacteria as biopreservative agents to control fungal spoilage of fresh cheese. Int Dairy J 56:87–91. doi:10.1016/J.IDAIRYJ.2016.01.006.

Shi, C., and M. Maktabdar. 2022. Lactic Acid Bacteria as Biopreservation Against Spoilage Molds in Dairy Products – A Review. Front Microbiol 12:819684. doi:10.3389/FMICB.2021.819684/BIBTEX.

Siedler, S., R. Balti, and A.R. Neves. 2019. Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. Curr Opin Biotechnol 56:138–146. doi:10.1016/J.COPBIO.2018.11.015.

Siedler, S., M.H. Rau, S. Bidstrup, J.M. Vento, S.D. Aunsbjerg, E.F. Bosma, L.M. Mcnair, C.L. Beisel, and A.R. Neves. 2020. Competitive exclusion is a major

bioprotective mechanism of *lactobacilli* against fungal spoilage in fermented milk products. Appl Environ Microbiol 86. doi:10.1128/AEM.02312-19/SUPPL_FILE/AEM.02312-19-S0001.PDF.

Singh, S., and R. Shalini. 2016. Effect of Hurdle Technology in Food Preservation: A Review. http://dx.doi.org/10.1080/10408398.2012.761594 56:641–649. doi:10.1080/10408398.2012.761594.

Sriramulu, D.D., M. Liang, D. Hernandez-Romero, E. Raux-Deery, H. Lünsdorf, J.B. Parsons, M.J. Warren, and M.B. Prentice. 2008. *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation. J Bacteriol 190:4559–4567. doi:10.1128/JB.01535-07.

Suriyarachchi, V.R., and G.H. Fleet. 1981. Occurrence and Growth of Yeasts in Yogurts. Appl Environ Microbiol 42:574–579. doi:10.1128/AEM.42.4.574-579.1981.

Talarico, T.L., I.A. Casas, T.C. Chung, and W.J. Dobrogosz. 1988. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. Antimicrob Agents Chemother 32:1854–1858. doi:10.1128/AAC.32.12.1854.

Talarico, T.L., and W.J. Dobrogosz. 1989. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. Antimicrob Agents Chemother 33:674–679. doi:10.1128/AAC.33.5.674.

Terán, L.C., G. Coeuret, R. Raya, M. Zagorec, M.C. Champomier-Vergès, and S. Chaillou. 2018. Phylogenomic Analysis of *Lactobacillus curvatus* Reveals Two Lineages Distinguished by Genes for Fermenting Plant-Derived Carbohydrates. Genome Biol Evol 10:1516–1525. doi:10.1093/GBE/EVY106.

Vimont, A., B. Fernandez, G. Ahmed, H.P. Fortin, and I. Fliss. 2019. Quantitative antifungal activity of reuterin against food isolates of yeasts and moulds and its potential application in yogurt. Int J Food Microbiol 289:182–188. doi:10.1016/J.IJFOODMICRO.2018.09.005.

Vogelxy, R.F., M. Lohmann, M. Nguyen, A.N. Weller, and W.P. Hammes. 1993. Molecular characterization of *Lactobacillus curvatus* and *Lact. sake* isolated from sauerkraut and their application in sausage fermentations. Journal of Applied Bacteriology 74:295–300. doi:10.1111/J.1365-2672.1993.TB03029.X.

Vollenweider, S., S. Evers, K. Zurbriggen, # And, and C. Lacroix. 2010. Unraveling the Hydroxypropionaldehyde (HPA) System: An Active Antimicrobial Agent against Human Pathogens. J. Agric. Food Chem 58:10315–10322. doi:10.1021/jf1010897.

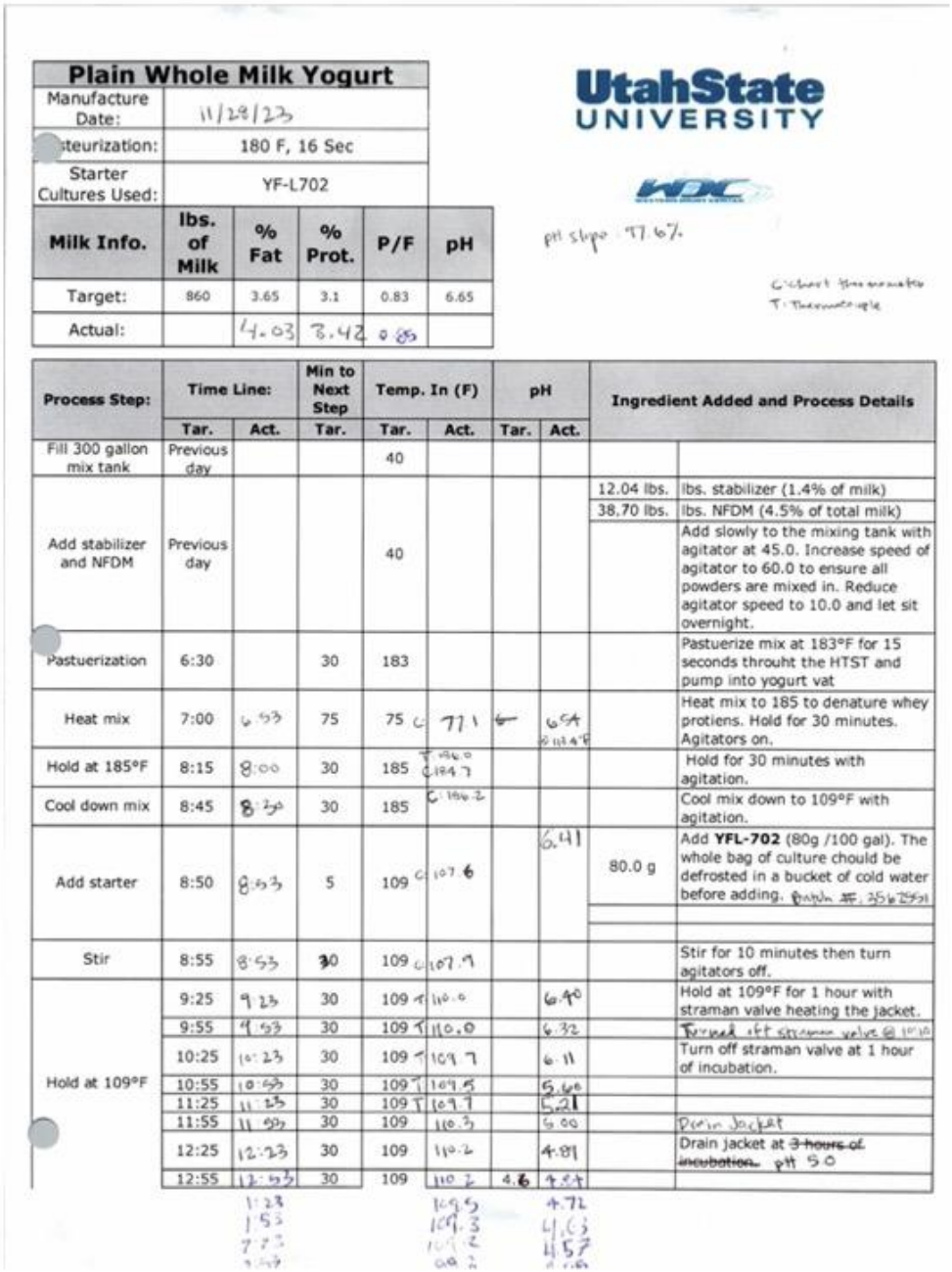
Vollenweider, S., and C. Lacroix. 2004. 3-Hydroxypropionaldehyde: Applications and perspectives of biotechnological production. Appl Microbiol Biotechnol 64:16–27. doi:10.1007/S00253-003-1497-Y/FIGURES/5.

Yang, J., P. Rainville, K. Liu, and B. Pointer. 2021. Determination of lactose in low-lactose and lactose-free dairy products using LC-MS. Journal of Food Composition and Analysis 100:103824. doi:10.1016/J.JFCA.2021.103824.

Yun, J.E., and D.G. Lee. 2016. A novel fungal killing mechanism of propionic acid. FEMS Yeast Res 16:89. doi:10.1093/FEMSYR/FOW089.

APPENDICES

Appendix A: Yogurt make sheets for all treatments



Plain Whole Milk Yogurt

UtahState UNIVERSITY

Manufacture Date:	11/29/23
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702

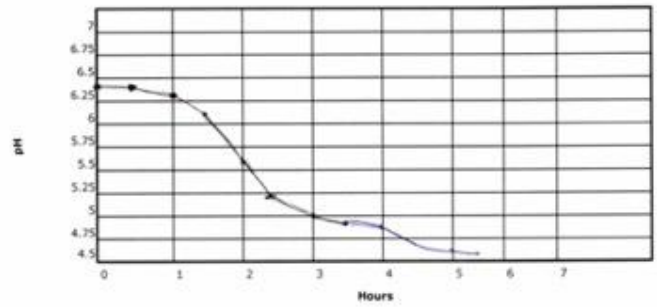
Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	860	3.65	3.1	0.83	6.65
Actual:		4.03	3.42	0.85	

pH slope: 77.67.

C: Chart thermometer
T: Thermocouple

Process Step:	Time Line: Tar.	Time Line: Act.	Min to Next Step Tar.	Temp. In (F) Tar.	Temp. In (F) Act.	pH Tar.	pH Act.	Ingredient Added and Process Details	
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				12.04 lbs.	lbs. stabilizer (1.4% of milk)
								38.70 lbs.	lbs. NFDM (4.5% of total milk)
									Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	6:53	75	75 C	77.1	6	6.54		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on.
Hold at 185°F	8:15	8:00	30	185	T: 186.0 C: 184.7				Hold for 30 minutes with agitation.
Cool down mix	8:45	8:30	30	185	C: 186.2				Cool mix down to 109°F with agitation.
Add starter	8:50	8:53	5	109 C	107.6		6.41	80.0 g	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding. Batch #: 25625931
Stir	8:55	8:53	30	109 C	107.9				Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:25	9:23	30	109 T	110.0		6.40		Hold at 109°F for 1 hour with straman valve heating the jacket.
	9:55	9:53	30	109 T	110.0		6.32		Turned off straman valve @ 10:10
	10:25	10:23	30	109 T	109.7		6.11		Turn off straman valve at 1 hour of incubation.
	10:55	10:53	30	109 T	109.5		5.60		
	11:25	11:23	30	109 T	109.7		5.21		
	11:55	11:53	30	109	110.3		5.00		Drain Jacket
	12:25	12:23	30	109	110.2		4.81		Drain jacket at ~~3 hours of incubation.~~ pH 5.0
	12:55	12:53	30	109	110.2	4.6	4.84		
		1:23			109.5		4.72		
		1:53			109.3		4.63		
		2:23			109.2		4.57		
		2:53			110.2		4.65		

Cool down mix	1:55	2:23	60	~~65~~ 109	169.2	4.6	4.57		Connect chill water lines to the jacket and cool the mix down to 65°F with agitation.
Hold in tank overnight	2:30			40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Pump yogurt through the smoothing valve	Next day 9:00	3:44	30	40-65	64.9	4.6	4.54		Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Flavor the mix	-		40	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		10	40-65			4.59		Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	12:00		5	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.1. Trial 1 Control yogurt make

Plain Whole Milk Yogurt					
Manufacture Date:	11/21/23				
Pasteurization:	180 F, 16 Sec				
Starter Cultures Used:	YF-L702 + WSU-1				
Milk Info.	**lbs. of Milk**	**% Fat**	**% Prot.**	**P/F**	**pH**
Target:	860 450	3.65	3.1	0.83	6.65
Actual:					

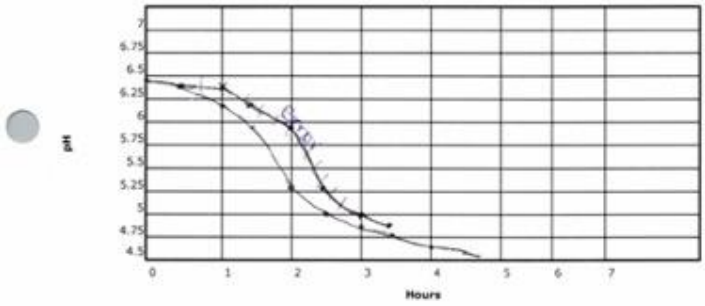
UtahState UNIVERSITY

165 gallon in Mixing tank 12-65 Stabilizer 40.65 NFDM

50 gallon ORing 6 5

Process Step:	Time Line:		Min to Next Step	Temp. In (F)		pH		Ingredient Added and Process Details	
	Tar.	Act.	Tar.	Tar.	Act.	Tar.	Act.		
Fill 300 gallon mix tank	Previous day			40			6.6 gallon sweet		
Add stabilizer and NFDM	Previous day			40			6.01 6.35	12.04 lbs. 38.70 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk) Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:07	1:15	75	534		6.69		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on.
Hold at 185°F	8:15	8:01	0:30	185	185.7		~~6.45~~		Hold for 30 minutes with agitation.
Cool down mix	8:45	8:31	0:30	185	184.3				Cool mix down to 109°F with agitation.
Add starter	9:15	8:57	0:00	109	107.2		6.45	80.0 g 80 ml 189 ml	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding. ~~Batch # 356251~~ WSU-1
Stir	9:15	8:57	0:30	109	107.2				Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:45	9:27	0:30	109	110.1		6.35		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	9:57	0:30	109	107.9		6.20		
	10:45	10:27	0:30	109	110.3		5.90		Turn off straman valve at 1 hour of incubation. 10:16 turned off
	11:15	10:57	0:30	109	110.3		5.30		
	11:45	11:27	0:30	109	111.1		4.99		Jacket Drained
	12:15	11:57	0:30	109	111.4		4.87		
	12:45	12:27	0:30	109	111.4		4.76		Drain jacket at ~~3 hours of incubation~~. pH 5.0
	13:15	12:57	0:30	109	110.9	4.6	4.65		
		1:27			110.6		4.61		
		1:37			110.3		4.57		

Cool down mix	13:45	1:37	1:20	65	65.3		4.57		Connect chill water lines to the jacket and cool the mix down to 65°F with agitation.
Pump yogurt through the smoothing valve	15:05	3:06	0:30	40-65	64.6	4.6	4.57		Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65			4.63		Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.2. Trial 1 WSU-1 yogurt

Plain Whole Milk Yogurt	
Manufacture Date:	11/30/23
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702 + WSU-1 + 200 mM glycerol

UtahState UNIVERSITY

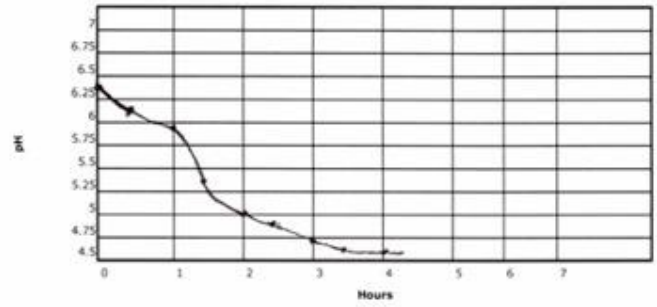
pH Store 96.9%.

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	860	3.65	3.1	0.83	6.65
Actual:					

Process Step:	Time Line:		Min to Next Step	Temp. In (F)		pH		Ingredient Added and Process Details	
	Tar.	Act.	Tar.	Tar.	Act.	Tar.	Act.		
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40			6.62 19.85	12.04 lbs. 38.70 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk) Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:00	1:15	75	79.0		6.54		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on.
Hold at 185°F	8:15	7:53	0:30	185	185.9				Hold for 30 minutes with agitation.
Cool down mix	8:45	8:23	0:30	185	182.5				Cool mix down to 109°F with agitation.
Add starter	9:15	8:46	0:00	109	107		823	80.0 g 189 mL	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding. WSU-1
Stir	9:15	8:46	0:30	109	107.0		6.39		Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:45	9:16	0:30	109	109.4		6.36		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	9:46	0:30	109	109.6		6.15		
	10:45	10:16	0:30	109	109.3		5.90		Turn off straman valve at 1 hour of incubation.
	11:15	10:46	0:30	109	108.9		5.36		
	11:45	11:16	0:30	109	109.4		5.04		Jacket drained
	12:15	11:46	0:30	109	109.7		4.87		
	12:45	12:16	0:30	109	109.3		4.72		Drain jacket at pH 5.0
	13:15	12:46	0:30	109	109.2	4.6	4.65		
		1:16			108.7		4.60		
		1:30			105.1		4.56		

all are equivalent

Break the mix and add glycerine	13:45	1:30	1:20	65	105.1 3.4kg 57	4.5		2.77L 5.48kg 0.73 gallons	Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05	2:24	0:30	40-65	56.9	4.6	4.54		Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65			4.63		Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.3. Trial 1 WSU-1 + glycerol yogurt make

Plain Whole Milk Yogurt	
Manufacture Date:	1-9-24
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	~~Batch # 3562351~~ YF-L702 + WSU-1

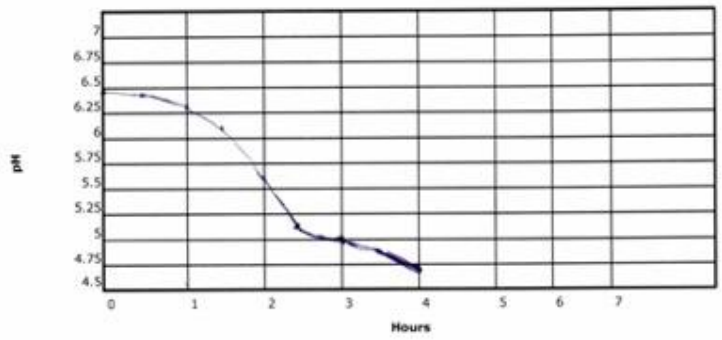
Pre Slope: 97.1%.

60gal

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	~~860~~ 430	3.65	3.1	0.83	6.65
Actual:					

Process Step:	Time Line:		Min to Next Step	Temp. In (F)		pH		Ingredient Added and Process Details	
	Tar.	Act.	Tar.	Tar.	Act.	Tar.	Act.		
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				12.04 lbs. 38.70 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk)
									Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:00	1:15	75	74.0				Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on. Straman @ 220°F
Hold at 185°F	8:15	7:59	0:30	185	~~185~~ 185.1				Hold for 30 minutes with agitation. Straman @ 200°F
Cool down mix	8:45	9:30	0:30	185	185.1				Cool mix down to 109°F with agitation.
Add starter	9:15	8:58	0:00	109	109.6		6.47	40.0g ~~80.0g~~	Add **YFL-702** (~~80g~~ 89 /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding. 189 mL WSU-1
Stir	9:15	8:58	0:~~30~~ 10	109	109.6				Stir for 10 minutes then turn agitators off. Straman @ 125°F
Hold at 109°F	9:45	9:08 ~~9:38~~	0:30	109	104.7 110.2		6.39		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	~~9:58~~	0:30	109	109.8		6.29		Straman valve turned off
	10:45	10:28	0:30	109	109.6		6:10		Turn off straman valve at 1 hour of incubation.
	11:15	~~10:58~~	0:30	109	109.3		5.61		
	11:45	~~11:28~~	0:30	109	109.5		5.19		
	12:15	~~11:58~~	0:30	109	110.0		4.99		Jacket drained
	12:45	~~12:28~~	0:30	109	109.8		~~4.84~~		Drain jacket at pH 5.0
	13:15	~~12:58~~	0:30	109	110.3	4.6	4.50		
		1:28			110.3		4.61		
		1:57			110.0		4.61		
		2:17							

Break the mix and add glycerine	13:45	2:12	1:20	65	49.5		4.55		Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05		0:30	40-65		4.6	4.57		Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65			4.61		Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.4. Trial 2 WSU-1 yogurt make

Plain Whole Milk Yogurt

UtahState UNIVERSITY

YFL702 Batch # 3717561 Produced 1.12.2023 Exp 1.12.2025

WDC

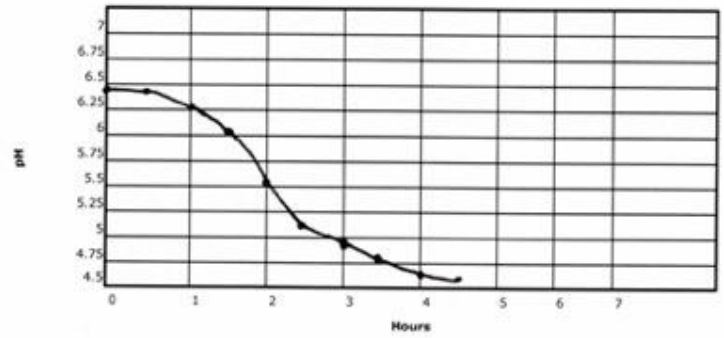
Manufacture Date:	1/12/24
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702 + LSU-1 1g glycerol 50gal

24 slope : 98.2%

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	~~850~~ 930	3.65	3.1	0.83	6.65
Actual:					

Process Step:	Time Line:		Min to Next Step	Temp. In (F)		pH		Ingredient Added and Process Details	
	Tar.	Act.	Tar.	Tar.	Act.	Tar.	Act.		
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				12.04 lbs. 38.70 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk) Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30	6:30	0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	~~8:02~~	1:15	75	82.6				Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on. Straman @ 225F
Hold at 185°F	8:15	7:47	0:30	185	184.9				Hold for 30 minutes with agitation. Straman @ 215°F
Cool down mix	8:45	8:17	0:30	185	184.2				Cool mix down to 109°F with agitation.
Add starter	9:15	8:40	0:00	109	111.3		6.40	~~40.0g~~ ~~80.0~~ g 41.0g	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding. 189mWSU-1
Stir	9:15	8:40	0:30	109					Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:45	9:10	0:30	109	110.3		6.38		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	9:40	0:30	109	109.6		6.27		
	10:45	10:10	0:30	109	109.5		6.04		Turn off straman valve at 1 hour of incubation. 9:40
	11:15	10:40	0:30	109	109.2		5.57		
	11:45	11:10	0:30	109	109.5		6.15		
	12:15	11:40	0:30	109	109.8		4.92		Jacket drained
	12:45	12:10	0:30	109	109.7		4.77		Drain jacket at pH 5.0
	13:15	12:40	0:30	109	110.0	4.6	4.66		
		1:10			110.2		4.60		
		1:40			110.5		4.54		—> Brose

Break the mix and add glycerine	13:45	1:40	1:20	65		4.54		3.48 KG	Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05	2:30	0:30	40-65		4.6			Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65		4.59			Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.5. Trial 2 WSU-1 + glycerol yogurt make

Plain Whole Milk Yogurt

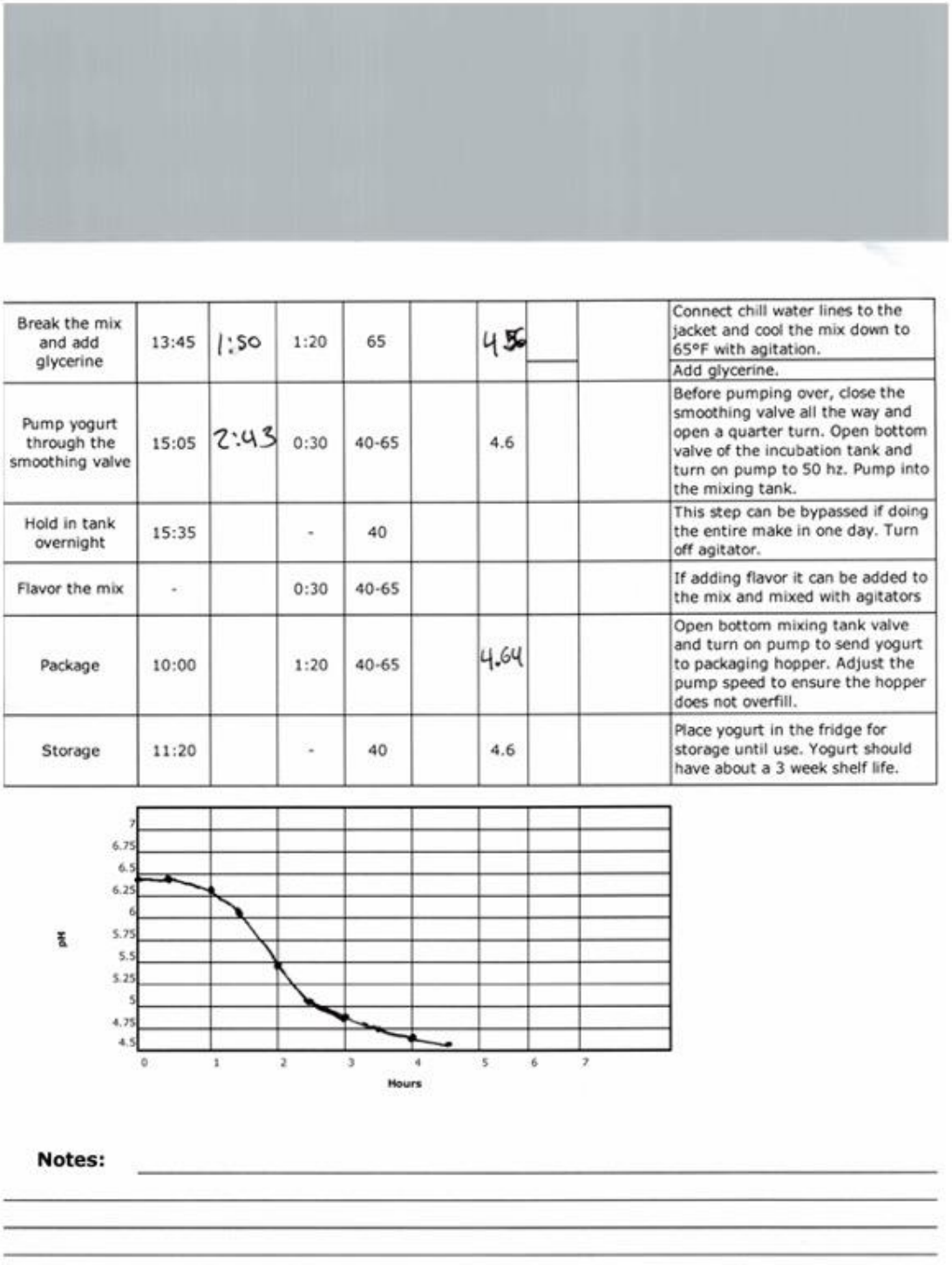
UtahState UNIVERSITY

Manufacture Date:	1/11/24
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702 Batch # 3717561

PH SLOPE 96.0%

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	860	3.65	3.1	0.83	6.65
Actual:					

Process Step:	Time Line: Tar.	Time Line: Act.	Min to Next Step	Temp. In (F) Tar.	Temp. In (F) Act.	pH Tar.	pH Act.	Ingredient Added and Process Details	
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				12.04 lbs.	lbs. stabilizer (1.4% of milk)
								38.70 lbs.	lbs. NFDM (4.5% of total milk)
									Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:00	1:15	75	72.1		6.61		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on. Straman @ 205°F
Hold at 185°F	8:15	8:08	0:30	185	185.0				Hold for 30 minutes with agitation. Straman @ 205°F
Cool down mix	8:45	8:38	0:30	185	185.8				Cool mix down to 109°F with agitation.
Add starter	9:15	8:58	0:00	109	109.9			80.0 g 82.	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding.
Stir	9:15	8:58	0:30	109	109.9		6.44		Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:45	9:28	0:30	109	110.8		6.40		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	9:58	0:30	109	110.4		6.32		
	10:45	10:28	0:30	109	110.3		6.05		Turn off straman valve at 1 hour of incubation.
	11:15	10:58	0:30	109	110.0		5.48		
	11:45	11:28	0:30	109	110.4		5.09		Jacket drained
	12:15	11:58	0:30	109	110.9		4.87		
	12:45	12:28	0:30	109	110.2		4.75		Drain jacket at pH 5.0
	13:15	12:58	0:30	109	111.1	4.6	4.66		
		1:28			110.5		4.60		
		1:50			110.5		4.56		Break



Break the mix and add glycerine	13:45	1:50	1:20	65		4.56			Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05	2:43	0:30	40-65		4.6			Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65		4.64			Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.

Notes:

Figure A.6. Trial 2 Control yogurt

Plain Whole Milk Yogurt

UtahState UNIVERSITY

WDC

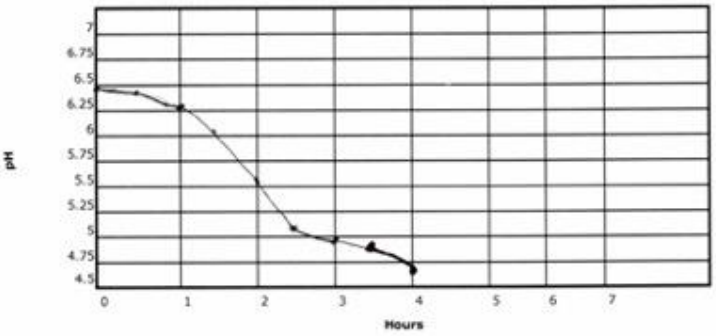
Manufacture Date:	2-13-24
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702 Batch # 371761

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	860	3.65	3.1	0.83	6.65
Actual:		4.24	3.43	0.81	6.58

pH slope 97.6%

Process Step:	Time Line: Tar.	Time Line: Act.	Min to Next Step Tar.	Temp. In (F) Tar.	Temp. In (F) Act.	pH Tar.	pH Act.	Ingredient Added and Process Details	
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				12.04 lbs. 38.70 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk) Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:00	1:15	75			6.98		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on.
Hold at 185°F	8:15	8:16	0:30	185	185.2				Hold for 30 minutes with agitation.
Cool down mix	8:45	8:46	0:30	185	186.1				Cool mix down to 109°F with agitation.
Add starter	9:15	9:06	0:00	109	109.8		6.98	80.0 g 80.0	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding.
Stir	9:15	9:16	0:30	109					Stir for 10 minutes then turn agitators off. Agitators off @ 9:16
Hold at 109°F	9:45	9:34	0:30	109	111.0		6.95		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	10:06	0:30	109	110.9		6.29		Straman off 10:12
	10:45	10:36	0:30	109	110.7		6.09	6.09	Turn off straman valve at 1 hour of incubation.
	11:15	11:06	0:30	109	110.8		5.56		
	11:45	11:36	0:30	109	110.2		5.17		
	12:15	12:06	0:30	109	110.8		4.97		
	12:45	12:36	0:30	109	111.3		4.82		Drain jacket at pH 5.0
	13:15	1:06	0:30	109	110.8	4.6	4.72		
		1:36			110.7		4.67		
		2:06			110.7		4.58		

Break the mix and add glycerine	13:45	2:13	1:20	65		4.58			Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05	3:17	0:30	40-65	64.8	4.6 4.61			Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40		4.60			This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65					Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.7. Trial 3 Control yogurt make

Plain Whole Milk Yogurt

UtahState UNIVERSITY

Manufacture Date:	2-14-24
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702 + WSU + tylyceva

YF-L707 Batch 371561

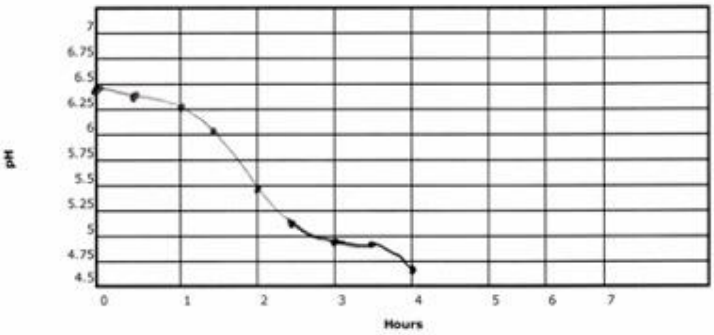
PH slope 98.0%.

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	430	3.65	3.1	0.83	6.65
Actual:					

Process Step:	Time Line: Tar.	Time Line: Act.	Min to Next Step Tar.	Temp. In (F) Tar.	Temp. In (F) Act.	pH Tar.	pH Act.	Ingredient Added and Process Details	
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				6.02 lbs. 19.35 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk) Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:01	1:15	75	84.5		6.65		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on.
Hold at 185°F	8:15	7:57	0:30	185	185.3				Hold for 30 minutes with agitation.
Cool down mix	8:45	8:27	0:30	185	186.3				Cool mix down to 109°F with agitation.
Add starter	9:15	8:47	0:00	109	104.5		6.42	40.0 g	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding.
Stir	9:15	8:57	0:30	109					Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:45	9:17	0:30	109	110.0		6.39		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	9:47	0:30	109	110.1		6.28		
	10:45	10:17	0:30	109	104.8		6.02		Turn off straman valve at 1 hour of incubation.
	11:15	10:47	0:30	109	109.7		5.47		
	11:45	10:17	0:30	109	109.3		5.10		
	12:15	10:47	0:30	109	109.9		4.98	Jacket Drained	
	12:45	12:17	0:30	109	110.3		4.86		Drain jacket at pH 5.0
	13:15	12:47	0:30	109	110.8	4.6	4.66		
		1:17			(10.)		4.62		

* Valve on the bottom was accidentally opened during

Break the mix and add glycerine	13:45	1:34	1:20	65		4.58		3.48 kg	Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05	2:15	0:30	40-65	60'	4.6			Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40					This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65		4.63			Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.8. Trial 3 WSU-1 + glycerol yogurt

Plain Whole Milk Yogurt	
Manufacture Date:	3/13/24
Pasteurization:	180 F, 16 Sec
Starter Cultures Used:	YF-L702 + WSU-1

UtahState UNIVERSITY

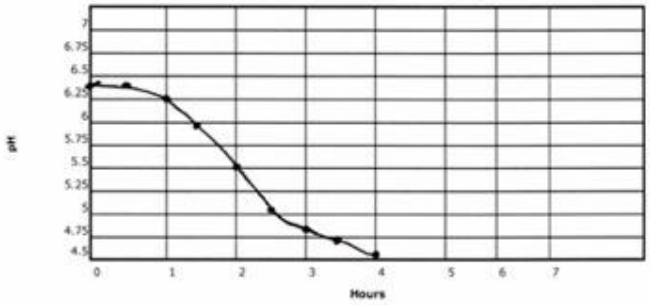
WDC

PH Slope 95.6%

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	430	3.65	3.1	0.83	6.65
Actual:					

Process Step:	Time Line:		Min to Next Step	Temp. In (F)		pH		Ingredient Added and Process Details	
	Tar.	Act.	Tar.	Tar.	Act.	Tar.	Act.		
Fill 300 gallon mix tank	Previous day			40					
Add stabilizer and NFDM	Previous day			40				6.02 lbs. 19.35 lbs.	lbs. stabilizer (1.4% of milk) lbs. NFDM (4.5% of total milk) Add slowly to the mixing tank with agitator at 45.0. Increase speed of agitator to 60.0 to ensure all powders are mixed in. Reduce agitator speed to 10.0 and let sit overnight.
Pastuerization	6:30		0:30	183					Pastuerize mix at 183°F for 15 seconds throuht the HTST and pump into yogurt vat
Heat mix	7:00	7:07	1:15	75	73.7		6.59		Heat mix to 185 to denature whey protiens. Hold for 30 minutes. Agitators on.
Hold at 185°F	8:15	8:53	0:30	185	185.9				Hold for 30 minutes with agitation.
Cool down mix	8:45	9:22	0:30	185	109.9				Cool mix down to 109°F with agitation.
Add starter	9:15	9:40	0:00	109	111.1		6.39	40.0 g 1.3-02	Add **YFL-702** (80g /100 gal). The whole bag of culture chould be defrosted in a bucket of cold water before adding. 189 mL WSU-1 Culture
Stir	9:15	9:40	0:30	109					Stir for 10 minutes then turn agitators off.
Hold at 109°F	9:45	10:00	0:30	109	108.4		6.39		Hold at 109°F for 1 hour with straman valve heating the jacket.
	10:15	10:40	0:30	109	109.1		6.26		
	10:45	11:10	0:30	109	108.9		5.96		Turn off straman valve at 1 hour of incubation.
	11:15	11:40	0:30	109	109.0		5.53		
	11:45	12:10	0:30	109	109.1		5.09		
	12:15	12:40	0:30	109	109.3		4.87		
	12:45	1:10	0:30	109	109.2		4.74		Drain jacket at pH 5.0
	13:15	1:40	0:30	109	110.0	4.6	4.66		
		2:10			110.4		4.54		chill on

Break the mix and add glycerine	13:45	2:10	1:20	65		4.59			Connect chill water lines to the jacket and cool the mix down to 65°F with agitation. Add glycerine.
Pump yogurt through the smoothing valve	15:05		0:30	40-65		4.6			Before pumping over, close the smoothing valve all the way and open a quarter turn. Open bottom valve of the incubation tank and turn on pump to 50 hz. Pump into the mixing tank.
Hold in tank overnight	15:35		-	40			4.73		This step can be bypassed if doing the entire make in one day. Turn off agitator.
Flavor the mix	-		0:30	40-65					If adding flavor it can be added to the mix and mixed with agitators
Package	10:00		1:20	40-65					Open bottom mixing tank valve and turn on pump to send yogurt to packaging hopper. Adjust the pump speed to ensure the hopper does not overfill.
Storage	11:20		-	40		4.6			Place yogurt in the fridge for storage until use. Yogurt should have about a 3 week shelf life.



Notes:

Figure A.9. Trial 3 WSU-1 yogurt

Appendix B: Mold Images

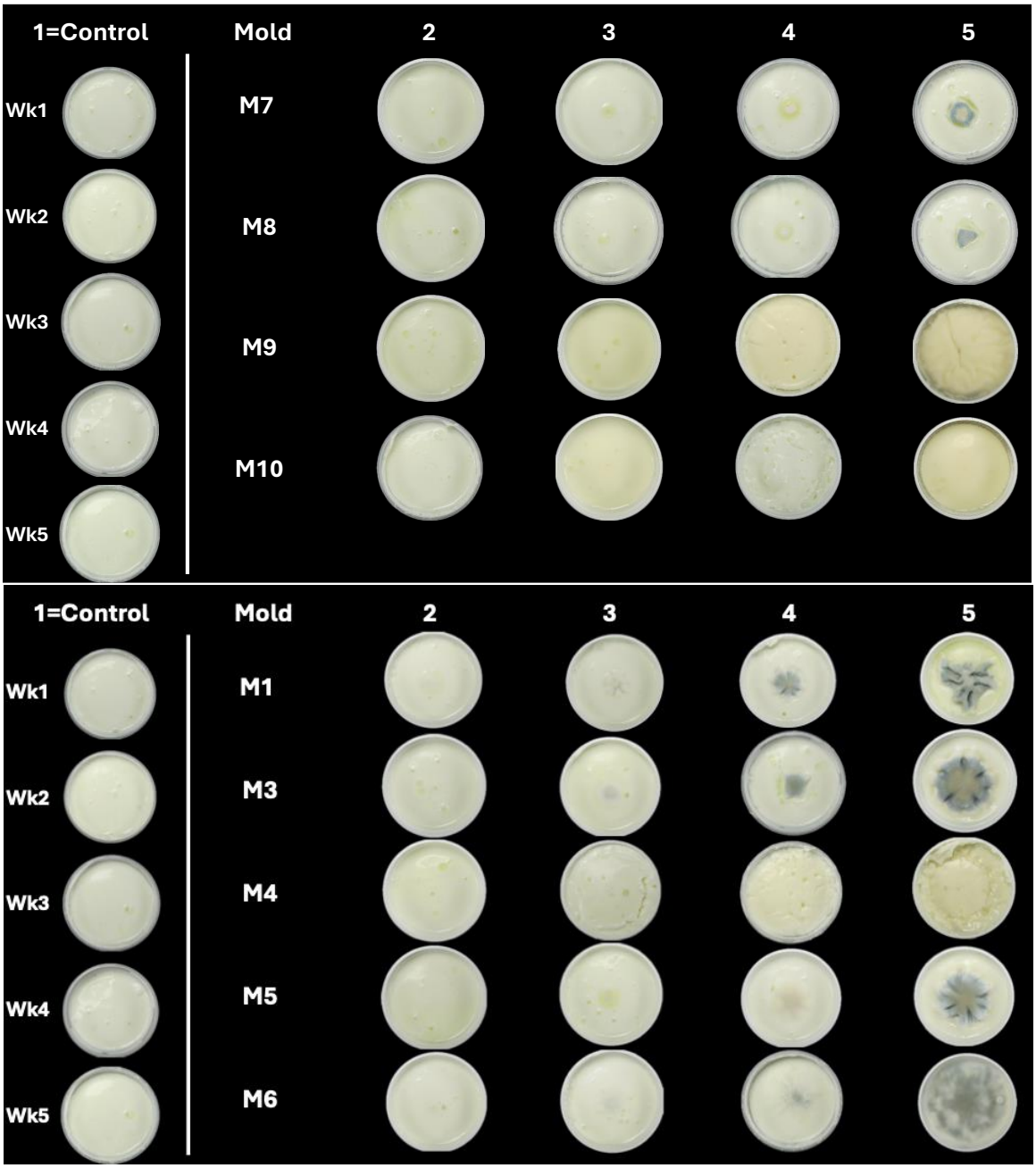


Figure B.1. Mold visual classification ranking reference

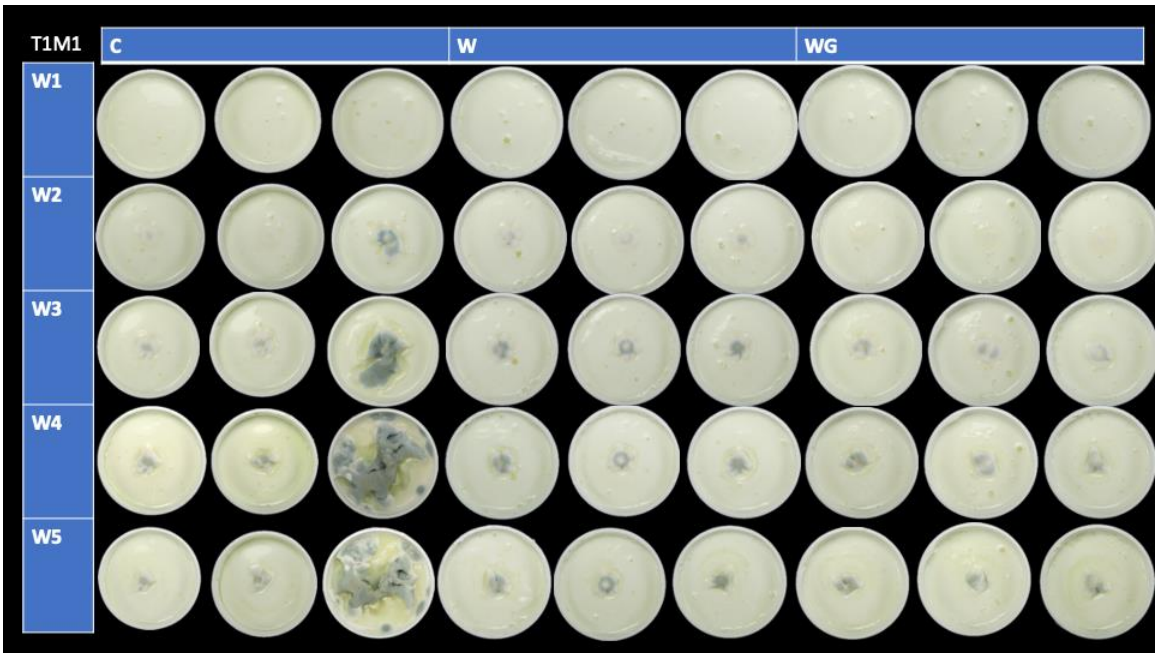


Figure B.2. First biological replicate of images of yogurt cups inoculated with *Penicillium commune.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

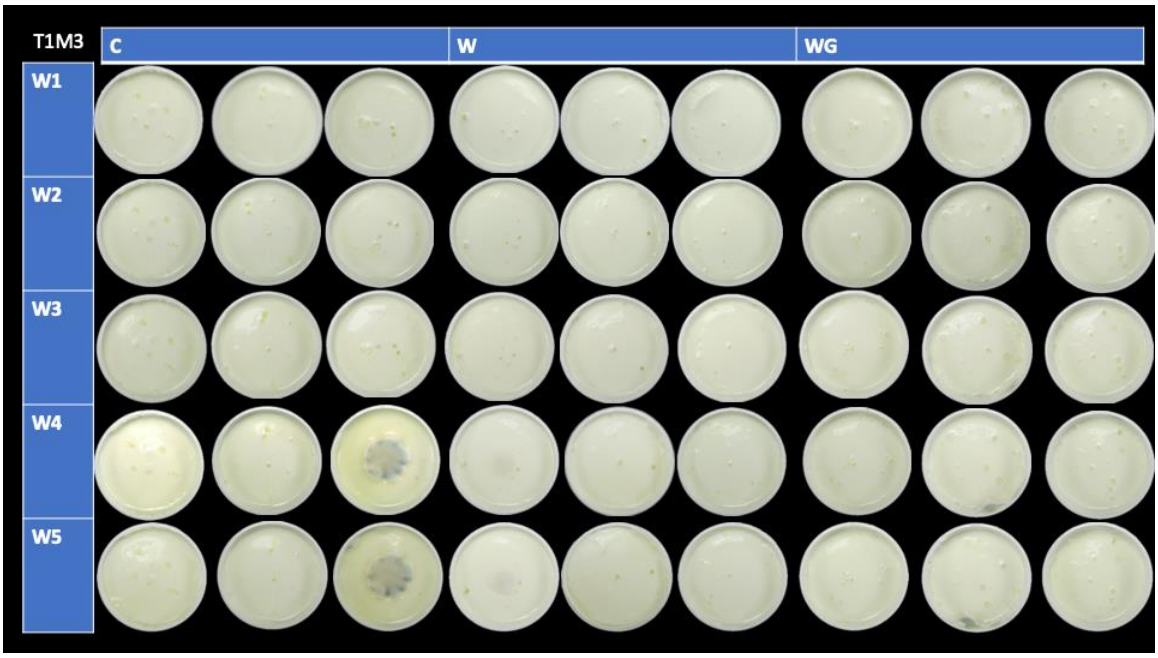


Figure B.3. First biological replicate of images of yogurt cups inoculated with *Penicillium citrinum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

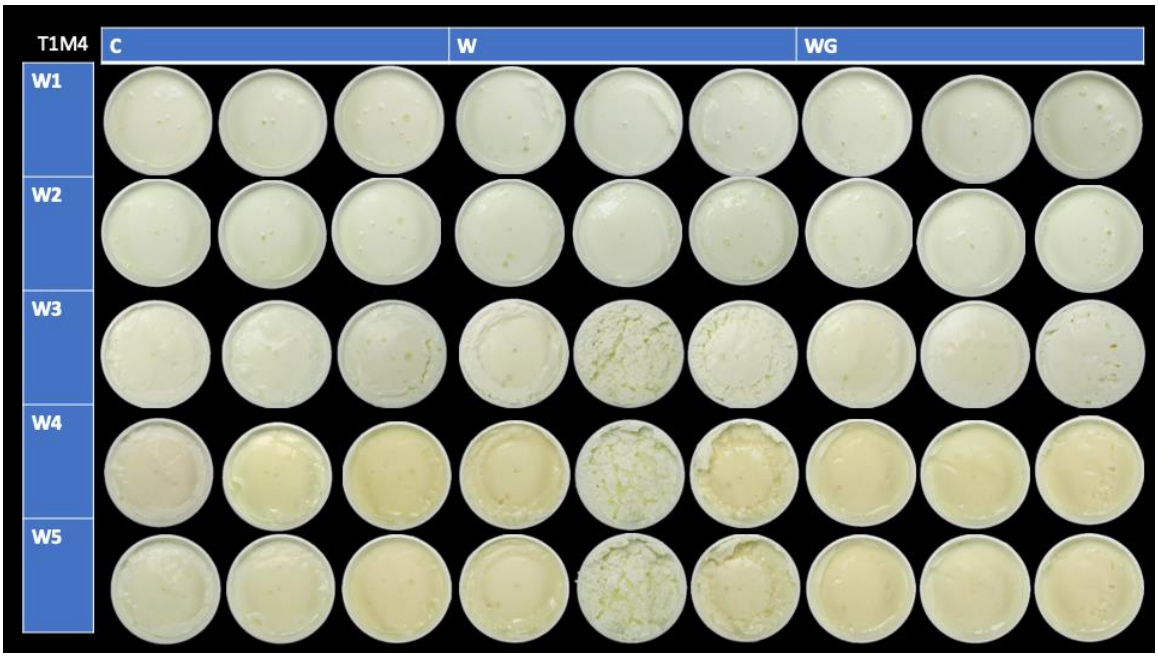


Figure B.4. First biological replicate of images of yogurt cups inoculated with *Penicillium decumbens.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

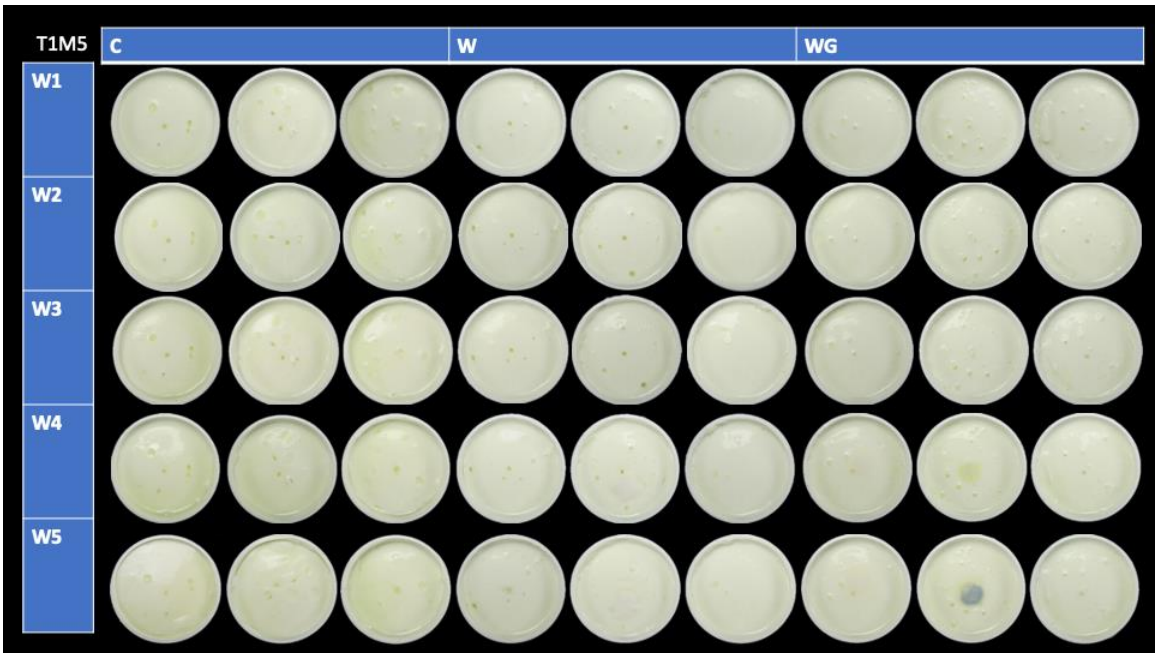


Figure B.5. First biological replicate of images of yogurt cups inoculated with *Aspergillus cibarius.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

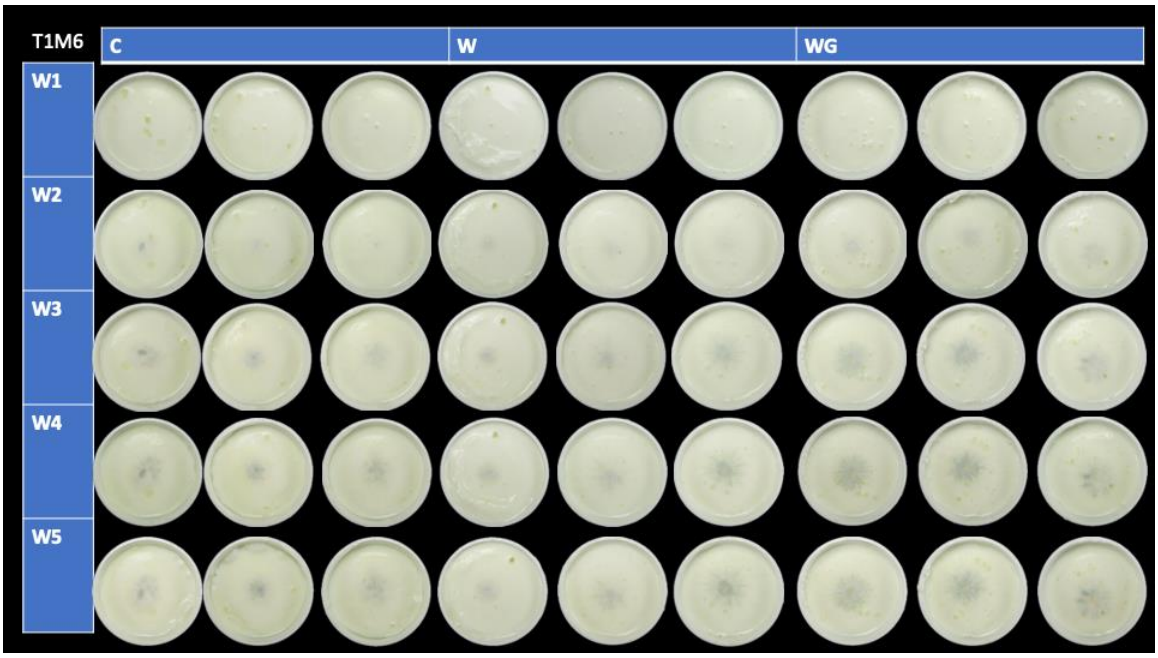


Figure B.6. First biological replicate of images of yogurt cups inoculated with *Penicillium roqueforti.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

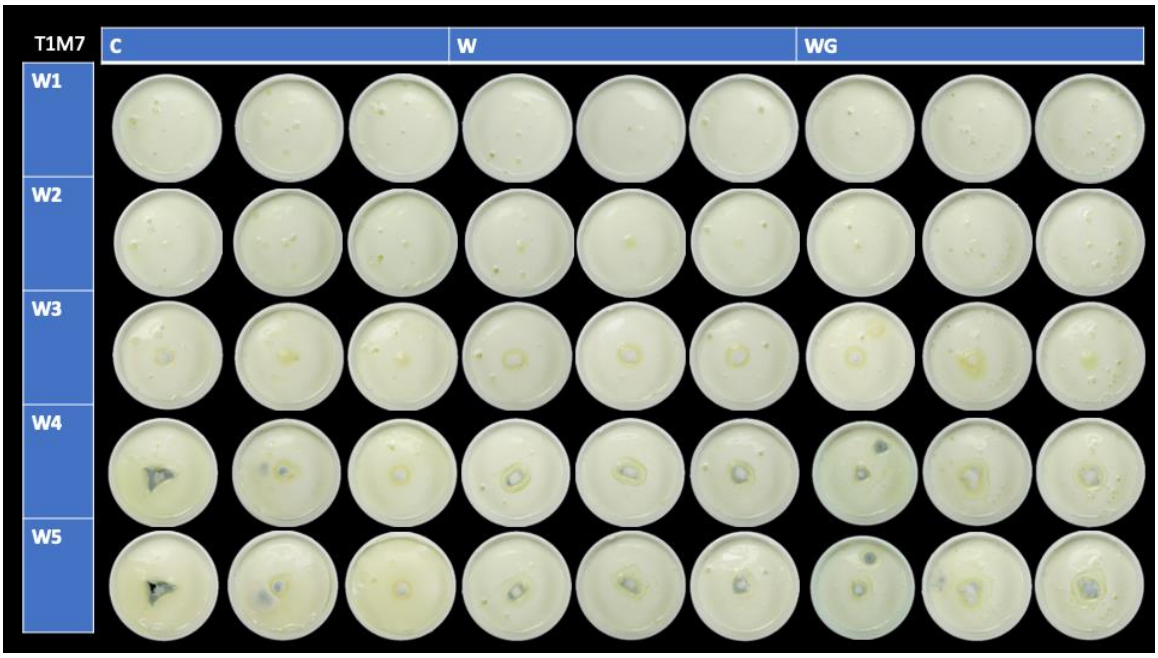


Figure B.7. First biological replicate of images of yogurt cups inoculated with *Penicillium chrysogenum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

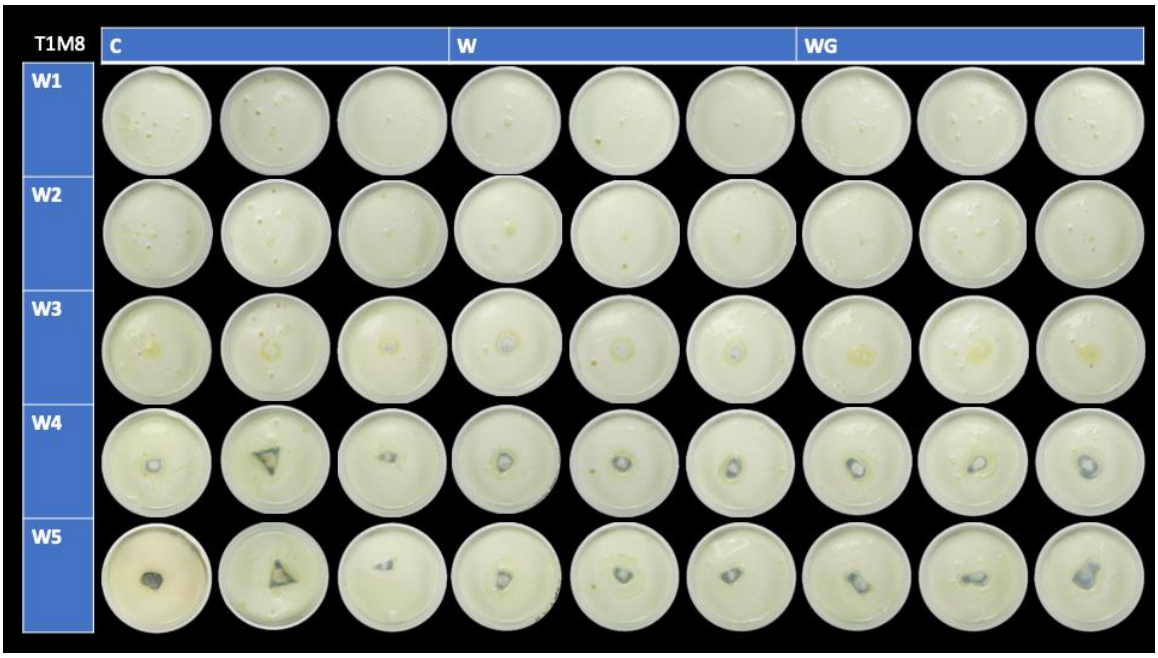


Figure B.8. First biological replicate of images of yogurt cups inoculated with *Phoma dimorpha.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

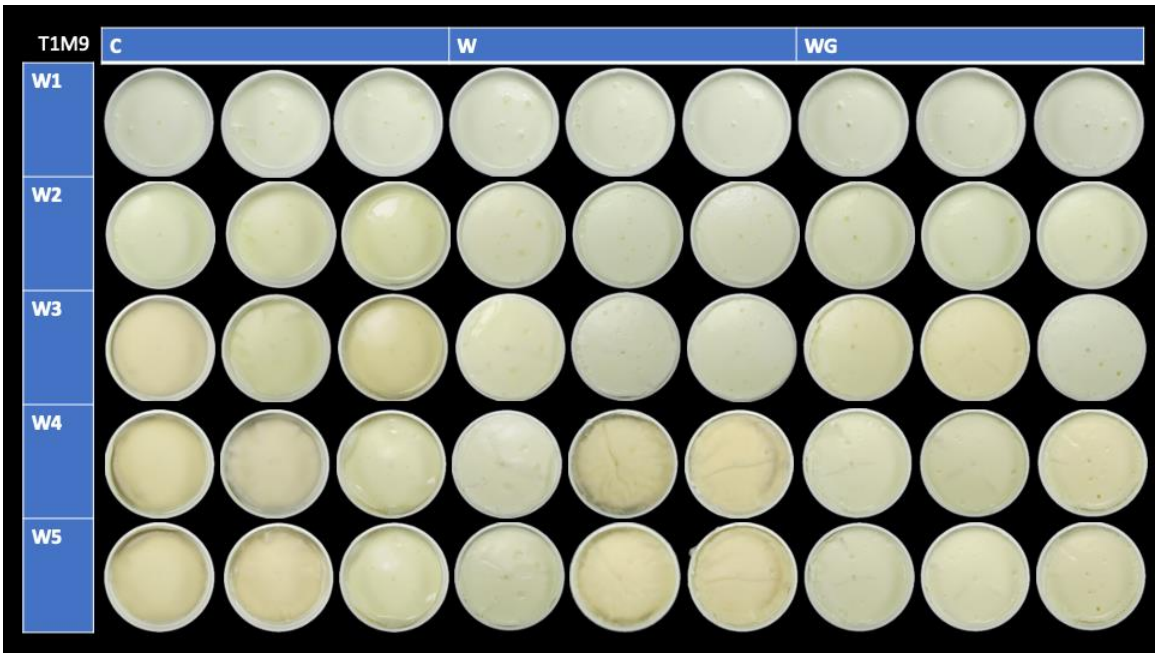


Figure B.9. First biological replicate of images of yogurt cups inoculated with *Mucor racemosus.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

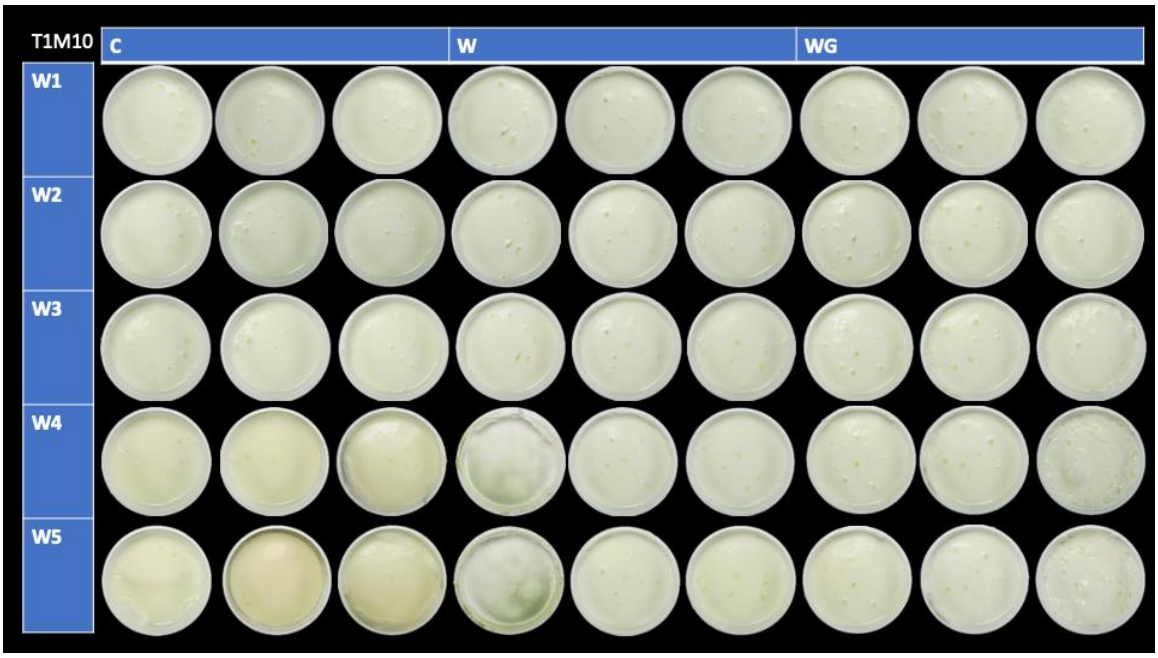


Figure B.10. First biological replicate of images of yogurt cups inoculated with *Trichoderma amazonicum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

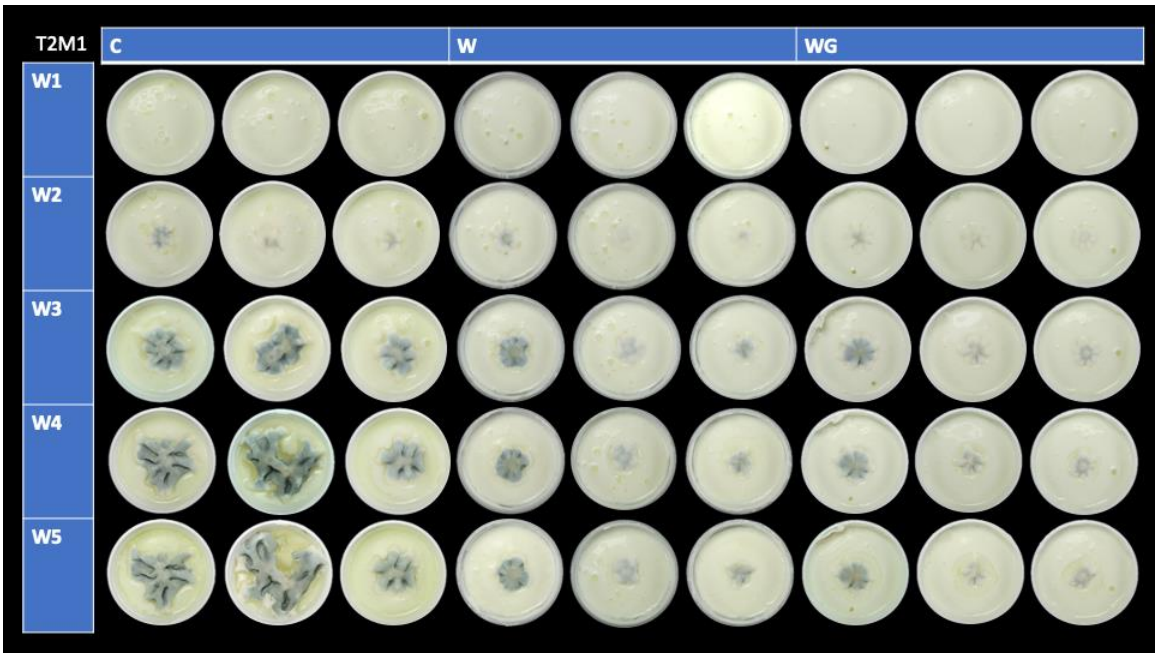


Figure B.11. Second biological replicate of images of yogurt cups inoculated with *Penicillium commune.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

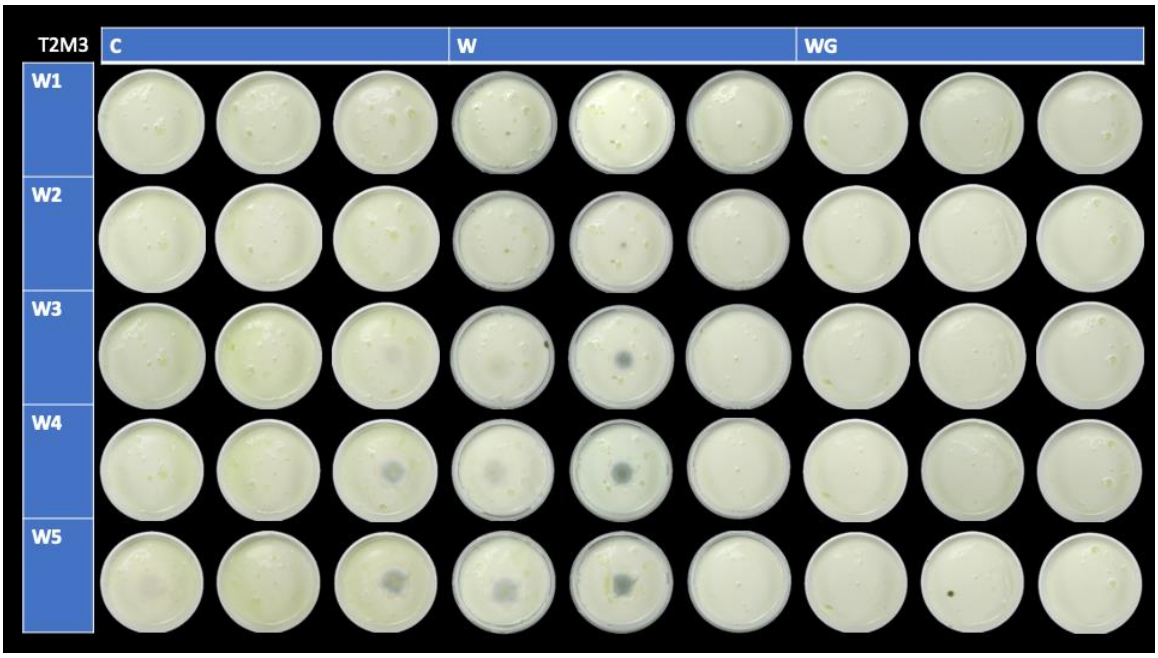


Figure B.12. Second biological replicate of images of yogurt cups inoculated with *Penicillium citrinum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

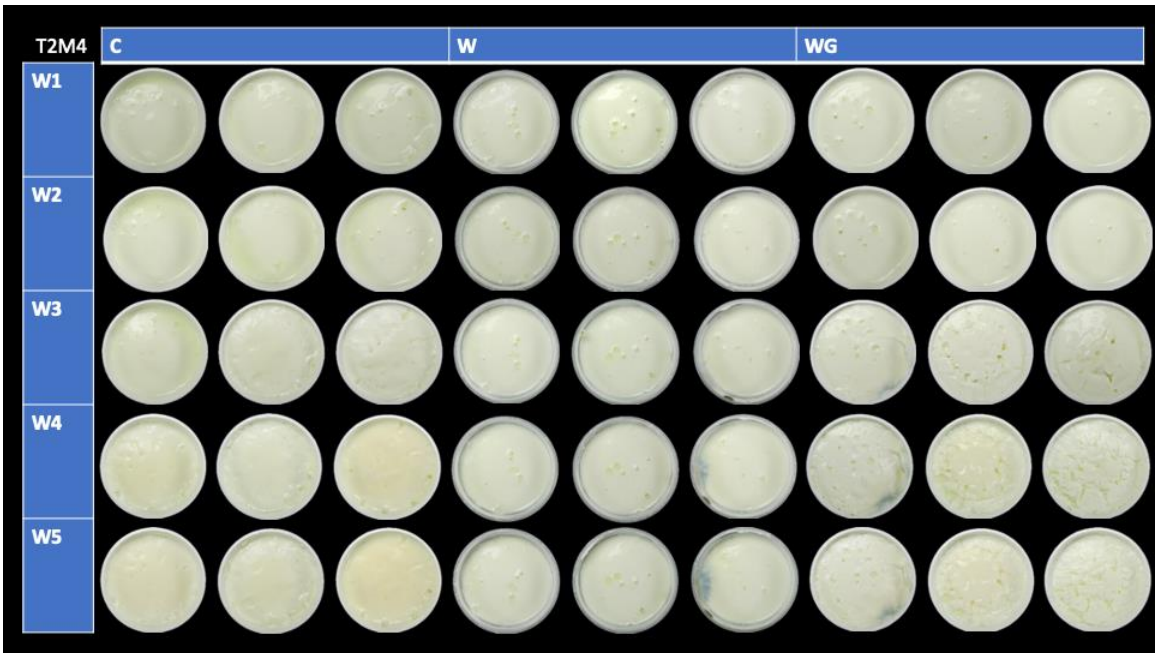


Figure B.13. Second biological replicate of images of yogurt cups inoculated with *Penicillium decumbens.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

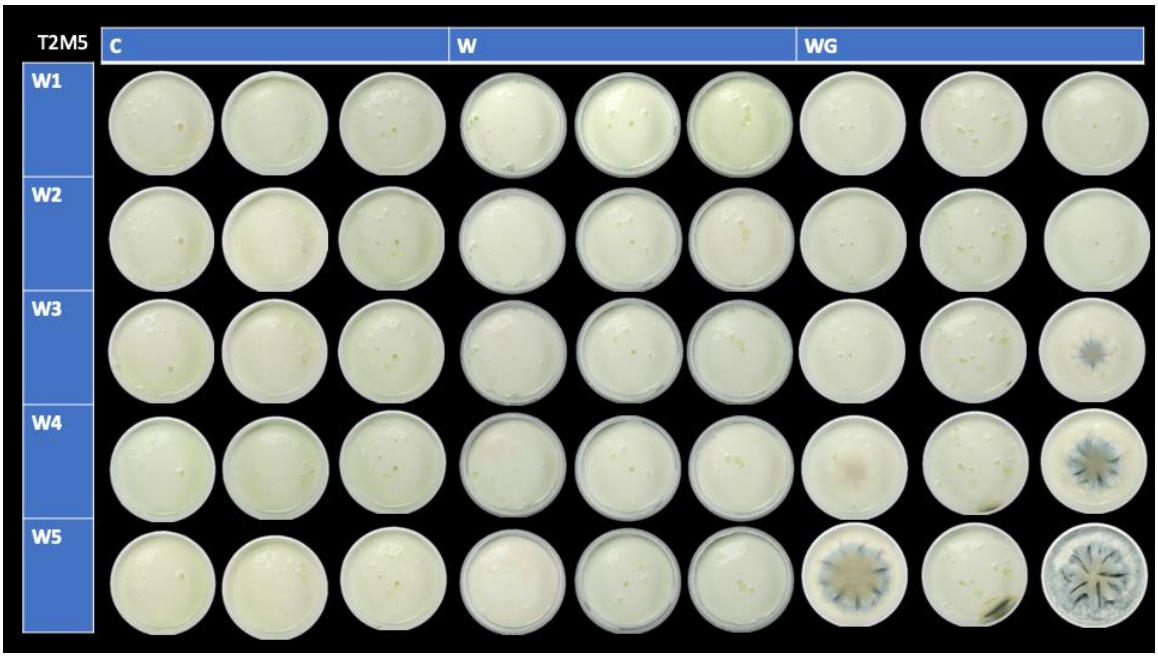


Figure B.14. Second biological replicate of images of yogurt cups inoculated with *Aspergillus cibarius.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

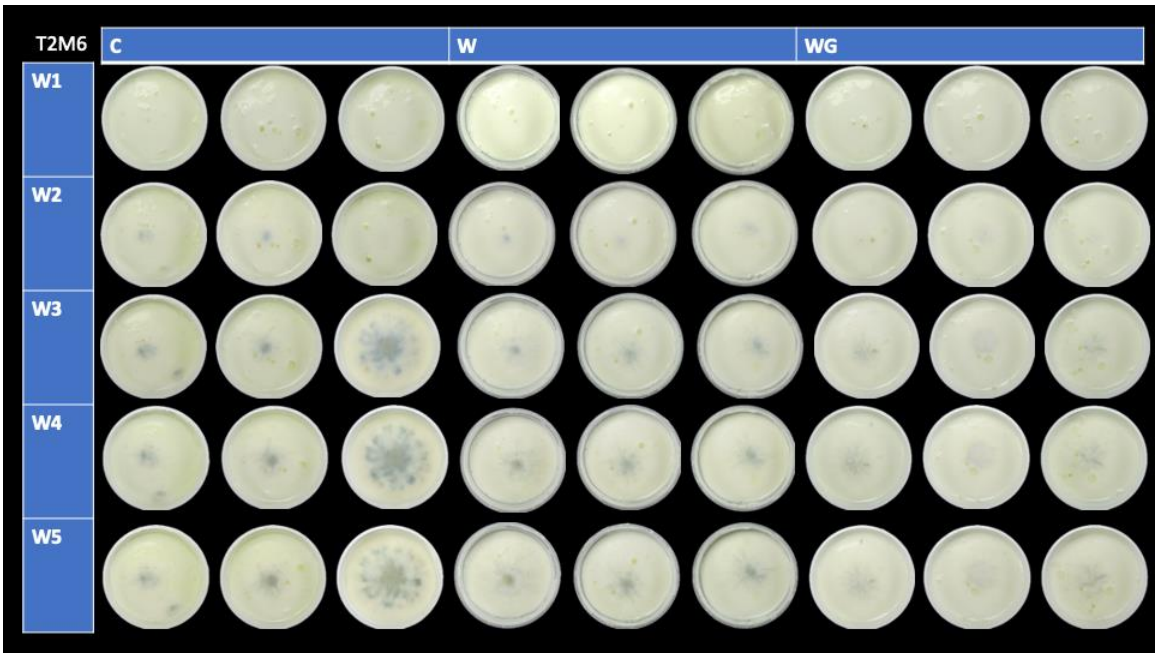


Figure B.15. Second biological replicate of images of yogurt cups inoculated with *Penicillium roqueforti.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

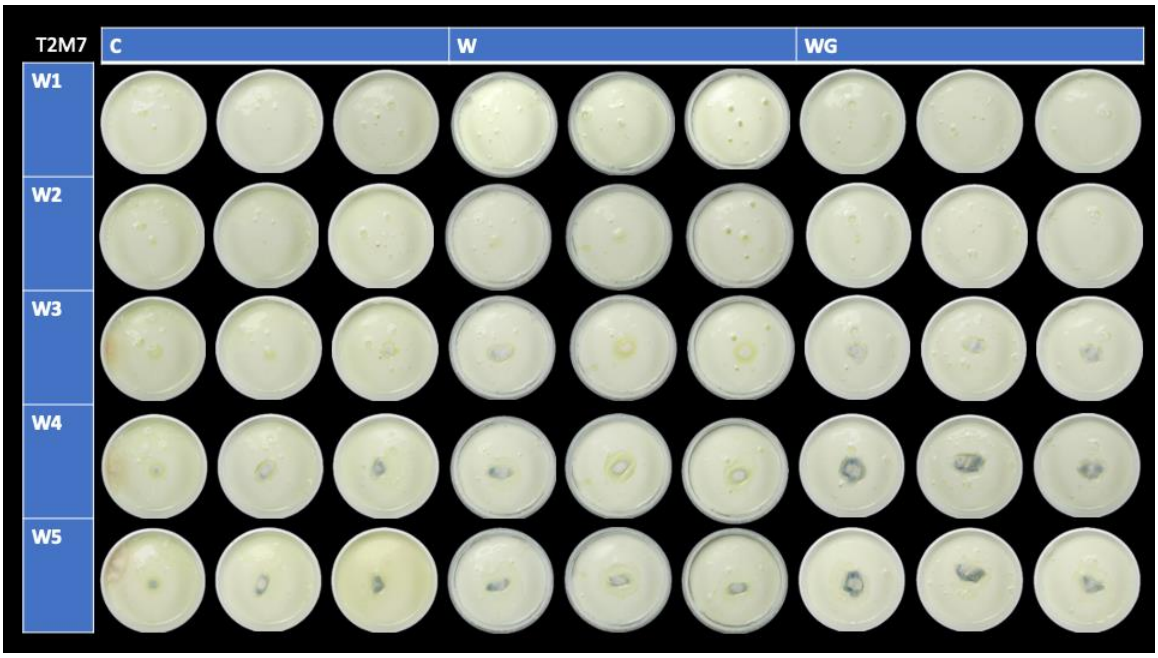


Figure B.16. Second biological replicate of images of yogurt cups inoculated with *Penicillium chrysogenum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

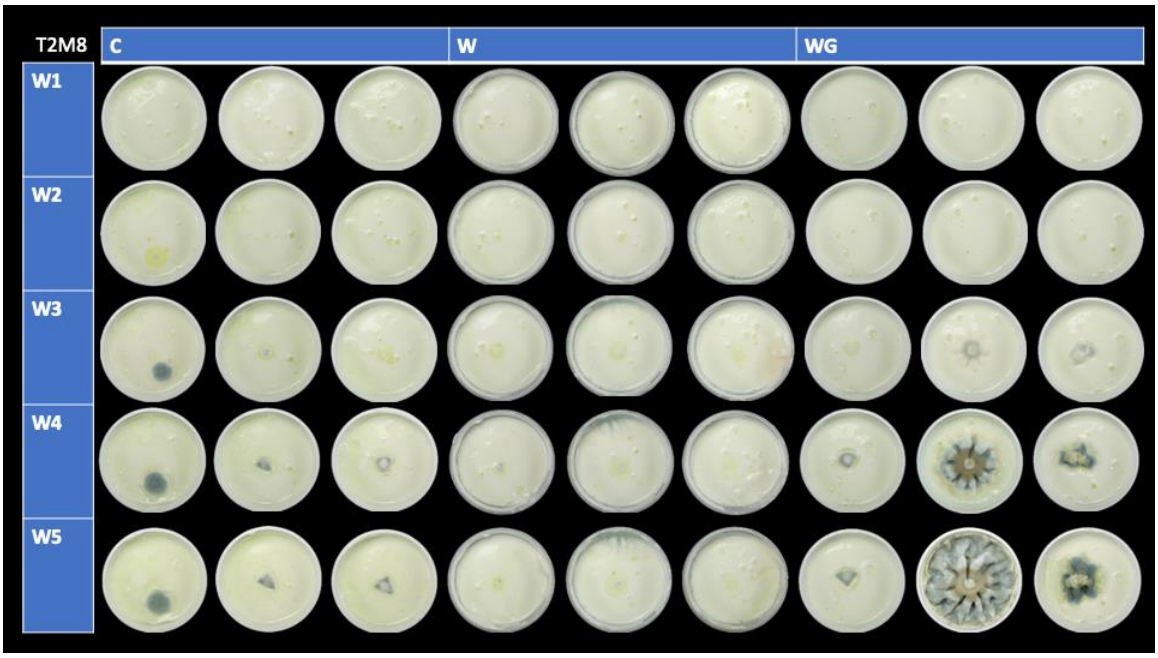


Figure B.17. Second biological replicate of images of yogurt cups inoculated with *Phoma dimorpha.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

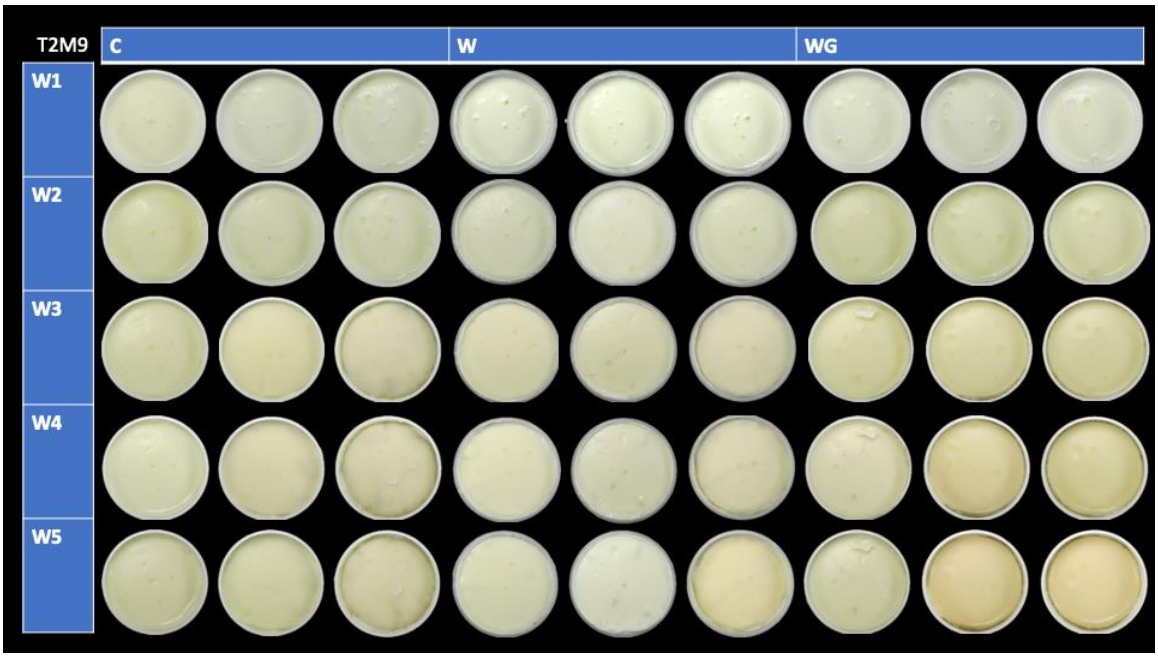


Figure B.18. Second biological replicate of images of yogurt cups inoculated with *Mucor racemosus.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

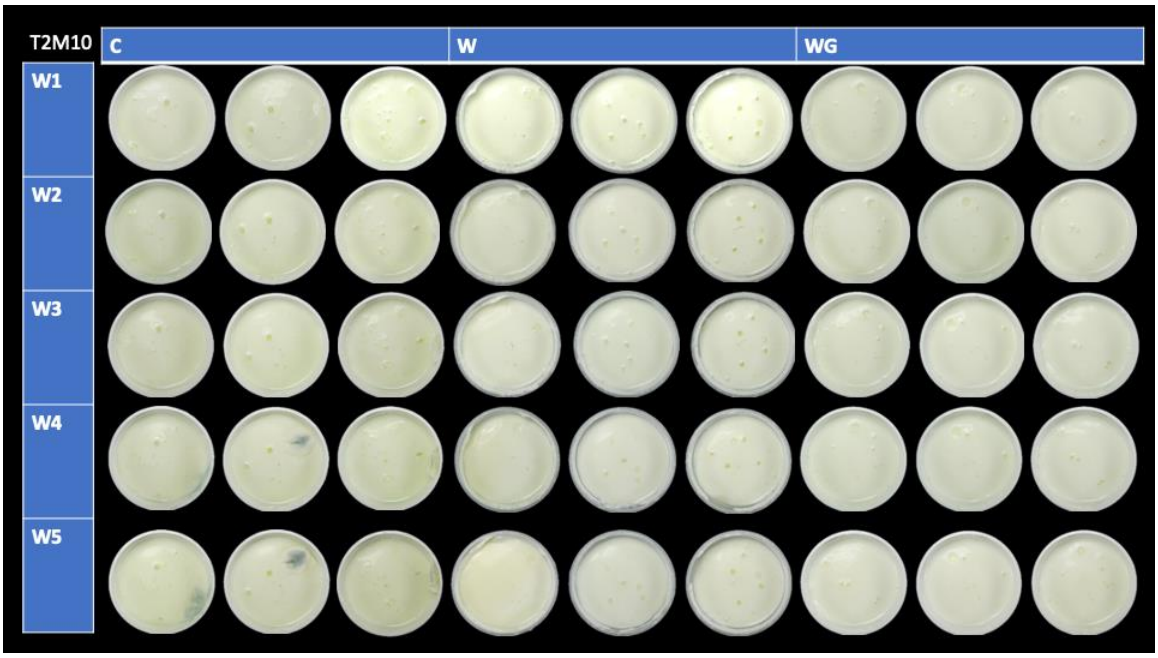


Figure B.19. Second biological replicate of images of yogurt cups inoculated with *Trichoderma amazonicum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

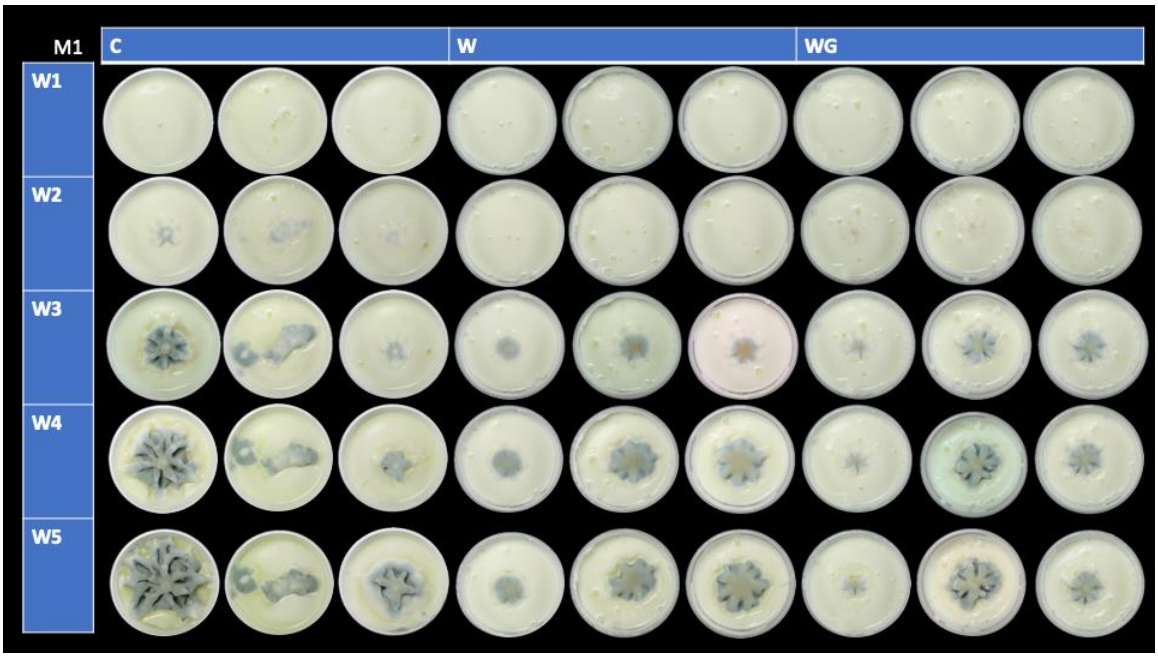


Figure B.20. Third biological replicate of images of yogurt cups inoculated with *Penicillium commune.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

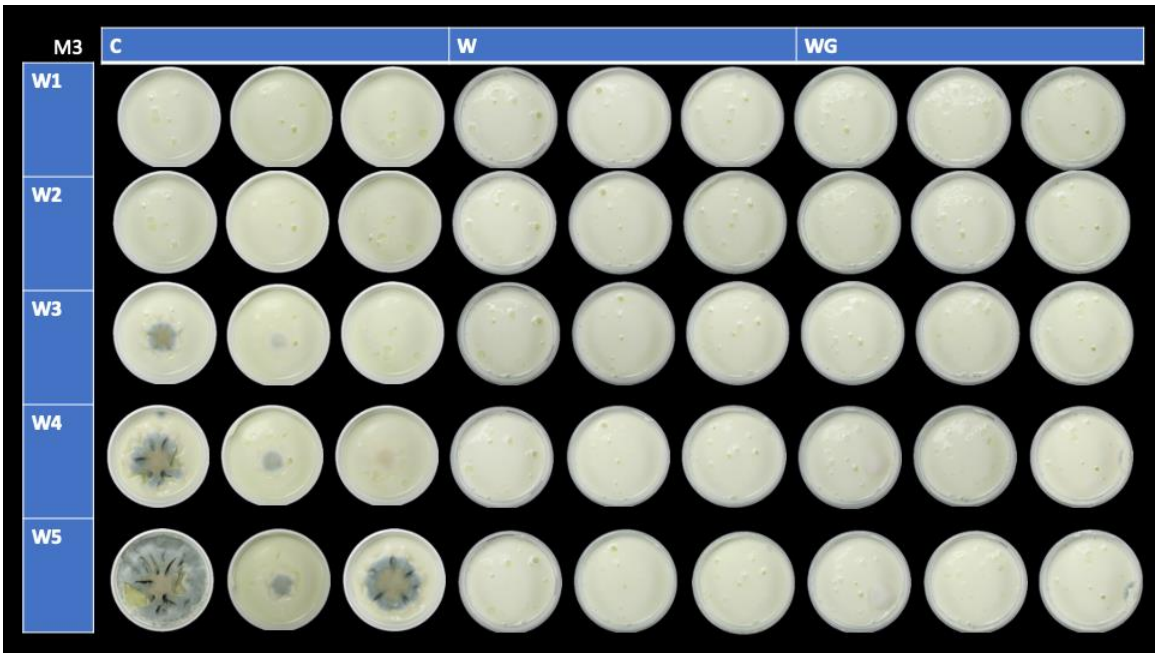


Figure B.21. Third biological replicate of images of yogurt cups inoculated with *Penicillium citrinum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

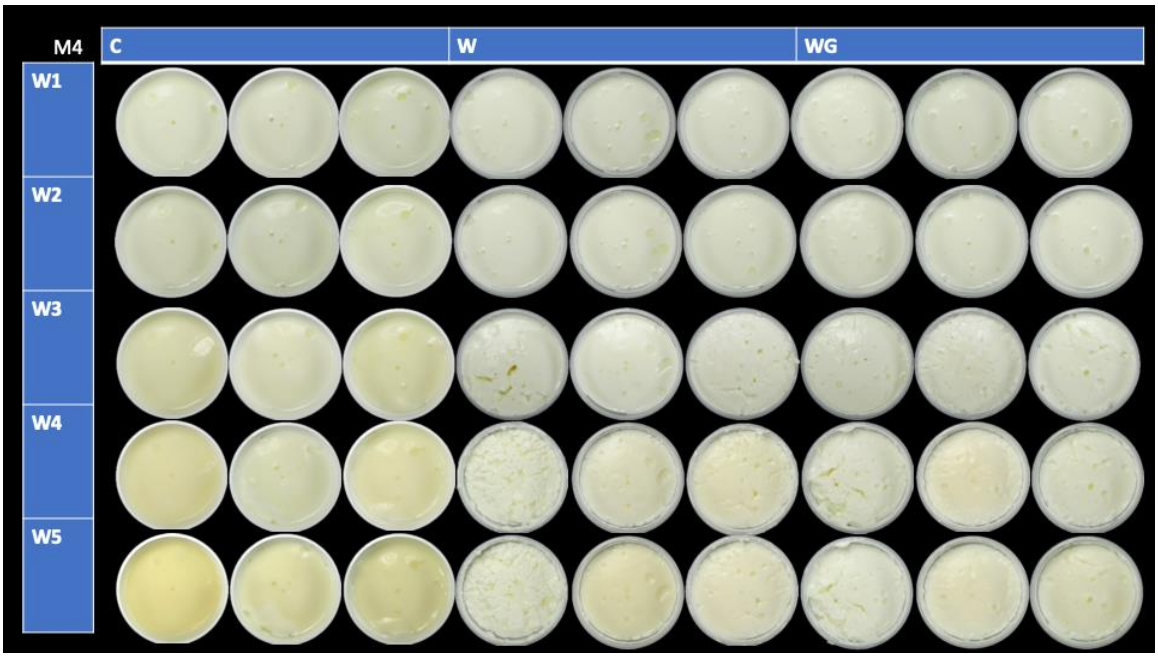


Figure B.22. Third biological replicate of images of yogurt cups inoculated with *Penicillium decumbens.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

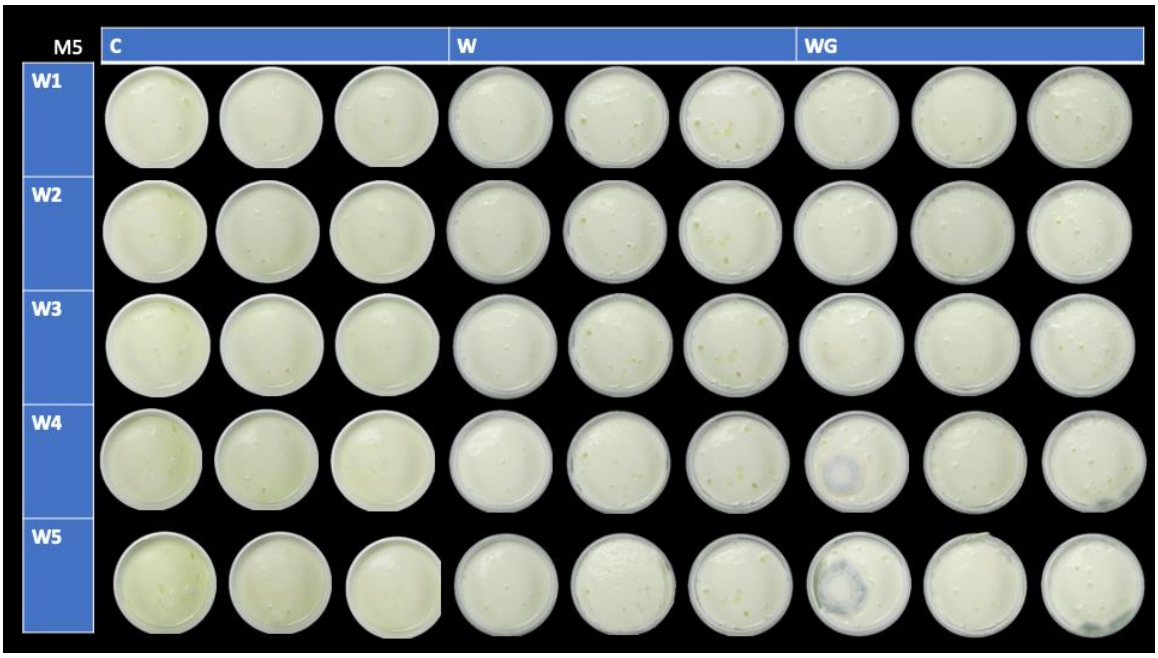


Figure B.23. Third biological replicate of images of yogurt cups inoculated with *Aspergillus cibarius.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

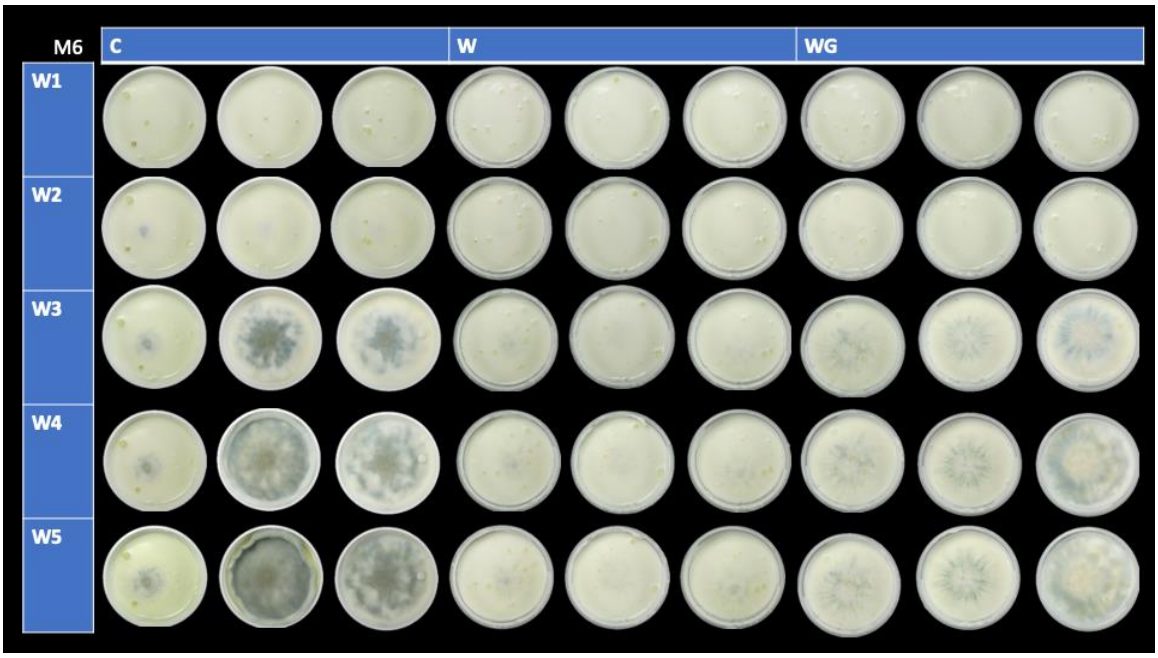


Figure B.24. Third biological replicate of images of yogurt cups inoculated with *Penicillium roqueforti.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

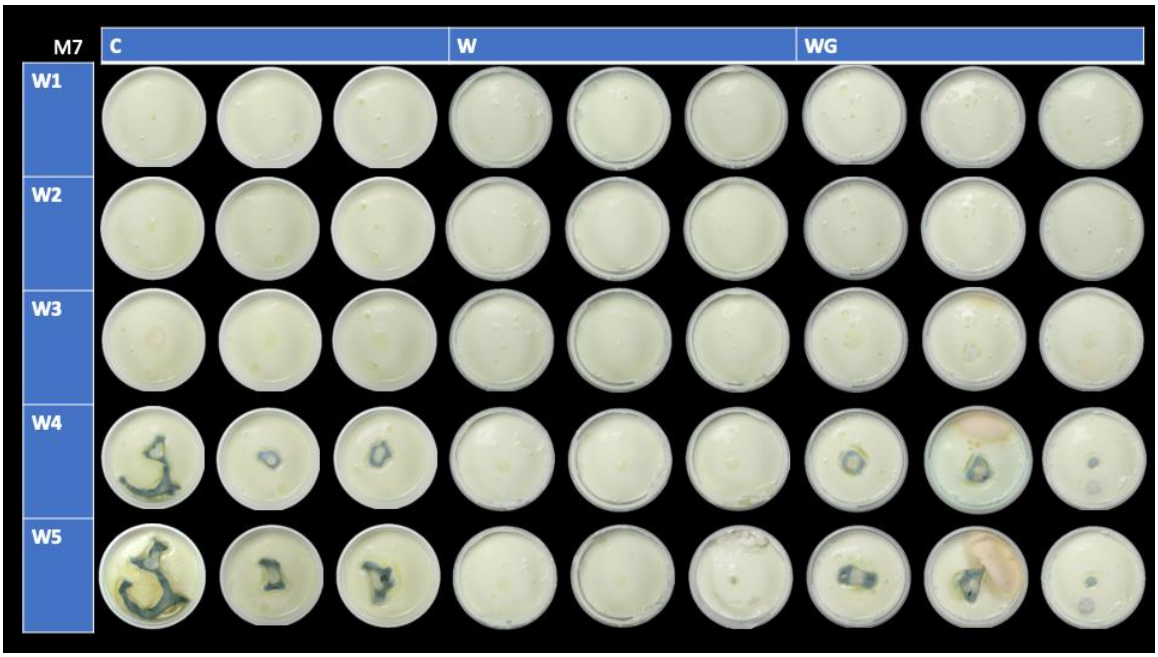


Figure B.25. Third biological replicate of images of yogurt cups inoculated with *Penicillium chrysogenum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

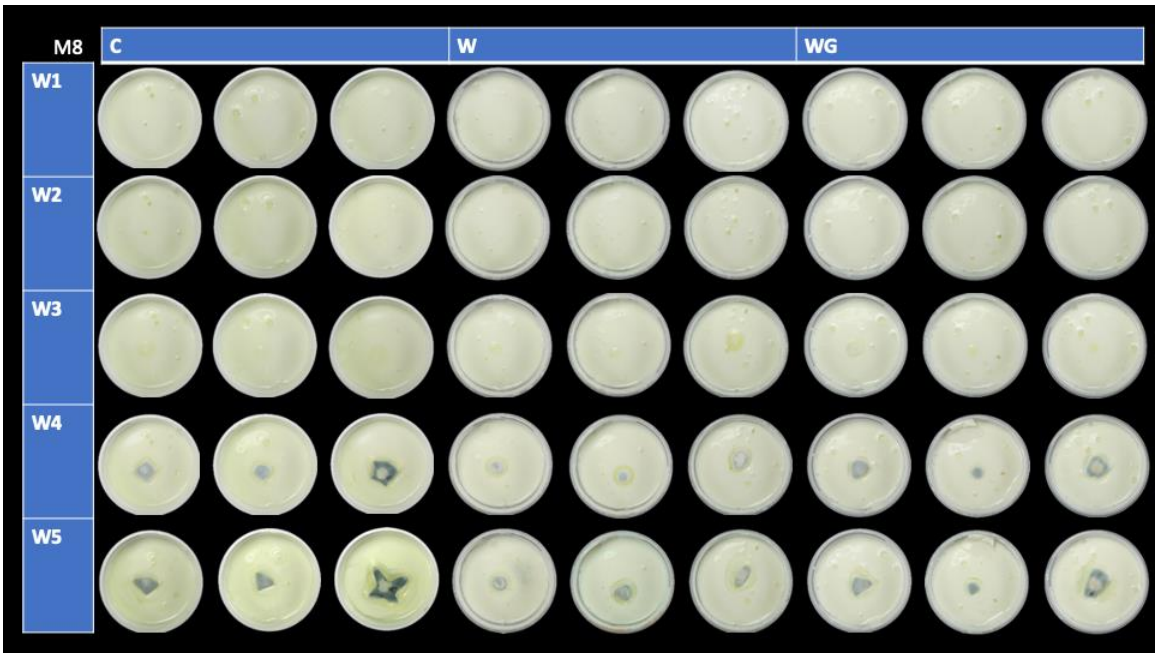


Figure B.26. Third biological replicate of images of yogurt cups inoculated with *Phoma dimorpha.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

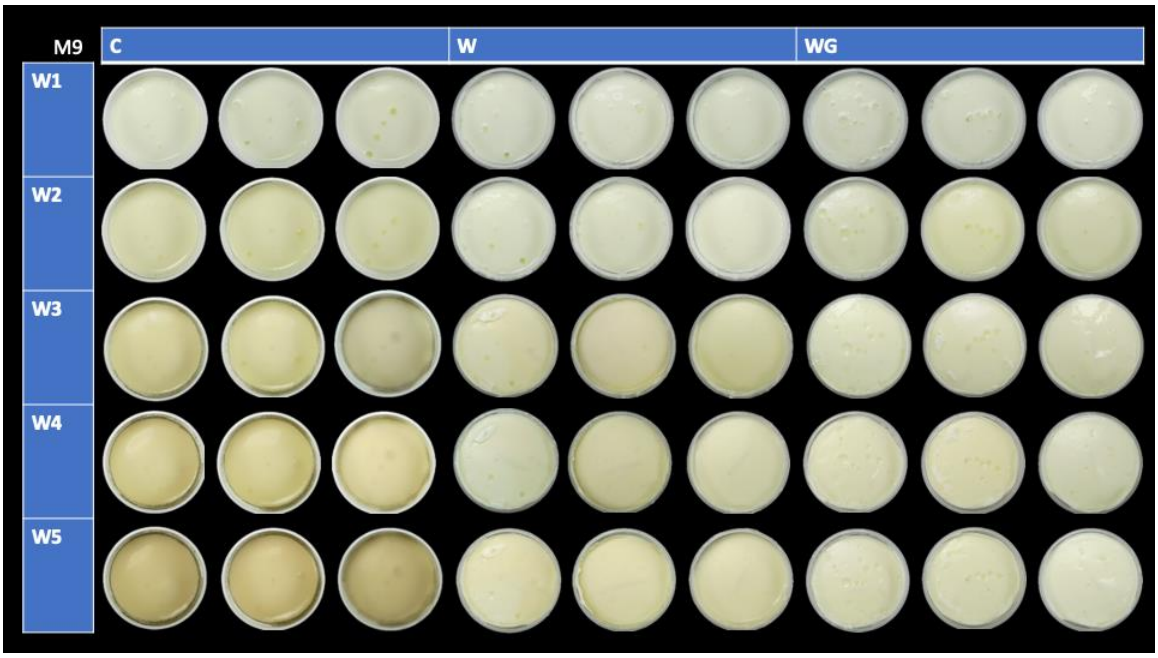


Figure B.27. Third biological replicate of images of yogurt cups inoculated with *Mucor racemosus.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

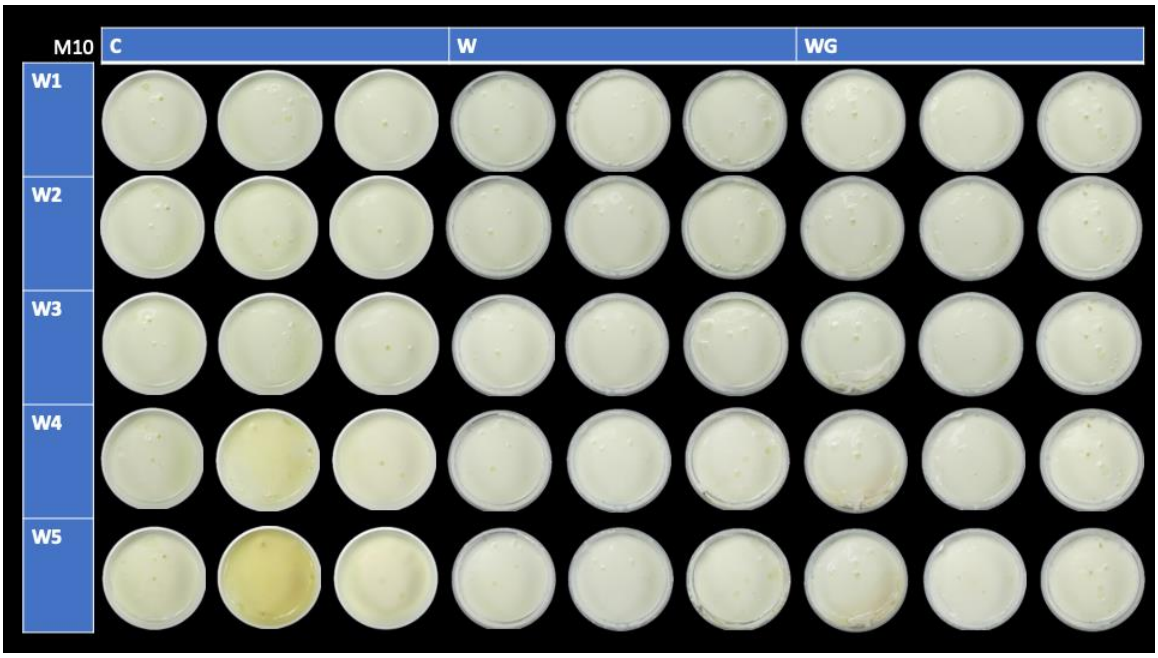


Figure B.28. Second biological replicate of images of yogurt cups inoculated with *Trichoderma amazonicum.* Cups are organized by treatment and shown over 5 weeks (W1-W5) which were conducted and shown triplicate. Treatments are as follows **C** = Control, **W** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 **WG** = yogurt with Log_{10} CFU/g *Lat. curvatus* WSU-1 and 200mM glycerol.

Appendix C: Sensory analysis supplementary information

SIMS TEST ROTATION PLAN: By Ballot # / Sample Set					
SIMS Definition Code: YOGURTCW					
SIMS Definition Description: 2-2024 Chase Yogurt Triangle Tests					
Sample 1: Control					
Sample 2: Treatment					
Rep: 1					
Ballot#	PanelistID	Panelist Name	Sample Order (Sample#/Sample Code)		
1	0000000001	Resp # 1	1-413	2-273	1-619
2	0000000002	Resp # 2	2-498	1-314	2-638
3	0000000003	Resp # 3	1-952	2-192	2-819
4	0000000004	Resp # 4	2-725	1-812	1-123
5	0000000005	Resp # 5	1-891	1-946	2-458
6	0000000006	Resp # 6	2-196	2-649	1-841
7	0000000007	Resp # 7	1-298	2-495	1-971
8	0000000008	Resp # 8	2-789	1-395	2-957
9	0000000009	Resp # 9	1-417	2-753	2-853
10	0000000010	Resp # 10	2-597	1-486	1-926
11	0000000011	Resp # 11	1-528	1-481	2-281
12	0000000012	Resp # 12	2-829	2-712	1-651
13	0000000013	Resp # 13	1-764	2-814	1-567
14	0000000014	Resp # 14	2-497	1-374	2-154
15	0000000015	Resp # 15	1-928	2-245	2-316
16	0000000016	Resp # 16	2-962	1-617	1-156
17	0000000017	Resp # 17	1-746	1-972	2-189
18	0000000018	Resp # 18	2-139	2-879	1-681
19	0000000019	Resp # 19	1-792	2-281	1-613
20	0000000020	Resp # 20	2-348	1-462	2-859
21	0000000021	Resp # 21	1-495	2-248	2-124
22	0000000022	Resp # 22	2-483	1-542	1-852
23	0000000023	Resp # 23	1-536	1-428	2-769
24	0000000024	Resp # 24	2-573	2-681	1-385
25	0000000025	Resp # 25	1-324	2-912	1-532
26	0000000026	Resp # 26	2-356	1-189	2-796
27	0000000027	Resp # 27	1-543	2-632	2-795
28	0000000028	Resp # 28	2-176	1-438	1-594
29	0000000029	Resp # 29	1-418	1-253	2-967
30	0000000030	Resp # 30	2-245	2-674	1-721
31	0000000031	Resp # 31	1-215	2-739	1-829
32	0000000032	Resp # 32	2-246	1-182	2-435
33	0000000033	Resp # 33	1-187	2-286	2-642
34	0000000034	Resp # 34	2-841	1-498	1-263
35	0000000035	Resp # 35	1-627	1-823	2-457
36	0000000036	Resp # 36	2-179	2-214	1-718

SIMS TEST ROTATION PLAN: By Ballot # / Sample Set					
SIMS Definition Code: YOGURTCW					
37	0000000037	Resp # 37	1-916	2-536	1-417
38	0000000038	Resp # 38	2-725	1-962	2-876
39	0000000039	Resp # 39	1-619	2-529	2-942
40	0000000040	Resp # 40	2-852	1-582	1-736
41	0000000041	Resp # 41	1-982	1-529	2-267
42	0000000042	Resp # 42	2-712	2-274	1-463
43	0000000043	Resp # 43	1-329	2-942	1-259
44	0000000044	Resp # 44	2-835	1-369	2-413
45	0000000045	Resp # 45	1-823	2-548	2-734
46	0000000046	Resp # 46	2-579	1-895	1-346
47	0000000047	Resp # 47	1-157	1-473	2-895
48	0000000048	Resp # 48	2-653	2-458	1-926
49	0000000049	Resp # 49	1-138	2-581	1-356
50	0000000050	Resp # 50	2-245	1-952	2-782
51	0000000051	Resp # 51	1-614	2-426	2-294
52	0000000052	Resp # 52	2-145	1-713	1-314
53	0000000053	Resp # 53	1-937	1-612	2-831
54	0000000054	Resp # 54	2-195	2-921	1-325
55	0000000055	Resp # 55	1-581	2-687	1-876
56	0000000056	Resp # 56	2-425	1-583	2-351
57	0000000057	Resp # 57	1-837	2-594	2-368
58	0000000058	Resp # 58	2-852	1-217	1-125
59	0000000059	Resp # 59	1-849	1-386	2-761
60	0000000060	Resp # 60	2-492	2-765	1-146
61	0000000061	Resp # 61	1-142	2-653	1-295
62	0000000062	Resp # 62	2-678	1-918	2-817
63	0000000063	Resp # 63	1-263	2-317	2-814
64	0000000064	Resp # 64	2-721	1-837	1-917
65	0000000065	Resp # 65	1-182	1-715	2-926
66	0000000066	Resp # 66	2-673	2-561	1-356
67	0000000067	Resp # 67	1-629	2-491	1-873
68	0000000068	Resp # 68	2-728	1-498	2-541
69	0000000069	Resp # 69	1-694	2-237	2-179
70	0000000070	Resp # 70	2-698	1-586	1-723
Rep: 2					
Ballot#	PanelistID	Panelist Name	Sample Order (Sample#/Sample Code)		
1	0000000001	Resp # 1	1-982	1-561	2-831
2	0000000002	Resp # 2	2-814	2-967	1-139
3	0000000003	Resp # 3	1-416	2-739	1-246
4	0000000004	Resp # 4	2-921	1-692	2-527
5	0000000005	Resp # 5	1-157	2-698	2-356
6	0000000006	Resp # 6	2-458	1-953	1-735

SIMS TEST ROTATION PLAN: By Ballot # / Sample Set					
SIMS Definition Code: YOGURTCW					
7	0000000007	Resp # 7	1-531	1-758	2-621
8	0000000008	Resp # 8	2-263	2-564	1-472
9	0000000009	Resp # 9	1-935	2-291	1-516
10	0000000010	Resp # 10	2-183	1-638	2-268
11	0000000011	Resp # 11	1-361	2-142	2-698
12	0000000012	Resp # 12	2-946	1-457	1-362
13	0000000013	Resp # 13	1-683	1-182	2-319
14	0000000014	Resp # 14	2-542	2-958	1-725
15	0000000015	Resp # 15	1-174	2-487	1-728
16	0000000016	Resp # 16	2-495	1-263	2-812
17	0000000017	Resp # 17	1-627	2-235	2-315
18	0000000018	Resp # 18	2-425	1-742	1-317
19	0000000019	Resp # 19	1-438	1-516	2-396
20	0000000020	Resp # 20	2-592	2-715	1-618
21	0000000021	Resp # 21	1-874	2-925	1-574
22	0000000022	Resp # 22	2-976	1-317	2-697
23	0000000023	Resp # 23	1-964	2-197	2-358
24	0000000024	Resp # 24	2-169	1-897	1-742
25	0000000025	Resp # 25	1-854	1-143	2-784
26	0000000026	Resp # 26	2-851	2-912	1-641
27	0000000027	Resp # 27	1-894	2-128	1-347
28	0000000028	Resp # 28	2-391	1-743	2-965
29	0000000029	Resp # 29	1-574	2-685	2-182
30	0000000030	Resp # 30	2-971	1-547	1-837
31	0000000031	Resp # 31	1-375	1-958	2-485
32	0000000032	Resp # 32	2-396	2-637	1-793
33	0000000033	Resp # 33	1-497	2-561	1-725
34	0000000034	Resp # 34	2-179	1-529	2-684
35	0000000035	Resp # 35	1-254	2-145	2-549
36	0000000036	Resp # 36	2-465	1-658	1-975
37	0000000037	Resp # 37	1-768	1-879	2-364
38	0000000038	Resp # 38	2-419	2-627	1-284
39	0000000039	Resp # 39	1-742	2-326	1-459
40	0000000040	Resp # 40	2-987	1-176	2-473
41	0000000041	Resp # 41	1-731	2-315	2-684
42	0000000042	Resp # 42	2-592	1-914	1-652
43	0000000043	Resp # 43	1-192	1-879	2-793
44	0000000044	Resp # 44	2-536	2-253	1-697
45	0000000045	Resp # 45	1-285	2-398	1-932
46	0000000046	Resp # 46	2-798	1-487	2-643
47	0000000047	Resp # 47	1-641	2-719	2-563
48	0000000048	Resp # 48	2-324	1-716	1-239
49	0000000049	Resp # 49	1-497	1-985	2-851
50	0000000050	Resp # 50	2-498	2-635	1-576
51	0000000051	Resp # 51	1-923	2-781	1-564
52	0000000052	Resp # 52	2-673	1-254	2-561

SIMS TEST ROTATION PLAN: By Ballot # / Sample Set					
SIMS Definition Code: YOGURTCW					
53	0000000053	Resp # 53	1-514	2-136	2-468
54	0000000054	Resp # 54	2-632	1-296	1-764
55	0000000055	Resp # 55	1-258	1-417	2-951
56	0000000056	Resp # 56	2-894	2-165	1-253
57	0000000057	Resp # 57	1-274	2-648	1-965
58	0000000058	Resp # 58	2-679	1-548	2-752
59	0000000059	Resp # 59	1-417	2-591	2-216
60	0000000060	Resp # 60	2-913	1-687	1-579
61	0000000061	Resp # 61	1-425	1-374	2-943
62	0000000062	Resp # 62	2-283	2-594	1-485
63	0000000063	Resp # 63	1-792	2-412	1-124
64	0000000064	Resp # 64	2-192	1-397	2-694
65	0000000065	Resp # 65	1-352	2-893	2-612
66	0000000066	Resp # 66	2-819	1-197	1-263
67	0000000067	Resp # 67	1-568	1-765	2-948
68	0000000068	Resp # 68	2-836	2-926	1-258
69	0000000069	Resp # 69	1-492	2-527	1-982
70	0000000070	Resp # 70	2-476	1-812	2-194

Figure C.1. Worksheet of randomized and balanced design for triangle tests

Thank you for participating in this yogurt sensory test. Please read all of the instructions before moving on:

1) You will be asked to complete TWO different sensory tests evaluating yogurt and you will receive TWO different trays.
2) Please confirm the numbers on the sample cups match the ones on the screen and taste the samples from left to right.
3) You will be asked to select which sample is different, if you can`t tell which is different you MUST guess.
4) Please take a bite of cracker and rinse with water between each sample.
5) Once you are done please lift the door, push the tray through, and receive your second tray and repeat the previous instructions.
6) If you have any questions you may ask the attendant at the door.

You are ready, please click on the hand to begin

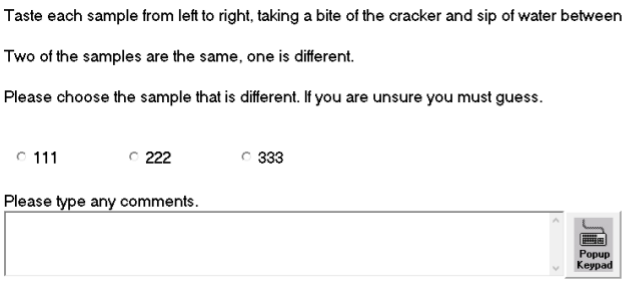


Figure C.2. Questionnaire for triangle tests

Ballot	Result	Comment - Comment
0000000001	Incorrect	
0000000002	Correct	
0000000003	Correct	this one was slightly runnier and a little sweeter than the other two
0000000004	Correct	527 and 921 tasted a little sweeter than 692
0000000005	Correct	I am pretty confident 157 was different. The other two samples were a lot more sweet. 157 was almost overwhelming when I first tasted it but the others were not.
0000000006	Correct	
0000000007	Correct	The flavor in 621 is less intense than the other two.
0000000008	Correct	472 was more sour than either the other two samples. It has a smoother look too, 263 and 564 look frothy as well
0000000009	Correct	291 was noticably thinner than the rest but tastes about the same but there is something different.
0000000010	Correct	I think that, in addition to tasting a lil` different, 638 was a little more viscous (my spoon slid in less easily)
0000000011	Correct	361 tatses the best, less sweet, no off flavor
0000000012	Correct	Sample was slightly sweeter
0000000013	Correct	
0000000014	Correct	542 and 958 had an interesting twang to it. and once again the consistency of 725 was slightly thicker
0000000015	Correct	all three had a similiar taste, but 487 had a slightly different appearance, it was less smooth than the other two.
0000000016	Incorrect	I think 495 is different because to me it tastes sweeter. Also, the dairy flavor is a lot milder. The other too taste very strong of dairy, while the 495 has a calming dairy taste. It also seems to be thinner in my mouth than the other two.
0000000017	Incorrect	627 - similar as the first test yogurts 235 - similar to the 627? not sure yet 315 - ohh this test is close. but i would say this one becasue its sweeter? Maybe
0000000018	Correct	
0000000019	Correct	felt this was easier to say which one is different the appearance also looks different
0000000020	Correct	2 samples were more sweeter than the 3rd one.
0000000021	Correct	tasted a little more sour also looked different.
0000000022	Correct	
0000000023	Incorrect	texture thinner,. a little brighter flavor
0000000024	Incorrect	
0000000025	Correct	784 tastes sweeter and less acidic
0000000026	Incorrect	
0000000027	Correct	
0000000028	Correct	
0000000029	Correct	
0000000030	Correct	
0000000031	Correct	485 is runnier and more bubbley. I do like the flavor more though. PAPI LIKEY!
0000000032	Incorrect	
0000000033	Correct	the one is not as thick and has less of an aftertaste
0000000034	Correct	529 is definitely sweeter and feels lighter than the others
0000000035	Correct	Smoother, I think.
0000000036	Correct	
0000000037	Incorrect	
0000000038	Incorrect	
0000000039	Correct	
0000000040	Correct	This one feels like it has a different taste.
0000000041	Correct	
0000000042	Correct	
0000000043	Correct	The different sample seemed to taste sweeter and less tart than the others.
0000000044	Correct	697 not as sweet, more plain, gifferent concictency when looking at and tasting
0000000045	Incorrect	
0000000046	Incorrect	Sample 798 tastes a bit sweeter
0000000047	Correct	
0000000048	Correct	I think 324 has a bit of different texture.
0000000049	Correct	this one tastes better than the last different one
0000000050	Incorrect	
0000000051	Correct	
0000000052	Correct	it seemed to be a different consistency than the other two
0000000053	Incorrect	I think 468 had a slight vanilla taste to it
0000000054	Incorrect	With the previous samples, when I tasted it, I mainly thought it tasted like normal yogurt, but for some reason the last sample made my mouth feel different.
0000000055	Correct	
0000000056	Incorrect	A little sweeter than the other 2
0000000057	Correct	648 is sweeter.
0000000058	Incorrect	I could tell the sample that was different from the texture, consistency, and look as well as taste
0000000059	Correct	
0000000060	Incorrect	
0000000061	Correct	
0000000062	Incorrect	
0000000063	Correct	
0000000064	Correct	

Figure C.3. Control vs. WSU-1 + glycerol yogurt sensory results and comments

Ballot	Result	Comment - Comment
0000000001	Correct	
0000000002	Correct	
0000000003	Correct	this one seemed a bit thicker, and tasted a bit cheesier than the others.
0000000004	Incorrect	
0000000005	Correct	Part of the reason I chose 458 as the one that was different was that it looked slightly different. It had some bubbles and lumps that the others did not have.
0000000006	Correct	
0000000007	Incorrect	298 has a stronger flavor.
0000000008	Incorrect	957 I believe was more sour than the other two samples
0000000009	Correct	They are all quite similar. The different one had a more pungent aftertaste which reminded me of aged cheese. The other two were clean like fresh milk.
0000000010	Incorrect	
0000000011	Incorrect	481 may have been slightly thicker. 281 seems more foamy, flavor is the same
0000000012	Incorrect	This one was slightly sweeter than the other two
0000000013	Correct	
0000000014	Correct	I think 374 had a thicker consistency and a lighter pungency.
0000000015	Correct	928 had a much stronger sour taste than the other two
0000000016	Incorrect	The other two samples kind of burn in the back of my throat. The 617 is a milder taste. It also seems to be thicker in my mouth than the other two.
0000000017	Incorrect	746 - too tart and sour 972 - less tart but still too much 189 - same as 972
0000000018	Correct	
0000000019	Correct	it was hard to choose which one is different, they taste very similar
0000000020	Correct	Not easy to to know which one is different from the 2 others.
0000000021	Correct	has like a bitter milkier taste
0000000022	Incorrect	
0000000023	Incorrect	A little more tart
0000000024	Incorrect	
0000000025	Correct	
0000000026	Incorrect	
0000000027	Incorrect	
0000000028	Incorrect	
0000000029	Correct	
0000000030	Incorrect	
0000000031	Incorrect	appeaerance is runnier, flavor is more subtle, and not quite as acidic
0000000032	Incorrect	
0000000033	Incorrect	less of an aftertaste
0000000034	Incorrect	They`re all very similar, I feel like 263 is a little sweeter.
0000000035	Correct	Tasted almost lumpier than the other ones.
0000000036	Incorrect	
0000000037	Correct	
0000000038	Correct	
0000000039	Incorrect	
0000000040	Correct	I feel like this sample was more sour? It also looks visually different to me.
0000000041	Incorrect	
0000000042	Incorrect	
0000000043	Incorrect	I`m not a culinary expert, so I don`t know the correct way to describe, but the different sample almost tastet butterier in a way.
0000000044	Correct	
0000000045	Correct	
0000000046	Incorrect	samples were very similar in test
0000000047	Correct	all are smooth and creamy
0000000048	Incorrect	All three samples tasted very similar but 458 might have had a slightly different taste,
0000000049	Incorrect	no comments
0000000050	Correct	rough
0000000051	Correct	
0000000052	Incorrect	i feel like 713 is not as tart as the other two
0000000053	Correct	I think 831 is smoother in taste. Less salty maybe?
0000000054	Correct	
0000000055	Incorrect	
0000000056	Incorrect	Taste is not good
0000000057	Correct	837 has a more tangy taste.
0000000058	Correct	I believe that I could tell the difference in the texture and look of the samples… also in the taste
0000000059	Incorrect	
0000000060	Correct	
0000000061	Incorrect	
0000000062	Incorrect	
0000000063	Correct	
0000000064	Correct	

Figure C.4. Control vs. WSU-1 yogurt sensory results and comments

Appendix D: Genomic information

WSU-1 Isolates 16S rRNA sequencing results in FASTA format

5-14, 27F primer

Trim length 757bp, Trimmed at 30 quality threshold, average quality 50.9

BLAST result: *Latilactobacillus curvatus* 99.6%

>27F_5-14_trim

GCAAGTCGAACGCACTCTCGTTAGATTGAAGAAGCTTGCTTCTGATTGATAACATTTGAGTGAGT
GGCGGACGGGTGAGTAACACGTGGGTAACTTGCCCTAAAGTGGGGGATAACATTTGGAAACAG
ATGCTAATACCGCATAAAACCTAGCACCGCATGGTGCAAGGTTGAAAGATGGTTTCGGCTATCACT
TTAGGATGGACCCGCGGTGCATTAGTTAGTTGGTGAGGTAAAGGCTCACCAAGACCGTGATGCAT
AGCCGACCTGAGAGGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAG
AAGGTTTTCGGATCGTAAAACTCTGTTGTTGGAGAAGAACGTATTTGATAGTAACTGATCAGGTAG
TGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAA
GCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGT
GGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGACACCAGTGGCGAAAGCGGCTG
TCTGGTCTGTAACTGACGCTGAGCTCGAAAGCATGGGTAGCAAACAGGA

5-14, 1492R primer (reverse compliment)

Trim length 739bp, Trimmed at 30 quality threshold, average quality 53.3

Blast result: *Latilactobacillus curvatus* 100%

>1492R_5-14_trim_reverse

CGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAGGATT
AGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAG
TGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG
AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT
TACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTTATTACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GATGGTACAACGAGTCGCGAGACCGCGAGGTTTAGCTAATCTCTTAAAACCATTCTCAGTTCGGAT
TGTAGGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGCC
GGTGAGGTAACCTTCGGGAGCCAGCCG

WSU-1 6-11, 27F primer

Trim length 571bp, Trimmed at 30 quality threshold, average quality 49.8

BLAST: *Latilactobacillus curvatus* 99.12%

>27F_6-11_trim

AGTCGAACGCACTCTCGTTAGATTGAAGAAGCTTGCTTCTGATTGATAACATTTGAGTGAGTGGC
GGACGGGTGAGTAACACGTGGGTAACCTGCCCTAAAGTGGGGGATAACATTTGGAAACAGATGC
TAATACCGCATAAAACCTAGCACCGCATGGTGCAAGGTTGAAAGATGGTTTCGGCTATCACTTTAG
GATGGACCCGCGGTGCATTAGTTAGTTGGTGAGGTAAAGGCTCACCAAGACCGTGATGCATAGCC
GACCTGAGAGGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGT
TTTCGGATCGTAAAACTCTGTTGTTGGAGAAGAACGTATTTGATAGTAACTGATCAGGTAGTGACG
GTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG
CGTTGTCCCGGATTTTATTGGGCGAAAAGCGAGCGCAGGCGGTTTTCTTTAAGT

WSU-1 6-11, 1492R primer (reverse compliment)

Trim length 599bp, trimmed at 30 threshold, quality average 46.6

BLAST: *Latilactobacillus curvatus* 99.83%

>1492R_6-11_trim_reverse
TGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG
AATTGACGGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTATTACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGG
AGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAAT
GGATGGTACAACGAGTCGCGAGACCGCGAGGTTTAGCTAATCTCTTAAAACCATTCTCAGTTCGG
ATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCG
GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAG
CCGGTGAGGTAACCTTC

WSU-1 Pac_seq1, 27F primer

Trim length 718bp, Trim threshold 30, quality average 50.2

BLAST: *Latilactobacillus curvatus* 99.86%

>27F_Pac_seq1_trim
TTGCTTCTGATTGATAACATTTGAGTGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCC
TAAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAAAACCTAGCACCGCATGGTGC
AAGGTTGAAAGATGGTTTCGGCTATCACTTTAGGATGGACCCGCGGTGCATTAGTTAGTTGGTGA
GGTAAAGGCTCACCAAGACCGTGATGCATAGCCGACCTGAGAGGGTAATCGGCCACACTGGGAC
TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTC
TGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTGGAGAAG
AACGTATTTGATAGTAACTGATCAGGTAGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGC
GCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACT
GGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATA
TGGAAGAACACCAGTGGCGAACGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCAT
GGGTAGCAAA

WSU-1 Pac_seq1, 1492R primer (reverse compliment)

Trim length 777bp, Trim threshold 30, quality average 49.9

BLAST: *Latilactobacillus curvatus* 100%

>1492R_Pac_seq1_trim_reverse

GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACG
CTGAGGCTCGAAAGCATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT
GAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCACTCCGCCT
GGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTA
GAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTACTAGTTGCCAGCATTTAGTTGG
GCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAAATCATCATGC
CCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTCGCGAGACCGCGAGGT
TTAGCTAATCTCTTAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGG
AATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGAGGTAACCTTCGGGAGCCAGCCGTCT

WSU-1, 9-22, 27F primer

Trim length 757, Trim threshold 30, quality 50.9

BLAST: *Latilactobacillus curvatus* 99.87%

>27F_9-22_trim

TGCAAGTCGAACGCACTTTCGTTAGATTGAAGAAGCTTGCTTCTGATTGATAACATTTGAGTGAGT
GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTAAAGTGGGGGATAACATTTGGAAACAG
ATGCTAATACCGCATAAAACCTAGCACCGCATGGTGCAAGGTTGAAAGATGGTTTCGGCTATCACT
TTAGGATGGACCCGCGGTGCATTAGTTAGTTGGTGAGGTAAAGGCTCACCAAGACCGTGATGCAT
AGCCGACCTGAGAGGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAG
AAGGTTTTCGGATCGTAAAACTCTGTTGTTGGAGAAGAACGTATTTGATAGTAACTGATCAGGTAG
TGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAA
GCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGT
GGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAACGCGGCT
GTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACA

WSU-1, 9-22, 1492R primer (reverse compliment)

Trim length 772, Trim threshold 30, quality 52.1

BLAST: *Latilactobacillus curvatus* 100%

>1492R_9-22_trim_reverse

GAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCT
GAGGCTCGAAAGCATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGA
GTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGG
GGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGA
GATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTACTAGTTGCCAGCATTTAGTTGGGCA
CTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCC

TTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTCGCGAGACCGCGAGGTTTA
GCTAATCTCTTAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAAT
CGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGAGAGTTTGTAACACCCAAAGCCGGTGAGGTAACCTTCGGGAGCCAGCCG

Fungal Isolates 18S rRNA sequencing results in FASTA format

M1

Trim length 573bp, Trimmed at 30 quality threshold, average quality 40

BLAST result: *Penicillium commune* 100%

>ITS1-F-M1

GTAGGTGACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGT
TTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGCTCACGCCCCCGGG
CCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCTGAGTGAAAATATAAAT
TATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT
ACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATT
CCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGT
CCTCCGATCTCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGT
ATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCA
GGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA

M3

Trim length 552bp, Trimmed at 30 quality threshold, average quality 41.4

BLAST result: *Penicillium citrinum* 100%

>ITS1-F-M3

GTAGGTGACCTGCGGAAGGATCATTACCGAGTGCGGGCCCCTCGGGGCCCAACCTCCCACCCGT
GTTGCCCGAACCTATGTTGCCTCGGCGGGCCCCGCGCCCGCCGACGGCCCCCCTGAACGCTGTCT
GAAGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCG
GCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCG
AGTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTG
CCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCCGGGGGGACGGGCCCGAAAGGC
AGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTAGTAGGCCCGG
CCGGCGCCAGCCGACCCCCAACCTTTAATTATCTCAGGTTGACCTCGGATCAGGTAGGGATACCC
GCTGAACTTAAGCATATCAATAAGCCGGAGGAAA

M4

Trim length 582bp, Trimmed at 30 quality threshold, average quality 40.2

BLAST result: *Penicillium decumbens* 99.31%

>ITS1-F-M4

CGTAGGTGACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTG
TCTATTGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGGCTTCTGCCCCCGGG
CCCGCGCCCGCCGAAGACACCATTGAACGCTGTCTGAAGATTGCAGTCTGAGCAATTAGCTAAAT
AAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGA
TACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGT

ATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTTAAGCACGGCTTATGTGTTGGGCCT
CCGTCCTTCCTTCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGC
GTATGGGGCTTCGTCACCCGCTCTGTAGGTCCGGCCGGCGCCTGCCGAACACATCAATCTTTTTTC
CAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAAAGGCAGGAGGA

M5

Trim length 523bp, Trimmed at 30 quality threshold, average quality 31.1

BLAST result: *Aspergillus cibarius* 86.36%

>ITS1-F-M5

GTGACCTGCGGAGGTTCATTACCGAGTGCGGGCCCTCTGGGTCCAACCTCCCATCCGTGTCTATCT
GTACCCTGTTGCTTCGGCGTGGCCACGGCCCGCCGAAGACTAACTTTTGAACACTGTCTGAATTT
TGCAGTCTGAGTTTTTAACTATGCAATAATTTTGTTTTCGAACGGATATCTTGGTTCTGCCATCTCG
GAAAAACGCACCTATAGGCGCCAATTTTAGTGAATTGCACAAATGATTGTGTCACCTCTTCTTTTTG
CACTCATTATCCGCCTTCGGTTTCCCCTGCATCCATGTTGGAAAGAGAGATTCCTTTCCTCAAAGCG
ATTGTGTGTTTTTTAAACCTTAAACTGCGTCTTCAGACGGGGTTCAAATGTTAGTCTTTCGGGGGG
GGCGCGCTCACGGCCGAAACCAAAGTCATACGCTTCCACTCGGTCGGGGTGGCCCTCTTCTCGCC
CCAATCTTTTTATCCAGGGTTGACCCTCTGACCATGCAGGGATACTCCGCTGAACTTTTA

M6

Trim length 576bp, Trimmed at 30 quality threshold, average quality 44.4

BLAST result: *Penicillium roqueforti* 99.65%

>ITS1-F-M6

TAGGTGACCTGCGGAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTTT
ATTTACCTTATTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGGTTTACACCCCCGGGCCC
GCGCCCGCCGAAGACACCCCGAACTCTGTCTGAAGAATGCAGTCTGAGAACAAATATAAATTATTT
AAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATACGT
AATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCG
GGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCTCGTCCT
CCGATTCTGGAGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGG
GGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGG

M7

Trim length 373bp, Trimmed at 30 quality threshold, average quality 38.8

BLAST result: *Penicillium chrysogenum* 94.64%

>ITS1-F-M7

GTGACCTGCGGAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTTTATT
TTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGCTTACCCCCCCGGGCCCGC
GCCCGCCTAACACACCCTCCAACTCTGTCTGAATATTGTACTCTGAGTGAAAATATAAATTATTTAAA
ACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATAACTAACGCAGCGAAATGCCCACGTAAAGT
GAATTGCAATTTCAGTGAATCAGCAATTCTTTGAACGCAGATTGCGCCCCCTGGTATTCCGGGGGG
GCATGCCTGTCCGATCGTCATTGCTGCCCTCAACCACGGCTTGG

M8

Trim length 539bp, Trimmed at 30 quality threshold, average quality 42.6

BLAST result: *Phoma* subspecies 98.89 %

>ITS1-F-M8

CGTAGGTGACCTGCGGAGGATCATTACCTAGAGTTGTAGGCTTTGCCTGCTATCTCTTACCCATGTC
TTTTGAGTACCTTCGTTTCCTCGGCGGGTTCGCCCGCCGATTGGACAATTTAAACCATTTGCAGTT
GCAATCAGCGTCTGAAAAAACTTAATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCTTC
AAGCTCTGCTTGGTGTTGGGTGTTTGTCTCGCCTCTGCGCGTAGACTCGCCTCAAAACAATTGGC
AGCCGGCGTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCATTCAGAACGACGACGTCCAA
AAGTACATTTTTACACTCTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA
AGGCGGAGGAAA

M9

Trim length 630bp, Trimmed at 30 quality threshold, average quality 49.4

BLAST result: *Mucor racemosus* 99.51%

>ITS1-F-M9

GTAGGTGACCTGCGGAGGATCATTAAATAATCAATAATCTTGGCTTGTCCATTATTATCTATTTACTG
TGAACTGTATTATTATTTGACATTCGAGGGATGTTTCAATGTTATAAGGATAGACATTGGAAATGTT
AACCGAGTCATAATCAGGTTTAGGCCTGGTATCCTATTATTATTTACCAAATGAATTCAGAATTAATA
TTGTAACATAGACCTAAAAAATCTATAAAACAACTTTTAACAACGGATCTCTTGGTTCTCGCATCGA
TGAAGAACGTAGCAAAGTGCGATAACTAGTGTGAATTGCATATTCAGTGAATCATCGAGTCTTTGA
ACGCAACTTGCGCTCATTGGTATTCCAATGAGCACACCTGTTTCAGTATCAAAACAAACCCTCTATC
CAACTTTTGTTGAATAGGATTATTGGGGGCCTCTCGATCTGTATTGATCTTGAAACCCTTGAAATGT
ACTAAGGCCTGAACTTGTTTAATGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGTAATTGAC
TTTGATGGGGCCTCCCAAATAAATCTCTTTAAATTTGATCTGAAATCAGGTGGGATTACCCGCTGA
ACTTAAGCATATCAATAAGCGGAGGAA

M10

Trim length 601bp, Trimmed at 30 quality threshold, average quality 45.6

BLAST result: *Trichoderma amazonicum* 99.33%

>ITS1-F-M10

GTGACCAGCGGAGGGACATTACCGAGTTTACAACTCCCAAACCCAATGTGAACGTTACCAAACTG
TTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCGCAGCCCCGGACCAAGGCGCCCGCCGGAGG
ACCAACCAAAACTCTTATTGTATACCCCCTCGCGGGTTTTTTTATAATCTGAGCCTTCTCGGCGCCC
CTCGTGGGCGTTTCGAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCC
CTCCGGGGGGTCGGCGTTGGGGATCGGCCCTCCCTCTGCGGGGGCCGTCTCCGAAATACAGTGG
CGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCACCGGGAGCGCGGCGCGTCC
ACAGCCGTTAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT
TAAGCATATCATAA

Y1
Trim length 444bp, Trimmed at 30 quality threshold, average quality 39.8
BLAST result: *Pichia fermentans* 98.97%
>ITS1-F-Y1
TTCGTAGGTGACCTGCGGAAGGATCATTACTGTGATTTATCCACCACACTGCGTGGGCGACACGA
AACACCGAAACCGAACGCACGCCGTCAAGCAAGAAATCCACAAAACTTTCAACAACGGATCTCTT
GGTTCTCGCATCGATGAAGAGCGCAGCGAAATGCGATACCTAGTGTGAATTGCAGCCATCGTGAA
TCATCGAGTTCTTGAACGCACATTGCGCCCGCTGGTATTCCGGCGGGCATGCCTGTCTGAGCGTC
GTTTCCTTCTTGGAGCGCAGCTTCAGACCTGGCGGGCTGTCTTTGTGGACGGCGCGCCCAAAGC
GAGGGGCCTTCTGCGCGAACTAGACTGTGCGCGCGGGGCGGCCGGCGAACTTATTACCAGCTCC
AACCCCAAACCAGGAAGAATAACCCGTGAACTTTAACCTATTCATAAGGCGAAGAA

Y3
Trim length 384bp, Trimmed at 30 quality threshold, average quality 38.8
BLAST result: *Clavispora lusitaniae* 98.96%
>ITS1-F-Y3
GTTTCCGTAGGTGACCTGCGGAGGATCATTAAAATAATACTTACACTTTGCATTTGCGAACAAAAA
AATAAATCTTTTATTCCAATTTCTTAATATCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCG
ATGAAGAACGCAGCGAATTGCGATACGTAGTATGACTTGCAGACGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCTCGAGGCATTCCTCGAGGCATGCCTGTTTGAGCGTCGCATCCCCTCTAACCCCC
GGTTAGGCGTTGCTCCGAAATATCAACCGCGCTGTCAAACACGTTTACAGCACGACATTTCGCCCT
CAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAAAAAGCGGAGGAA

Y4
Trim length 634bp, Trimmed at 30 quality threshold, average quality 42.2
BLAST result: *Debaryomyces hansenii* 99.37%
>ITS1-F-Y4
GTGACCTGCGGAAGGACATTACAGTATTCTTTTTGCCAGCGCTTAATTGCGCGGCGAAAAAACCT
TACACACAGTGTTTTTTGTTATTACAAGAACTTTTGCTTTGGTCTGGACTAGAAATAGTTTGGGCC
AGAGGTTTACTGAACTAAACTTCAATATTTATATTGAATTGTTATTTATTTAATTGTCAATTTGTTGAT
TAAATTCAAAAAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCTTCGGGTTTG
GTATTGAGTGATACTCTTAGTTGAACTAGGCGTTTGCTTGAAATGTATTGGCATGAGTGGTACTGG
ATAGTGCTATATGACTTTCAATGTATTAGGTTTATCCAACTCGTTGAATAGTTTAATGGTATATTTCTC
GGTATTCTAGGCTCGGCCTTACAATATAACAAACAAGTTTGACCTCAAATCAGGTAGGATTACCCG
CTGAACTTAAGCATATCAATAAAGCCGGAGGAAA

Y5
Trim length 425bp, Trimmed at 30 quality threshold, average quality 39.4
BLAST result: *Debaryomyces* 98.82 %
>ITS1-F-Y5
CGTAGGTGACCTGCGGAAGGACATTACAGTATTCTTTTTGCCAGCGCTTAATTGCGCGGCGAAAA
AACCTTACACACAGTGTTTTTTGTTATTACAAGAACTTTTGCTTTGGTCTGGACTAGAAATAGTTTG

GGCCAGAGGTTTACTGAACTAAACTTCAATATTTATATTGAATTGTTATTTATTTAATTGTCAATTTGT
TGATTAAATTCAAAAAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCTTCTGG
TTTGGGATTGAGTGATAATCTTAAT

Y6
Trim length 606bp, Trimmed at 30 quality threshold, average quality 46.4
BLAST result: *Candida zeylanoides* 96.72%
>ITS1-F-Y6
GTAGGTGACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAATTGCGCGGCGAAAAA
CCTTACACACTATGTTTTTTTGATTTGAAACTTTTGCTTTGGTCTGACTTAGAAATGAGTTGGGCCA
AAGGTTTTATACTAAAACTTCAATTTTATTATTGAATTGTTAATTAATTATATTGTCAATTTGTTGATTA
AATTCAAAAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGC
CCTATGGTATTCCATAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAATCTTCGGATTTGGTTTT
GAGTGATACTCTTAGTCAGACTAAGCGTTTGCTTGAAATGTATTGGCATGAGTGGTACTACATAGT
GCTAAACTGTTTCAATGTATTAAGCTTATCCAACTCGTTGACCAGTATAGTATTTGTTTATTTACACA
GGGTCGGCCTTACAACTGTAAAGGATAGTTTGACCTCTCATCTAGGAGAGACTACCCCGCTGAAC
TTAA

Y8
Trim length 562bp, Trimmed at 30 quality threshold, average quality 45.4
BLAST result: *Rhodotorula mucilaginosa* 100%
>ITS1-F-Y8
TCATTAGTGAATATAGGACGTCCAACTTAACTTGGAGTCCGAACTCTCACTTTCTAACCCTGTGCAC
TTGTTTGGGATAGTAACTCTCGCAAGAGAGCGAACTCCTATTCACTTATAAACACAAAGTCTATGA
ATGTATTAAATTTTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
ACCTTGCGCTCCATGGTATTCCGTGGAGCATGCCTGTTTGAGTGTCATGAATACTTCAACCCTCCTC
TTTCTTAATGATTGAAGAGGTGTTTGGTTTCTGAGCGCTGCTGGCCTTTACGGTCTAGCTCGTTCG
TAATGCATTAGCATCCGCAATCGAACTTCGGATTGACTTGGCGTAATAGACTATTCGCTGAGGAATT
CTAGTCTTCGGACTAGAGCCGGGTTGGGTTAAAGGAAGCTTCTAATCAGAATGTCTACATTTTAAG
ATTAGATCTCAAATCAGGTAGGACTACCCG

Y9
Trim length 598bp, Trimmed at 30 quality threshold, average quality 41.4
BLAST result: *Meyerozyma guilliermondii* 99.50%
>ITS1-F-Y9
TTCCGTAGGTGACCTGCGGAAGGACATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCGGCGAA
AAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGG
GCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAATTTTGAATTAATCTTCAA
AACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
ATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAG

GGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGT
CGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTGGATAGTGCTGTCGACCTCTCA
ATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCT
TACAACAACCAAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCATA
AG

Y10
Trim length bp, Trimmed at 30 quality threshold, average quality 43.8
BLAST result: *Torulaspora delbrueckii* 99.87 %
>ITS1-F-Y10
TTCCGTAGGTGACCTGCGGAAGGATCATTAGAGAAATCTATATGAATGAAGTTAGAGGACGTCTAA
AGATACTGTAAGAGAGGATCTGGTTCAAGACCAGCGCTTAATTGCGCGGTTGCGGCTTGGTTCGC
CTTTTGCGGAACATGTCTTTTCTCGTTGTTAACTCTACTTCAACTTCTACAACACTGTGGAGTTTTC
TACACAACTTTTCTTCTTTGGGAAGATACGTCTTGTGCGTGCTTCCCAGAGGTGACAAACACAAAC
AACTTTTTATTATTATAAACCAGTCAAAACCAATTTCGTTATGAAATTAAAAATATTTAAAACTTTCA
ACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTG
CAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCAGGGGGCATGC
CTGTTTGAGCGTCATTTCCTTCTCAAACAATCATGTTTGGTAGTGAGTGATACTCTGTCAAGGGTTA
ACTTGAAATTGCTAGCCTGTTATTTGGTTGTGATTTTGCTGGCTTGGATGACTTTGTCCAGTCTAGC
TAATACCGAATTGTCGTATTAGGTTTTACCAACTTCGGCAGACTGTGTGTTGGCTCGGGCGCTTTA
AAGACTTTGTCGTAAACGATTTATCGTTTGTTTGAGCTTTTCGCATACGCAATCCGGCGAACAATAC
TCTCAAAGTTTGACCTCAAATCAGTAGGAATACCCGCTGAACTTAAGC

Appendix E: R Code

WSU-1 ANOVA Code

```
# Read data from the CSV file
your_data <- read.csv()

# Convert factors to appropriate types
your_data$Treatment <- as.factor(your_data$Treatment)
your_data$Replication <- as.factor(your_data$Replication)
your_data$Week <- as.factor(your_data$Week)

# Load necessary libraries
library(tidyverse)

# Create a function to run ANOVA and Tukey HSD for a specific week
run_anova_tukey <- function(week_data, week_number) {
  anova_results <- aov(Yeast.Count ~ Treatment + Replication, data = week_data)

  print(paste("ANOVA for Week", week_number, ":"))
  print(summary(anova_results))

  # Tukey HSD post-hoc test
  tukey_results <- TukeyHSD(anova_results)
  print("Tukey HSD post-hoc:")
  print(tukey_results)

  # Boxplot for visualization
  boxplot(Count ~ Treatment, data = week_data, main = paste("WSU-1", week_number),
col = "lightblue")
}

# Run one-way ANOVA for each week
for (week in unique(your_data$Week)) {
  subset_data <- subset(your_data, Week == week)

  # Call the function for each week
  run_anova_tukey(subset_data, week)
}
```

WSU-1 Line Graph Code

```
# Load necessary libraries
library(ggplot2)
```

```
# Specify the name of your dataset
your_data <- WSU_1_Count  # Replace WSU-10 with the name of your dataset

# Create a function to compute mean and standard error
compute_mean_se <- function(data) {
  mean_value <- mean(data)
  se_value <- sd(data) / sqrt(length(data))
  return(c(mean = mean_value, se = se_value))
}

# Calculate mean and standard error for each Treatment and Day combination
mean_se_data <- aggregate(Count ~ Treatment + Day, data = your_data, FUN =
compute_mean_se)

# Extract mean and standard error values
mean_se_data$mean <- mean_se_data$Count[, "mean"]
mean_se_data$se <- mean_se_data$Count[, "se"]

# Define the name of the dataset
dataset_name <- "WSU-1_Count"  # Change this to the name of your dataset

# Create line plot with error bars
p <- ggplot(mean_se_data, aes(x = Day, y = mean, group = Treatment)) +
  geom_line(linewidth = 0.3,
            color = "black") +
  geom_point(aes(shape = Treatment),
             size = 2,
             color = "black") +
  ylim(0,8) +
  geom_errorbar(aes(ymin = mean - se, ymax = mean + se), width = 0.6) +
  labs(title = paste("WSU-1 Count Over Time"),
       x = "Day",
       y = expression(Log[10] * "(CFU/g)")
  ) +
  theme(axis.text.y = element_text(color = "black", size = 15, face = "bold"),
        axis.text.x = element_text(color = "black", size = 15, face = "bold"),
        axis.title.x = element_text(size = 15, face = "bold", color = "black"),
        axis.title.y = element_text(size = 15, face = "bold", color = "black"),
        panel.background = element_blank(),
        axis.line = element_line(),
        legend.key = element_blank(),
        legend.text = element_text(size = 15, face = "bold"),
        legend.title = element_text(size = 15, face = "bold"),
        plot.title = element_text(hjust = 0.5, size = 18, face = "bold"),) +
  scale_x_continuous(breaks = c(5, 12, 19, 26))
```

```
# Print the plot
print(p)
```

Yogurt Trial Yeast ANOVA Code

```
#load in data to a variable
your_data <- Y1_Trial_1

#convert columns to factor type
your_data$Treatment <- as.factor(your_data$Treatment)
your_data$Replication <- as.factor(your_data$Replication)
your_data$Week <- as.factor(your_data$Week)

#create empty dataframes to add anova and tukey hsd values to
anova_results_table <- data.frame(Week=factor(), anova_p_value=numeric())
tukey_results_table <- data.frame(Week=factor(), comparison=factor(),
tukey_p_value=numeric())
tukey_results_table_replication <- data.frame(Week=factor(), comparison=factor(),
tukey_p_value=numeric())


# Create a function to run ANOVA and Tukey HSD for a specific week
run_anova_tukey <- function(week_data, week_number) {
  anova_results <- aov(Yeast_Count ~ Treatment + Replication, data = week_data)

  #print(paste("ANOVA for Week", week_number, ":"))
  #print(summary(anova_results))
  capture_a <- summary(anova_results)
  capture.output(capture_a, file = paste(week_number, "_anovaresults.txt", sep=""))
  #this retrieves just the p-value from anova
  anova_p_value <- summary(anova_results)[[1]]$`Pr(>F)`[1]
  #this populates the data frame with the p-values and week number
  anova_results_table <<- rbind(anova_results_table, data.frame(Week = week_number,
anova_p_value = anova_p_value))

  # Tukey HSD post-hoc test
  tukey_results <- TukeyHSD(anova_results)
  #print("Tukey HSD post-hoc:")
  #print(tukey_results)
  treatment_results <- data.frame(tukey_results$Treatment)
  p_adj_values <- treatment_results[, "p.adj"]
  comparison_levels <- rownames(treatment_results)
  tukey_df <- data.frame(comparison = comparison_levels, p.adj = p_adj_values)
```

```
  tukey_df$Week <- week_number
  tukey_results_table <<- rbind(tukey_results_table, tukey_df)
  replicate_results <- data.frame(tukey_results$Replication)
  p_adj_values_replication <- replicate_results[, "p.adj"]
  comparison_levels_replicates <- rownames(replicate_results)
  tukey_df_replicates <- data.frame(comparison = comparison_levels_replicates, p.adj =
p_adj_values_replication)
  tukey_df_replicates$Week <- week_number
  tukey_results_table_replication <<- rbind(tukey_results_table_replication,
tukey_df_replicates)

  # Boxplot for visualization
  boxplot(Yeast_Count ~ Treatment, data = week_data, main = paste("Y1 Week",
week_number), col = "lightblue")
}

# Run one-way ANOVA for each week
for (week in unique(your_data$Week)) {
  subset_data <- subset(your_data, Week == week)

  # Call the function for each week
  run_anova_tukey(subset_data, week)
}

#write results to csv
write.csv(anova_results_table, "anova_results.csv", row.names = FALSE)
write.csv(tukey_results_table, "tukey_results.csv", row.names = FALSE)
write.csv(tukey_results_table_replication, "tukey_results_replicates.csv", row.names =
FALSE)
```

Yogurt Trial Yeast Combined Line Graph Code

```
# Load necessary libraries
library(ggplot2)
library(dplyr)

# List of datasets
datasets <- list(Y1 = Y1, Y3 = Y3, Y4 = Y4, Y5 = Y5, Y6 = Y6, Y8 = Y8, Y9 = Y9, Y10
= Y10)

# Create a function to compute mean and standard error
compute_mean_se <- function(data) {
  mean_value <- mean(data)
  se_value <- sd(data) / sqrt(length(data))
  return(c(mean = mean_value, se = se_value))
}
```

```
# Process each dataset and combine them into a single dataframe
combined_data <- do.call(rbind, lapply(names(datasets), function(name) {
  data <- datasets[[name]]
  mean_se_data <- aggregate(Yeast_Count ~ Treatment + Day, data = data, FUN =
compute_mean_se)
  mean_se_data$mean <- mean_se_data$Yeast_Count[, "mean"]
  mean_se_data$se <- mean_se_data$Yeast_Count[, "se"]
  mean_se_data$Dataset <- name
  return(mean_se_data)
}))

# Convert Dataset to a factor with the desired order
combined_data$Dataset <- factor(combined_data$Dataset, levels = c("Y1", "Y3", "Y4",
"Y5", "Y6", "Y8", "Y9", "Y10"))

# Create line plot with error bars and faceting
p <- ggplot(combined_data, aes(x = Day, y = mean, group = Treatment)) +
  geom_line(linewidth = 0.3, color = "black") +
  geom_point(aes(shape = Treatment), size = 2, color = "black") +
  ylim(0,8) +
  geom_errorbar(aes(ymin = mean - se, ymax = mean + se), width = 0.6) +
  labs(x = "Day", y = expression(Log[10] * "(CFU/g)")) +
  theme(axis.text.y = element_text(color = "black", size = 12, face = "bold"),
        axis.text.x = element_text(color = "black", size = 12, face = "bold"),
        axis.title.x = element_text(size = 15, face = "bold"),
        axis.title.y = element_text(size = 15, face = "bold"),
        panel.background = element_blank(),
        axis.line = element_line(),
        legend.key = element_blank(),
        legend.text = element_text(size = 12, face = "bold"),
        legend.title = element_text(size = 15, face = "bold"),
        legend.position = c(1, 0),
        legend.justification = c("right", "bottom"),
        legend.margin = margin(1, 1, 1, 1),
        legend.background = element_blank(),
        legend.box.background = element_rect(color = "black"),
        plot.title = element_text(hjust = 0.5, size = 18, face = "bold"),
        strip.text = element_text(size = 12, face = "bold", color = "black")) +
  scale_x_continuous(breaks = c(0, 5, 12, 19, 26)) +
  facet_wrap(~ Dataset, ncol = 3)  #Adjust the number of columns as needed

# Print the plot
print(p)
```

Mold Krukal Wallis, Dunn Post Hoc Code

```
# Load necessary libraries
library(tidyr)
library(FSA)
library(dplyr)

# Read the CSV file
data <- M10
dataset_name <- "M10"  # Set the dataset name
week_number <- 5  # Set the week number

# Convert Treatment types to factors
data$Treatment <- as.factor(data$Treatment)

# Filter out the week of interest
WeekData <- subset(data, Week == week_number)

# Kruskal-Wallis test
kruskal_result <- kruskal.test(Score ~ Treatment, data = WeekData)

# Print Kruskal-Wallis test results
print(kruskal_result)

# Save Kruskal-Wallis test results to a file
sink(paste0(dataset_name, "_Week_", week_number, "_kruskal_wallis_results.txt"))
print(kruskal_result)
sink()

# If the Kruskal-Wallis test is significant, perform Dunn's test
if (kruskal_result$p.value < 0.05) {
  dunn_result <- dunnTest(Score ~ Treatment, data = WeekData, method = "bonferroni")

  # Print Dunn's test results
  print(dunn_result)

  # Save Dunn's test results to a file
  write.table(dunn_result$res, file = paste0(dataset_name, "_Week_", week_number,
"_dunn_test_results.txt"), sep = "\t", row.names = FALSE)
} else {
  print("Kruskal-Wallis test is not significant, no post-hoc test performed.")

  # Save message to a file
  sink(paste0(dataset_name, "_Week_", week_number, "_dunn_test_results.txt"))
  cat("Kruskal-Wallis test is not significant, no post-hoc test performed.")
  sink()
}
```

Mold Combined Bar Graph Code

```
# Load necessary libraries
library(ggplot2)
library(dplyr)

# Assuming your dataset names and file names for each dataset
dataset_names <- c("M1", "M3", "M4", "M5", "M6", "M7", "M8", "M9", "M10")
file_names <- c("M1", "M3", "M4", "M5", "M6", "M7", "M8", "M9", "M10")

# Function to compute mean and standard error
compute_mean_se <- function(data) {
  mean_value <- mean(data)
  se_value <- sd(data) / sqrt(length(data))
  return(c(mean = mean_value, se = se_value))
}

# List to store aggregated dataframes
aggregated_data <- list()

# Process each dataset
for (i in seq_along(dataset_names)) {
  # Load dataset
  data <- get(file_names[i])

  # Aggregate data to compute mean and standard error
  aggregated <- data %>%
    group_by(Treatment, Week) %>%
    summarise(Score_mean = mean(Score),
              Score_se = sd(Score) / sqrt(n())) %>%
    mutate(Dataset = dataset_names[i])

  # Store aggregated dataframe
  aggregated_data[[i]] <- aggregated
}

# Combine all aggregated datasets into one dataframe
combined_data <- bind_rows(aggregated_data)

# Ensure Dataset factor levels are in the desired order
combined_data$Dataset <- factor(combined_data$Dataset, levels = dataset_names)

# Create bar plot with error bars and faceting
p <- ggplot(combined_data, aes(x = as.factor(Week), y = Score_mean, fill = Treatment))
+
```

```
  geom_bar(stat = "identity", position = "dodge", width = 0.8) +
  geom_errorbar(aes(ymin = Score_mean - Score_se, ymax = Score_mean + Score_se),
                position = position_dodge(width = 0.8), width = 0.25) +
  labs(x = "Week", y = "Average Score") +
  scale_fill_manual(values = c('gray25', 'gray50', 'gray75')) +
  theme_classic() +
  theme(axis.text.x = element_text(size = 12, face = "bold", color = "Black"),
        axis.text.y = element_text(size = 12, face = "bold", color = "Black"),
        axis.title.x = element_text(size = 15, face = "bold", color = "Black"),
        axis.title.y = element_text(size = 15, face = "bold", color = "Black"),
        legend.position = "bottom",  # Place legend at the bottom
        legend.title = element_blank(),# Remove legend title
        legend.box.background = element_rect(color = "black", linewidth = 1),
        strip.text = element_text(size = 12, face = "bold", color = "Black"),
        strip.background = element_blank()) +
  facet_wrap(~ Dataset, ncol = 3)  # Adjust the number of columns as needed

# Print the plot
print(p)
```

Yogurt Sugar analysis code

```
# Assuming the data frame is already loaded as Yogurt_Galactose

# Convert ID and Week to factors (if not already)
Yogurt_Galactose$ID <- as.factor(Yogurt_Galactose$ID)
Yogurt_Galactose$Week <- as.factor(Yogurt_Galactose$Week)

# Perform ANOVA
anova_result <- aov(Concentration ~ ID + Week, data = Yogurt_Galactose)

# Display the ANOVA result summary
anova_summary <- summary(anova_result)
print(anova_summary)

anova_table <- anova_summary[[1]]
# Extract the p-value
p_value <- anova_summary[[1]][["Pr(>F)"]][1]
write.csv(anova_table, "ANOVA_table.csv", row.names = FALSE)
# Perform Tukey's HSD test

tukey_result <- TukeyHSD(anova_result)
print(tukey_result)

# Extract and format Tukey HSD results
tukey_df <- as.data.frame(tukey_result[[1]])
```

```
# Extract comparisons and format as data frame
comparisons <- data.frame(comparison = rownames(tukey_df))
tukey_df <- cbind(comparisons, tukey_df)

# Save Tukey HSD results to CSV file
write.csv(tukey_df, "TukeyHSD_table.csv", row.names = FALSE)

# Load necessary libraries
library(ggplot2)
library(dplyr)

# Calculate means and confidence intervals
summary_stats <- Yogurt_Galactose %>%
  group_by(ID, Week) %>%
  summarise(
    Mean = mean(Concentration),
    SD = sd(Concentration),
    N = n(),
    SE = SD / sqrt(N),
    CI95 = qt(0.975, N-1) * SE
  )

# Create the bar graph with error bars and custom colors
bar_plot <- ggplot(summary_stats, aes(x = interaction(ID, Week), y = Mean, fill =
Week)) +
  geom_bar(stat = "identity", position = position_dodge(), color = "black") +
  geom_errorbar(aes(ymin = Mean - CI95, ymax = Mean + CI95),
                width = 0.2, position = position_dodge(0.9)) +
  labs(title = "Yogurt Galactose Concentration",
       x = "Sample ID and Week",
       y = "Galactose (g/L)",
       fill = "Week") +
  scale_fill_manual(values = c('gray50', 'gray25')) +  # Apply custom colors
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1, face = 'bold', size =12),
        plot.title = element_text(hjust = 0.5, face = 'bold', size = 15),
        panel.background = element_blank(),
        panel.grid = element_blank(),
        axis.title.x = element_text(size = 15),
        axis.title.y = element_text(size = 15),
        axis.text.y = element_text(face = 'bold', size = 12),
        legend.text = element_text(size = 12),
        legend.title = element_text(size =15),
        plot.background = element_rect(fill = "white")) +
  scale_y_continuous(breaks = c(0, 10, 20, 30, 40, 50))
```

```
print(bar_plot)

ggsave("bar_plot.png", plot = bar_plot, width = 5, height = 6)
```

Delta E Code

```
# Load necessary library
library(farver)

# Define the LAB values for each treatment and time point
data <- data.frame(
  Treatment = c("C", "W", "WG"),
  L1 = c(91.79, 91.69, 91.55),
  L4 = c(91.59, 91.59, 91.33),
  a1 = c(-2.82, -2.84, -2.90),
  a4 = c(-2.76, -2.70, -2.84),
  b1 = c(8.68, 8.58, 8.66),
  b4 = c(8.67, 8.45, 8.71)
)

# Function to calculate delta E
calculate_deltaE <- function(L1, a1, b1, L2, a2, b2) {
  lab1 <- matrix(c(L1, a1, b1), ncol = 3)
  lab2 <- matrix(c(L2, a2, b2), ncol = 3)
  deltaE <- farver::compare_colour(from = lab1, to = lab2, from_space = "lab", method =
"CIE2000")
  return(deltaE)
}

# Calculate delta E for W compared with C at Week 1 and Week 4
deltaE_W_C_1 <- calculate_deltaE(data$L1[2], data$a1[2], data$b1[2], data$L1[1],
data$a1[1], data$b1[1])
deltaE_W_C_4 <- calculate_deltaE(data$L4[2], data$a4[2], data$b4[2], data$L4[1],
data$a4[1], data$b4[1])

# Calculate delta E for WG compared with C at Week 1 and Week 4
deltaE_WG_C_1 <- calculate_deltaE(data$L1[3], data$a1[3], data$b1[3], data$L1[1],
data$a1[1], data$b1[1])
deltaE_WG_C_4 <- calculate_deltaE(data$L4[3], data$a4[3], data$b4[3], data$L4[1],
data$a4[1], data$b4[1])

# Combine the results into a data frame
results <- data.frame(
  Comparison = c("W vs C at Week 1", "W vs C at Week 4", "WG vs C at Week 1", "WG
vs C at Week 4"),
```

```
  DeltaE = c(deltaE_W_C_1, deltaE_W_C_4, deltaE_WG_C_1, deltaE_WG_C_4)
)

# Export the results to a CSV file
write.csv(results, "deltaE_results.csv", row.names = FALSE)

# Print results
cat("Delta E for W compared with C at Week 1:", deltaE_W_C_1, "\n")
cat("Delta E for W compared with C at Week 4:", deltaE_W_C_4, "\n")
cat("Delta E for WG compared with C at Week 1:", deltaE_WG_C_1, "\n")
cat("Delta E for WG compared with C at Week 4:", deltaE_WG_C_4, "\n")

# Confirmation message
cat("Results have been exported to 'deltaE_results.csv'\n")
```