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Use of Protective Lactic Acid Bacteria Adjunct Cultures to Decrease the Incidence of Gas Defects in Cheddar Cheese

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USE OF PROTECTIVE LACTIC ACID BACTERIA ADJUNCT CULTURES TO DECREASE THE INCIDENCE OF GAS DEFECTS IN CHEDDAR CHEESE

by

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ABSTRACT

The Use of Protective Lactic Acid Bacteria Adjunct Cultures to Decrease the Incidence of Gas Defects in Cheddar Cheese Production.

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Heterofermentative non-starter lactic acid bacteria (NSLAB) can pose a major problem in the dairy industry by causing late-stage gas formation defects in Cheddar cheese, which are characterized by slits, cracks, and blown bags. Slits and cracks make the cheese more difficult to shred and slice, and, along with the blown bags, cause the cheese to be less appealing to the customer. These defects can also cause the cheese to be downgraded to a lower margin product, which reduces manufacturer's profits. Heterofermentative NSLAB have the ability use six-carbon sugars, like galactose, to produce carbon dioxide. Recently, starter cultures like *Streptococcus thermophilus* have been used to increase the rate of acid production during Cheddar cheese production, which increases the risk of gas production due to its inability to ferment galactose. The primary objective of this research was to manufacture cheese using previously identified galactose positive and lactose negative protective adjunct cultures, as well as other protective adjunct cultures that we believed could decrease the amount of gas produced by heterofermentative NSLAB. The adjunct cultures effect on gas production was

determined by challenging them with *Levilactobacillus brevis* 277-1, *Limosilactobacillus fermentum* 305-1, *Lentilactobacillus parabuchneri,* and *Paucilactobacillus wasatchensis* WDCO4. These four cultures are all known gas producing heterofermentative NSLAB.

The selected protective adjunct cultures were *Lacticaseibacillus rhamnosus* 20DK04, *Lacticaseibacillus paracasei* 20DK06, *Pediococcus acidilactici* 23F, and *Latilactobacillus curvatus* WSU1. These protective adjunct cultures were added to the milk at the beginning of the cheese make along with the *St. thermophilus* and *Lactococcus lactis* starter cultures. The following day the cheese was ground and inoculated with individually grown cultures of the heterofermentative NSLAB in duplicate for a final concentration of 10^4 CFU/g in the cheese. This yielded a total of 10 samples including controls. These 10 samples were then pressed back into blocks, after which each sample was cut into seven 450 g sub-samples and vacuum sealed. Gas levels were checked weekly for 16 weeks for all samples. This process was done in duplicate for every protective adjunct culture.

The results showed there is potential for using protective adjunct cultures to reduce late gas production in Cheddar cheese. Most notably, there were reductions in gas production when *Lat. curvatus* WSU1 was challenged with *Lev. brevis* 277-1, and when *Lcb. rhamnosus* 20DK04 and *Lcb. paracasei* 20DK06 were each challenged with *Pa. wasatchensis* WDC04. However, there was an increase in gas production and the gas production rate when *P. acidilactici* 23F was challenged with *Pa. wasatchensis* WDC04. When comparing the number of subsamples in each sample group that were able to reach certain levels of gas, there were also many differences, and many protective adjunct cultures reduced this number between of 50% and 100%. Again, however, in some cases

it increases the number of subsamples that reached the selected levels of gas. The selected adjunct cultures showed promise, especially for a primary cause of decreasing late-stage gas production. The protective adjunct culture that is best suited for each nonstarter Heterofermentative Lactic acid bacteria is strain specific, pointing for the need of a cocktail of cultures for the best results. (88 pages)

PUBLIC ABSTRACT

The Use of Protective Lactic Acid Bacteria Adjunct Cultures to Decrease the Incidence of Gas Defects in Cheddar Cheese Production

Rhees Crompton

Gas production in cheese making is becoming increasingly prevalent in the dairy industry. This gas is produced by microbes that are naturally found in the cheese, and when they metabolize sugar or other sources of energy, they can produce gas. This gas causes slits and cracks in the cheese, which causes the cheese to be worth less and causes issues during slicing and shredding. There are many microbes that cause unwanted gas in cheese, this research focuses on four know gas producers and five other protective microbes that use the same energy sources or have the ability to inhibit the gas producers in some way. Each protective microbe was challenged with all four of the gas producing microbes. The BUILD Dairy program of the Western Dairy Center granted the funding, so that this research could take place. This funding was used to support graduate students as well as for laboratory and manufacturing expenses.

The Information that this study yielded data about how the use of protective adjunct cultures can be used to reduce gas production caused by gas producing microbes has been presented at national dairy and cheese industry conferences.

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First and foremost, I would like to thank the wonderful people of the BUILD Dairy Program and the Western dairy center for helping me complete this research. I would also like to thank each of my committee members for their years of guidance, and support. I would not be where I am today without their input, patience and without them constantly pushing me in the right direction.

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LIST OF ABBREVIATION

SLAB = Starter lactic acid bacteria

NSLAB = Non-starter lactic acid bacteria

ALAB = Adjunct lactic acid bacteria

LAB = Lactic acid bacteria

CFU/mL= Colony forming units/milliliter

MRS = de Man, Rogosa, and Sharpe media

MRS+R = de Man, Rogosa, and Sharpe media supplemented with 1% ribose

M17+L = M17 media supplemented with 1% Lactose.

OD = OD_{600} = optical density at 600 nm

OD:1 = optical density 1.00 at 600 nm

INTRODUCTION

Problem Statement

Late gas formation, which causes splits and cracks in Cheddar cheese, is a major problem in the dairy industry. Carbon dioxide production in cheese can be caused by heterofermentative non-starter lactic acid bacteria (NSLAB) as they metabolize residual hexose sugars producing CO_2 as a byproduct in aging cheese. These gas defects are most commonly manifest as splits, cracks, and a general openness in the body of the cheese. These defects are becoming more prevalent in the dairy industry as manufacturers try to increase the speed of cheese production by using starter cultures that produce acid at a faster rate. One example is the use of *Streptococcus thermophilus* instead of, or in addition to, the more traditional *Lactococcus* strains. One consequence of using *S. thermophilus* as a starter culture is that it only ferments the glucose portion of lactose while exporting the galactose moiety out of the cell into the cheese. (Michel, V., & Martley, F. G. 2001). Heterofermentative NSLAB can then metabolize the galactose producing CO_2 as a byproduct. As the CO_2 accumulates, it causes splits and cracks, which makes slicing more difficult, and blown packaging that decreases consumer acceptance. These factors can eventually cause the cheese to be downgraded to a lower margin product.

Previous research has investigated the potential of using lactic acid bacteria (LAB) adjunct cultures that metabolize the residual galactose but cannot ferment lactose, which ensures that starter culture activity is not affected. Several cultures were identified that ferment the residual galactose without utilizing lactose, then reducing the amount of gas produced by *Paucilactobacillus wasatchensis* WDCO4 in vitro (Green et al., 2021).

This decrease in gas production was determined by competitive growth studies utilizing Durham fermentation tubes in carbohydrate restricted MRS broth. This is not the only way that cultures for this study were chosen however, some of the cultures in this study were chosen for their ability to sequester ions and for their ability to produce antimicrobial compounds.

The aim of this research is to test the use of previously identified lactic acid bacteria adjunct cultures (ALAB) to reduce gas production in Cheddar cheese. The cultures selected for this project as protective adjuncts are *Lacticaseibacillus rhamnosus* 20DK04, *Lacticaseibacillus paracasei* 20DK06, *Pediococcus acidilactici* 23F, and *Latilactobacillus curvatus* WSU1. The gas producing challenge cultures selected were *Limosilactobacillus fermentum* 305-1, *Levilactobacillus brevis* 277-1, *Lentilactobacillus parabuchneri,* and *Paucilactobacillus wasatchensis* WDC04.

HYPOTHESIS AND OBJECTEVES

Hypothesis – Adding adjunct cultures during cheese production will cause inhibition of heterofermentative non-starter lactic acid bacteria growth and gas production through competitive inhibition and other factors.

Objectives.

1. Manufacture Cheddar two vats of cheese for each selected adjunct cultures to determine which will have the most potential for reducing late-stage gas production by using blend of *St. thermophilus* and *L. lactis* as a starter culture and adding Lcb. *rhamnosus* 20DK04, *Lcb. paracasei* 20DK06, *P. acidilactici* 23F, or *Lat. curvatus* WSU1 as an adjunct culture.
2. Challenge the ALAB by adding heterofermentative NSLAB known to cause gas, resulting in splitting and cracking defects in aged Cheddar cheese by grinding and then inoculating the ALAB containing cheese with selected heterofermentative NSLAB, making sure that the challenge culture in uniformly added to the cheese.
3. Determine how the addition of each ALAB affects microbial inhibition and gas production in Cheddar cheese by vacuum sealing the cheese and measuring how much gas is produced in the headspace above the cheese during aging.

LITERATURE REVIEW

Cheddar Cheese

Cheddar cheese is a semi-hard cheese variety which has a smooth body, close texture, and clean nutty flavor (Varnam and Sutherland, 1994). The color of Cheddar cheese will vary from region to region but should generally be a very light straw color in natural cheese and a yellow orange for colored cheese. The desired body for Cheddar cheese has been described as a solid, close-knit plug, possessing smoothness, meatiness, waxiness, and silkiness, and should be void of gas holes. (Agricultural economics and Management, 2021). The desired flavor characteristics depend on where you are in the world. A study by Drake et al. (2008) determined American consumers prefer their Cheddar cheese to have flavor characteristics of umami, sulfur, and meat soup. Irish and Asian consumers, on the other hand, prefer sweet fruit-like flavors (McEwan et al., 1989; Wang et al., 2021).

During cheese manufacture, starter lactic acid bacteria (SLAB) are added and allowed to ripen after which the milk is coagulated/set using rennet (chymosin). The set vat is then cut into curd particles, continuously stirred, and cooked until it reaches the desired pH. This stirring and working process helps expel whey from the curd particles. After the cheese curd has been cooked, the whey is removed, and the Cheddaring process begins. Briefly, the curd particles knit together into cheese slabs, which are flipped and stacked, allowing time for whey expulsion and lactic acid production until the matted cheese reaches the desired pH. The cheese is then milled, salted, and pressed. Aged Cheddar cheese is usually ripened for at least 3 months and upwards of 12 months (Papademas and Bintisis, 2017)

Lactic Acid Bacteria and Starter Lactic Acid Bacteria

Lactic acid bacteria (LAB) along with other microorganisms are naturally found in raw milk and have been shown to survive pasteurization. These LAB are known as non-starter lactic acid bacteria (NSLAB) and are very important in cheese production because they provide enzymes that hydrolyze casein, which produces flavor compounds. Milk is an ideal environment for microbial growth due to its neutral pH, high water activity, and nutrient density, which is why most commercial dairy product producers choose to pasteurize the raw milk prior to cheese making (Montville and Matthews, 2017; Green et al., 2021). Milk that has been completely sterilized and is void of NSLAB produces very bland flavored cheese, so only pasteurization temperatures that kill potentially pathogenic microorganisms are used. This allows the NSLAB and some enzymes to remain active for the production of many desired and unique flavor compounds found in Cheddar cheese (Hutkins, 2006). The pasteurization process uses temperatures that kill harmful bacteria and is required by law in many cases. Pasteurization also has the added benefit of inactivating many enzymes that can produce off flavors and reduces the number of NSLAB found in the milk, which allows for more consistent ripening outcomes (Tillocca et al., 2020).

After pasteurization, SLAB are deliberately added to milk at the beginning of the cheese manufacturing process before rennet addition. Starter cultures are preferred by most commercial manufacturers because they allow for a quicker and more predictable cheese making process. Starter cultures can be added in many different forms but in recent years frozen direct vat set cultures have become the norm. Not only does this reduce the risk of contamination, but it also helps to reduce the number of dead vats and

can be a more viable option for certain manufacturers (Hutkins, 2006). After SLAB addition, an initial ripening step allows the starter to regain its metabolic activity and start replicating. If the proper concentration of SLAB is not reached or the initial ripening step is skipped, the metabolic activity of the SLAB could be reduced causing the lactic acid production to be slower, which could potentially increase the amount of time for the pH of the milk to drop to the desired level or stop it from reaching the proper final pH entirely. Starter cultures also play an important role in protein breakdown and texture development of the final product (Orsi and Zambrini, 2017).

Traditionally, SLAB used for Cheddar cheese were a mix of *Lactococcus lactis* and *Lactococcus cremoris* strains. More recently there has been a shift towards other SLAB that produce acid at a faster rate. One example is *Streptococcus thermophilus* which was traditionally used for yogurt, Swiss and Italian cheese production. With an optimal growth temperature between 35 - 47^{o}C, it maintains faster acid production after cooking when compared to the traditional *L. lactis* which prefers temperatures between 30 - 34^{o}C (Green et al, 2021; Harnett, 2011). Because of this, *S. thermophilus* allows for a much faster make time during cheese manufacture and it also provides resistance to *L. lactis* phage. Interestingly, it lacks the tagatose-6-P or Leloir pathway which means it cannot ferment galactose as a form of energy (Michel and Martley, 2001; Wu et al., 2015). In order to prevent the buildup of galactose intracellularly, *S. thermophilus* excretes the galactose into the cheese matrix through a lactose-galactose antiporter. The expelled galactose can cause some flavor and texture defects, and can later be utilized for energy by NSLAB, which can produce CO_2 as a metabolic byproduct (Harnett, 2011; Hutkins, 2006; De Vos and Vaughn, 1994).

Gas producing heterofermentative NSLABS

During the first few weeks of cheese ripening, the lactose levels are between 0.7% and 1.7%, but are quickly depleted after which the SLAB start to die off (Turner and Thomas, 1980). The NSLAB that are present in the milk or gain access to the milk during processing start to become more prevalent by using other carbohydrates besides lactose as an energy source eventually growing to high numbers (Naylor and Sharpe, 1958; Peterson and Marshall, 1990; Martley and Crow, 1993; Somers et al., 2000; Williams et al, 2000). When milk is pasteurized for commercial cheese making, this reduces the number of undesirable NSLAB that can cause defects during the ripening process. Unfortunately, some unwanted NSLAB either survive pasteurization or the milk might be inoculated post pasteurization by a contaminated processing environment. Sources of NSLAB contamination include the air, processing equipment, and biofilms found in cheese vats or pipes used to transport the milk (Crow et al., 1995). Nonstarter lactic acid bacteria can become a problem if they are heterofermentative, meaning they lack the fructose diphosphate aldolase enzyme. This enzyme cleaves hexose sugars into glyceraldahyde-3-phosphate and dihydroxy-acetone-phosphate, and without this enzyme these LAB are prevented from using the Embden-Myerhoff pathway. Instead, heterofermentative NSLAB must utilize the pentose phosphate pathway, which metabolizes hexose sugars through pyruvate and acetyl-phosphate intermediates producing lactic acid as well as CO_2 and ethanol. Cheddar cheese made with starter cultures like *S. thermophilus* are at greater risk for gas defects because certain heterofermentative NSLAB can ferment the built-up galactose left in the cheese matrix by *S. thermophilus* (Jordan and Cogan, 1993; Crow et al., 2001; Banks and Williams,

2004; Ortakci et al., 2015).

The end products of hexose sugar metabolism by NSLAB can produce undesirable flavors as well as body defects like splits and cracks caused from the accumulation of gas in the cheese (Hayek and Brahim 2013; Kahilid and Marth, 1990). Studies on microorganisms responsible for late gas defect in cheese are abundant and many microorganisms responsible for the gas defect in cheese have been identified (Bassi et al., 2015). When the conditions are suitable (e.g., higher ripening temperatures and high initial numbers compared to other adventitious lactobacilli), heterofermentative NSLAB grow to, and remain at, high cell densities ($>10^7$ CFU/g) throughout cheese ripening. This is a major problem in the dairy industry as unwanted gas formation in Cheddar cheese is recurrent and widespread (Mullan, 2000; Ortakci et al, 2014).

There have been many ways proposed to control heterofermentative NSLAB, but these organisms are extremely versatile and can utilize many different substrates (Beresford and Williams, 2004). For example, *Pa. wasatchensis* WDC04 has been found to utilize ribose, galactose, and sodium gluconate, so even if producers control one risk factor, there could be another that was unforeseen (Green et al., 2021; Oberg et al., 2021). Additionally, most NSLAB can also grow at low pH and lack salt inhibition, which are common features of aging cheese that prevent continued growth of other microorganisms (Jordan and Cogan, 1993).

Factors that cause downgrading to Lower Margin Products

Lacticaseibacillus casei/paracasei, Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus, Levilactobacillus brevis 277-1, *Latilactobacillus curvatus* WSU1, and *Limosilactobacillus fermentum* 305-1 are typically grouped together as

NSLAB. As a group, these NSLAB are often considered to intensify cheese flavor, and hard cheeses containing selected strains of these NSLAB as cultures are generally superior to pasteurized milk cheeses without them, and are described as being closer to raw milk cheeses but with a cleaner milder flavor (Dacre, 1958; Fryer and Sharpe, 1966; Chapmen and Sharpe, 1990; Broome, Krause and Hickey, 1990; Jordan and Cogan, 1993; Broadbent et al., 2004). Although NSLAB do produce flavor that is desirable, they can produce inconsistent flavor development in the Cheddar cheese if left unchecked. In addition, off flavors commonly associated with NSLAB growth that can cause downgrading are pungent, sour, or acidic, cardboard, soapy, bitter, and rancid caused by excessive lipolysis (Deeth, 2006).

The body and texture of the cheese plays a major role in the grading process. It should be solid, compact, and free of any holes or cracks. Nonstarter lactic acid bacteria which are salt tolerant can cause the development of undesirable texture and body defects including gas accumulation in Cheddar-style and brine salted cheeses (Laleye et al., 1987; Khalid and Marth, 1990; Dacre, 2009; Sheehan, 2011) Slits and cracks in Cheddar cheese are often attributed to the growth of heterofermentative lactobacilli during aging. In most cases these slits are caused by the formation of CO_2 through the fermentation of citrate, lactose, galactose, amino acids, or a combination of these substrates during cheese ripening by heterofermentative NSLAB (McMahon et al, 2022). *Latilactobacillus curvatus* WSU1, *Lim. fermentum* 305-1, *Lev. brevis* 277-1, *Len. parabuchneri*, and *Pa. wasatchensis* WDC04 are common heterofermentative NSLAB found in commercial Cheddar cheese which exhibit the slit defect and this excessive CO_2 can even cause unwanted gas pockets and blowing of packaged cheeses in the retail environment (Hayek

& Ibrahim 2013). A change in the finish and color of the cheese can also cause downgrading to lower margin products. Some common criticisms for these categories are cracked rinds, light spots, moldy, uneven surfaces, seamy and wavy (USDA 1956).

The accumulation of gas in Cheddar cheese caused by heterofermentative NSLAB causes Cheddar cheese to develop splits and cracks leading to issues with cutting or slicing this cheese. This requires downgrading the cheese to lower margin products due to consumer acceptability. Heterofermentative NSLAB can also cause off flavors due to the volatile flavor compounds that they produce. Cheese made using starter cultures like *S. thermophilus* are more prone to these defects because they do not utilize the residual galactose in the cheese, allowing it to be utilized by heterofermentative NSLAB. There are several ways that this can be controlled. These include lowering the ripening temperature to allow the *L. lactis* to do more of the fermentation, using only lactose positive and galactose positive starters, increasing pasteurization temperatures to eliminate more of the NSLAB, and eliminating the use of gluconate which can increase gas production. The problem with these options is that they all impact the manufacturing and storage of the cheese, so a microbial remedy for the unwanted gas production would be very valuable.

MATERIALS AND METHODS

Cultures

Adjunct cultures *Lcb. rhamnosus* 20DK04, *Lcb. paracasei*, and *P. acidilactici* 23F were previously identified as galactose positive and lactose negative. The fourth protective ALAB *Lat. curvatus* WSU1 was selected because it produces potential antimicrobial compounds (Green et al., 2021). Freezer stocks were made by growing the cultures in MRS broth for 12 h and then transferring 500 µL of the culture into 2 mL screw top cryotubes along with 500 µL of a sterile 50% glycerol solution. This was done for all cultures and the tubes were stored at -80ºC. As needed these freezer stocks were used to inoculate MRS broth and incubated at 32ºC for 12 h. The same procedure was followed for the heterofermentative NSLAB *Lim. fermentum* 305-1, *Lev. brevis* 277-1, and *Len. parabuchneri*. However, *Pa. wasatchensis* WDC04 was grown using MRS+R and incubated at 25ºC 12 h.

After the initial 12 h incubation, the heterofermentative NSLAB challenge cultures were standardized by back diluting the samples with MRS until they reached an OD_{600} = 1.0 on the spectrophotometer to ensure the cultures started at the same concentration each time. The newly standardized cultures were then placed in the incubator for another 12 hrs. The standardized challenge cultures were then serially diluted to determine the CFU/mL. This data was then used to calculate how much inoculum would need to be added to 10 ml of BPW so that the final concentration of challenge cultures would be 10^4 CFU/g in the cheese. The 10 mL of culture was put in aseptic spray bottles which would be used to inoculate the cheese.

Vancomycin Resistance and Petrifilm Plating Method

Once the cultures were confirmed as pure and viable, all cultures were tested for vancomycin resistance. This was done by growing each culture in MRS broth and on MRSA plates that contained 50 µg/ml of vancomycin. To confirm that the selected LAB would grow on 3M LAB 338XH5 Petrifilm in the presence of vancomycin, 0.15g vancomycin was added to 10 mL Buffered Peptone Water (BPW). For each LAB, 0.5 mL of the BPW plus vancomycin solution was combined with 1 mL of the diluted LAB grown from the freezer stock and the full 1.5 mL was plated on the LAB Petrifilm. To confirm that the protective ALAB and heterofermentative NSLAB would grow together on the Petrifilm, each ALAB was combined with each of the challenge cultures and plated using the same process described above.

This plating method served two purposes. First, it selected for the ALAB and the challenge cultures because they were vancomycin resistant while inhibiting SLABS because they are vancomycin sensitive and, secondly by adding 1.5 mL of solution onto the Petrifilm any problems with water activity that has been previously observed when using Petri films was mitigated. The challenge cultures were differentiated from the ALAB by the gas bubbles they produce around the colonies. This allowed us to count both heterofermentative LAB and ALAB cultures from the cheese samples on the same Petrifilm.

Cheese Manufacture

The cheese was produced at the Gary Haight Richardson Dairy Products Laboratory at Utah State University and the milk was obtained from the Utah State University George B. Caine Dairy Research and Teaching Center (Wellsville, UT). Once the milk was delivered, it was standardized to a protein-to-fat ratio of 0.83

and pasteurized according to the PMO. Two Tetra Scherping horizontal cheese vats (Tetra Pak Cheese & Powder Systems, Inc., Winsted, MN) were filled with 680 kg of milk each.

As seen in in the make sheets (Appendix A), Vat 1 was immediately heated to 31°C and 61 g of thawed and homogenous starter containing both *St. thermophilus* and *L. lactis* (A3040; Chr. Hansen Inc., Milwaukee, WI) was used to inoculate the milk along with the indicated ALAB, which had been grown under controlled conditions in a biofermentor, at the beginning of the cheese manufacture. After a 5-minute ripening time, 0.073 mL/kg double strength (~650 International milk clotting units/ml) chymosin rennet (Maxiren; DSM Food Specialties USA Inc., Eagleville, PA) was added. The milk was then stirred for eight minutes and allowed to set for 30 minutes. After the milk was set, cut, and healed for 15 minutes, the curd and whey were continuously stirred and cooked by gradually heating the vat to 39°C over 35 minutes and then stirred for 15 minutes at that temperature.

The curd and whey were pumped to the drain table (Kusel Equipment Co., Watertown, WI) and mechanically agitated. Once the curd reached a pH of 6.30, the whey was drained off and the remaining curd stirred for 10 more passes of the agitator. The curd was then allowed to mat together and Cheddared until the curd pH reached 5.40. The curd was then milled and salted to a final salt concentration of 1.7% w/w by manually adding the salt 1/3 at a time and waiting five minutes between each application. Finally, 6 hoops containing 10.4 kg of curd were pressed into blocks for three hours, vacuum sealed, and placed in the cooler.

After the curd and whey from Vat 1 was pumped to the drain table, the make for Vat 2 was started and followed the same procedure outlined above with the drain table being cleaned and sanitized before the second vat of the day was pumped over.

Challenge Culture Inoculation

The following day after the cheese was cooled, one block was set aside as a control and the rest of the cheese blocks were comminuted to less than 4 mm size pieces using the medium cutting head on a Comitrol Processor Model 3640 (Urschel Laboratories Inc.). The comminuted cheese was then split into 10 equal samples and each sample was inoculated with one of the challenge organisms in duplicate, yielding 2 biological replicates per challenge culture. This was achieved by spraying the cheese while agitating with each culture for a final concentration of 10^4 CFU/g. The inoculated ground cheese was then pressed back into blocks for 3 hours, vacuum sealed and placed in the cooler overnight. The following day each of the newly formed blocks were cut into seven 450 g blocks and vacuum sealed. This produced a total of 7 samples for gas measurement of each of the two replicates for each of the challenge cultures per vat (Table 1). Three additional samples per replicate were also retained for microbiological testing at 1, 8, and 16 weeks to determine starter and non-starter lactic acid bacteria counts. Cultures used as ALAB were *Lcb. rhamnosus* 20DK04, *Lcb. paracasei, P. acidilactici* 23F, and *Lat. curvatus* WSU1, while gas-producing challenge cultures were *Lim. fermentum* 305-1, *Lev. brevis* 277-1, *Len. parabuchneri,* and *Pa. wasatchensis* WDC04.

Gas Measurement

Utilizing the method described by McMahon et al. (2022), the distance from the block of cheese to the position where the two sides of the vacuum sealed bag were still held together by the vacuum seal was measured. The gas levels of each cheese were measured in mm and recorded every week.

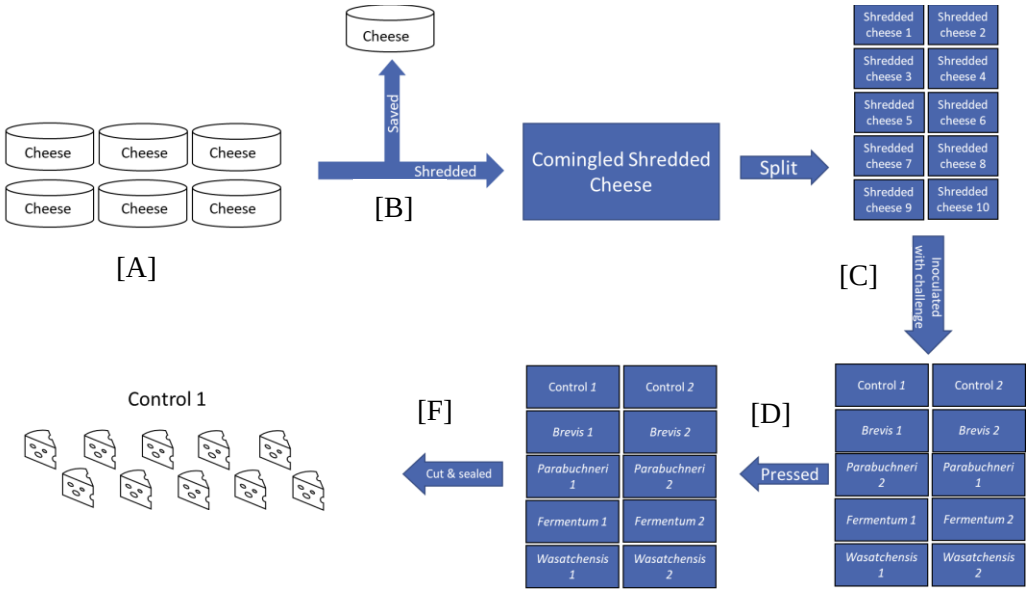

Figure 1. Heterofermentative non-starter lactic acid bacteria inoculation into cheese flow chart, totaling 7 subsamples for gas measurement and 3 for microbiological testing per group. [A] = Cheese blocks from previous day, [B] = cheese comminuted to less than 4 mm size pieces using the medium cutting head on a Comitrol Processor Model 3640 (Urschel Laboratories Inc.) [C]= cheese inoculated with biological replicates of challenge cultures, [D]= Samples were pressed back into blocks for 3 hours then vacuum sealed and stored in the cooler overnight, [F]= Cheese cut into 450 g blocks and vacuum sealed.

Table 1. Randomization of the application of the gas producing challenge cultures to each vat and the day each was performed. (Vat 1 & Vat 6 = no protective culture, Vat 2 & Vat 3 = *Lacticaseibacillus rhamnosus* 20DK04, Vat 4 & Vat 9 = *Latilactobacillus curvatus* WSU1, Vat 5 & Vat 8 = *Lacticaseibacillus paracasei* 20DK06, Vat 7 & Vat 10 = *Pediococcus acidilactici* 23F)

	Day 1		Day 2		Day 3		Day 4		Day 5	
Challenge Culture	Vat 1	Vat 2	Vat 3	Vat 4	Vat 5	Vat 6	Vat 7	Vat 8	Vat 9	Vat 10
Control	10	9	8	9	9	3	6	1	10	3
Control	4	10	9	4	8	5	10	5	8	7
Lev. brevis 277-1	3	6	6	6	6	9	3	4	4	8
Lev. brevis 277-1	8	2	2	7	1	4	4	10	9	9
Lim. fermentum 305-1	6	3	10	1	2	8	2	7	2	1
Lim. fermentum 305-1	2	7	3	5	7	1	7	2	3	4
Len. parabuchneri	9	5	1	8	10	7	8	9	7	5
Len. parabuchneri	1	8	7	10	3	2	5	6	5	2
Pa. wasatchensis WDC04	7	1	4	3	4	10	1	8	1	10
Pa. wasatchensis WDC04	5	4	5	2	5	6	9	3	6	6

Microbial testing

Each cheese replicate was tested on week 1, 8, and 16. The cheese was first diluted by adding 11g of cheese to 99 mL of sterile buffered peptone water (BPW) and then stomached at 260 bpm for 2 min. The stomached sample was diluted to 10^{-6} using 9 mL BPW dilution blanks, then spread plated on two sets of M17 plus lactose (M17+L), and aerobically incubated at 40^{o}C and 32^{o}C for thermophilic and mesophilic starters, respectively. Samples were also plated on MRS plates and incubated in anerobic jars at 32^{o}C for total NSLAB enumeration.

The challenge and ALAB were counted using 3M LAB Petri films. After the

samples were diluted out to 10^{-6}, 1 mL of sample was combined with 0.5 mL of sterile BPW containing vancomycin (150 µg/mL), then the full 1.5 mL was added to the Petri film following manufacture's protocol.

Week 1

- 10^{-6} and 10^{-7} MRS spread plates inoculated anaerobically at 32ºC to determine the number of total NSLAB.
- Two sets of 10^{-6} and 10^{-7} M17+lactose spread plates using the control replicates for each vat were incubated at 32ºC and 42ºC to determine the SLAB counts in duplicate.
- Two sets of 10^{-2} and 10^{-3} Petrifilms were incubated aerobically to determine the number gas producing bacteria and ALAB present.

Week 8

- 10^{-5} and 10^{-6} MRS spread plates inoculated anaerobically at 32ºC to determine the number of total NSLAB.
- Two sets of 10^{-5} and 10^{-6} M17+lactose spread plates using the control replicates for each vat were incubated at 32ºC and 42ºC to determine the SLAB counts.
- Two sets of 10^{-2} and 10^{-3} Petrifilms were incubated aerobically to determine the number gas producing bacteria and ALAB cultures present.

Week 16

- 10^{-4} and 10^{-5} MRS spread plates inoculated anaerobically at 32ºC to determine the number of total NSLAB.
- Two sets of 10^{-5} and 10^{-6} M17+lactose spread plates using the control replicates for each vat were incubated at 32ºC and 42ºC to determine the SLAB counts.
- Two sets of 10^{-3} and 10^{-4} Petrifilms were incubated aerobically to determine the number

gas producing bacteria and ALAB cultures present.

Proximate Analysis

Cheese samples were taken from every vat of cheese produced and frozen after manufacture at -20ºC until analyzed. Cheese pH was measured on week 1 cheese along with cheese composition. Moisture was measured in triplicate by drying 2 to 4 g of shredded cheese for 18 hours in a hot air oven at 95ºC. The fat content of the cheese was determined by using the Babcock method (Wehr and Frank, 2004). Cheese pH was measured using a glass electrode after blending shredded cheese with distilled water in a 2:1 ratio. The salt was measured by adding 5.00 g of cheese into 98.20 g of distilled water, which was stomached at 260 rpm for 4 min. The sample was allowed to rest for 15 minutes and then the slurry was filtered to remove any cheese particles. Salt content was measured using a chloride analyzer (Model 926; Corning). Cheese samples used for determining galactose and lactose concentrations were prepared using the same procedure as for salt determination. Lactose and galactose were measured using the enzyme analysis kit LACGR (Megazyme Inc.).

Statistical Analysis & Experimental Design

There were five vats made in duplicate for a total of ten vats, which resulted in two vats for each protective ALAB culture and two control vats. There were five challenge cultures per vat with those five cultures propagated independently in duplicate for a total of ten samples per vat. Finally, each of the ten samples inoculated with the challenge cultures were cut and packaged into seven 450 g subsamples. Gas production for each subsample was measured each week for 16 weeks. This experimental design

provides a total of four repetitions and five treatments. The research project was designed in this way because it was the most cost-effective way to determine how the ALAB and challenge cultures would affect each other.

To analyze the gas production data, we took the weekly data from each subsample and first removed the high and low for each week, then calculated the average of the remaining five subsamples. The number of days it took each sample group to reach selected levels of gas was calculated through extrapolation and statistical analysis was performed through SAS using an ANOVA and T test. (JMP 2016).

RESULTS AND DISCUSSION

Proximate Cheese Composition

Because different protective ALAB cultures were added to the cheese on different days there was some variation in the salt, moisture, fat, and pH of the cheese when measured at week one after the manufacture. These variations fell within the target ranges for each category so it was concluded that these variations would not influence the growth of the ALAB or challenge cultures (Table 2).

Salt and Moisture

Salt and moisture levels were determined as they are better indicators of the salt level in the water phase of the cheese. The salt level for each vat of cheese produced was within the selected target range with means from 1.56 to 1.63%. The moisture levels for each vat were in the range of Cheddar cheese, except the mean for the cheese containing *P. acidilactici* 23F (37.86%) which was just outside the target range of 35.50 to 37.50%. Because this cheese had the highest moisture and one of the lowest salt percentages, it had a S/M of 3.95 which was just outside of the S/M target range of 4.0 to 4.5 (Table 2). However, there was no observed reduction of growth in any of the LAB at this lower S/M, thus it was determined that this did not affect the results. The higher moisture could have been caused by decreased time on the drain table due to the speed at which the SLAB lowered the pH once the curd had been pumped over the drain table. This decreased the amount of time for whey expulsion, which in turn, increased the final moisture.

Table 2. Mean initial proximate cheese composition and substrate levels. (Salt measured using Corning chloride analyzer 926. Moisture measured using oven drying method. Fat measured using Babcock method. Galactose and Lactose measured using Megazyme assay kit).

	Proximate				
Protective Cultures	%Salt	%Moisture	S/M	Fat	Galactose%
Control	1.56	36.48	4.10	33.12	0.27
Lcb. rhamnosus 20DK04	1.59	35.78	4.26	33.34	0.30
Lcb. paracasei 20DK06	1.63	36.63	4.26	33.67	0.30
P. acidilactici 23F	1.56	37.86	3.95	34.16	0.29
Lat. curvatus WSU1	1.56	36.93	4.07	34.75	0.29
Target range	1.50-1.80	35.5-37.5	4.0-4.5	33-35	0.25-0.5

Starter LAB

As seen in Figure 2, SLAB levels were measured at week one, eight and sixteen. The *St. thermophilus* starter levels performed as expected and showed a reduction over time from 10^{-8} CFU/gram at week one to 10^{-7} CFU/gram at week sixteen. There was an increase in streptococcal starter culture death in the cheese that had the protective culture *Lat. curvatus* WSU1. These cheeses reached 10^{-6} CFU/gram by week sixteen, which was statistically significantly different when compared to the other cheeses ($P = 0.005$).

Lactococcal starter levels were measured at the same time intervals, and they also showed a reduction over time from 10^{8} CFU/gram at week one to 10^{7} CFU/gram at week sixteen. None of the samples from the control cheeses that had been treated with protective cultures differed in lactococcal starter concentrations when compared with the untreated control. (Figure 3).

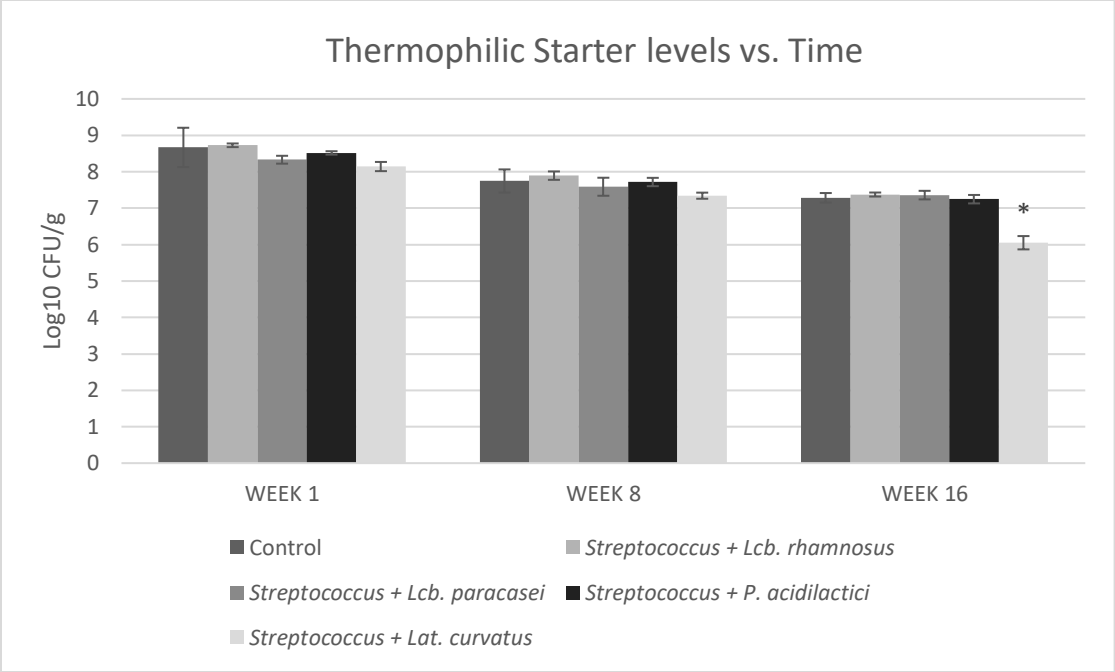

Figure 2. Concentration of thermophilic *Streptococcus thermophilus* starter culture over time. as measured using plate counting (Log10 CFU/gram). * indicates statistical significance within that time point compared to the other groups. Error bars=SE, n=4.

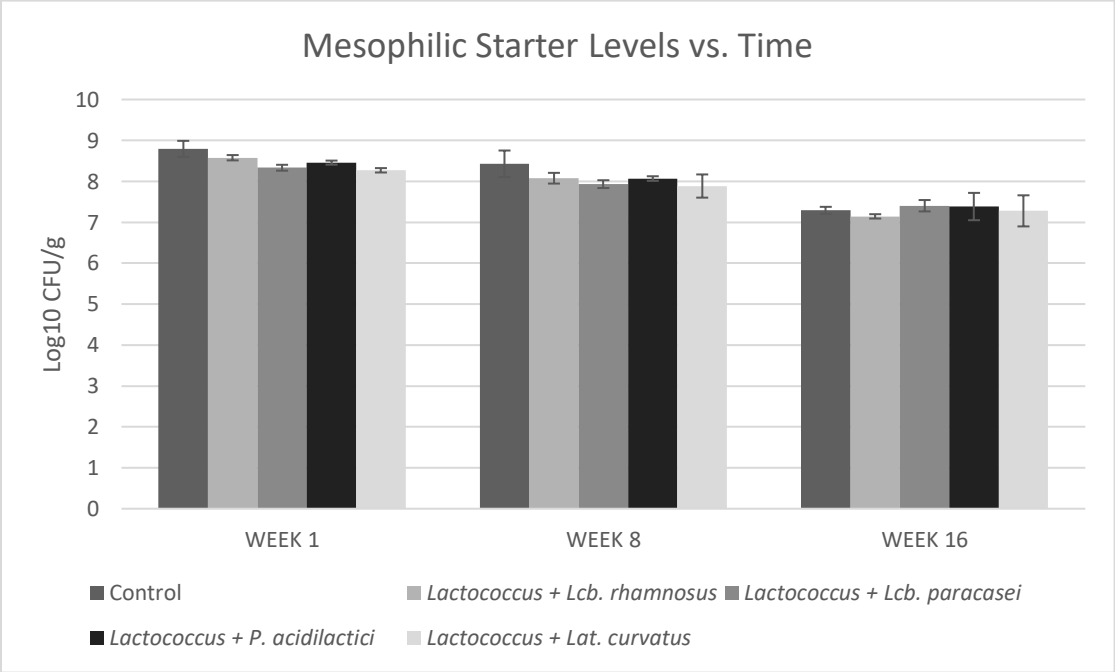

Figure 3. Concentration of mesophilic *Lactococcus* starter cultures over time. as measured using plate counting (Log10 CFU/gram). * indicates statistical significance within that time point compared to the other groups, Error bars=SE, n=4. No statistically significant differences within each time point.

Protective adjunct culture levels

After inoculation, all protective ALAB were able to grow to 10^8 CFU/g by week 1. From week 1 to week 16 the protective culture that showed the greatest reduction was *Lcb. rhamnosus* 20DK04, which showed a full log reduction from 10^8 to 10^7 CFU/g in all samples with one exception. When *Lcb. rhamnosus* 20DK04 was challenged with *Pa. wasatchensis* WDC04 it showed a reduction in viable cell counts from 10^8 to 10^6 CFU/g at week 16. During the first week of ripening when compared to other *Lcb. rhamnosus* 20DK04 + challenge culture cheeses only *Len. parabuchneri* showed a significant effect on the viable cell counts of *Lcb. rhamnosus* 20DK04 ($P = 0.022$). However, this reduction in cell counts was resolved by week 8 where only *Lcb. rhamnosus* 20DK04 + *Lim. fermentum* 305-1 cheeses showed a significant reduction in cell counts compared to the other cheeses. By week 16, there was no significance between any of the *Lcb. rhamnosus* 20DK04 protective culture cheeses (Figure 4).

Both *Lcb. paracasei* 20DK06 and *P. acidilactici* 23F showed a similar reduction in cell counts over time with a few exceptions. When *Lcb. paracasei* 20DK06 was challenged with *Lev. brevis* 277-1 and *Lim. fermentum* 305-1, it showed an even greater reduction in cell counts from week 1 to week 16 (Figure 5). Conversely, *P. acidilactici* 23F had an even reduction over time for all challenge cultures except *Pa. wasatchensis* WDC04, which showed less reduction in cell number (Figure 6). When comparing how different challenge cultures affect *Lcb. paracasei*, there was no initial difference in cell counts at week one, but by week 8 *Lcb. paracasei* 20DK06 + Control and *Lcb. paracasei* 20DK06 + *Len. parabuchneri* were significantly different from each other ($P = 0.020$), with the latter having higher *Lcb. paracasei* 20DK06 cell counts then all other

combinations. Week 16 shows that *Lcb. paracasei* 20DK06 samples containing *Len. parabuchneri* and *Pa. wasatchensis* WDC04 contained the highest number of *Lcb. paracasei* 20DK06 cell counts with samples containing *Lim. fermentum* 305-1 having significantly lower *Lcb. paracasei* 20DK06 levels ($P = 0.018$) and ($P = 0.035$) when compared, respectively (Figure 5).

Similarly, *P. acidilactici* showed no difference in CFU/g at the week 1, but started to differentiate by week 8, and by week 16 *P. acidilactici* 23F levels in samples containing *Paucilactobacillus wasatchensis* WDC04 were significantly higher than those samples containing *Lev. brevis* 277-1 ($P = 0.005$) and *Lim fermentum* ($P = 0.016$) (Figure 6).

Latilactobacillus curvatus WSU1 showed a one-half log reduction which was the least amount of reduction from week 1 to week 16 for any of the protective cultures. When *Lat. curvatus* WSU1 was challenged by any of the different gas producing challenge cultures there was no change in viable cells (Figure 7).

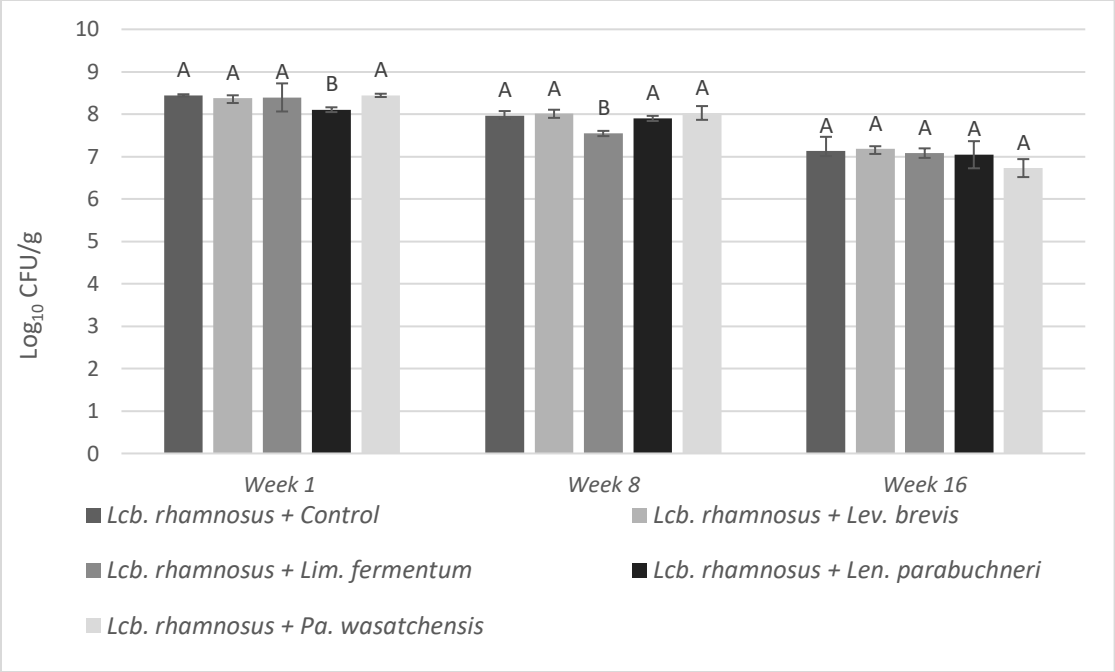

Figure 4. Growth of *Lacticaseibacillus rhamnosus* 20DK04 on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04 BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4.

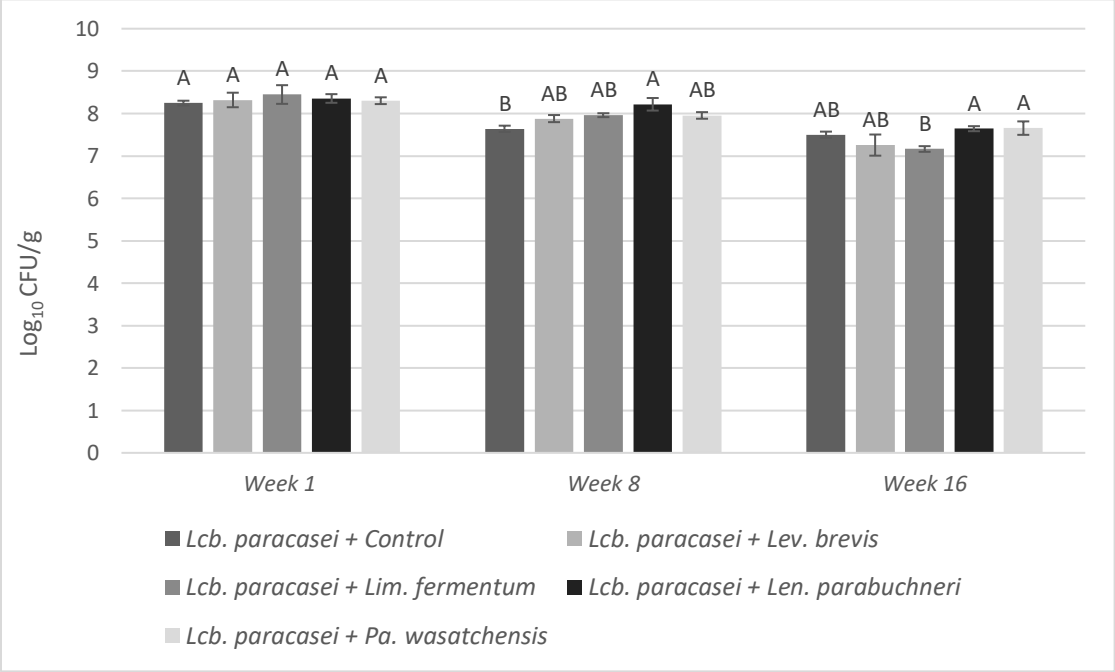

Figure 5. Growth of *Lacticaseibacillus paracasei* 20DK06 on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04 BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4.

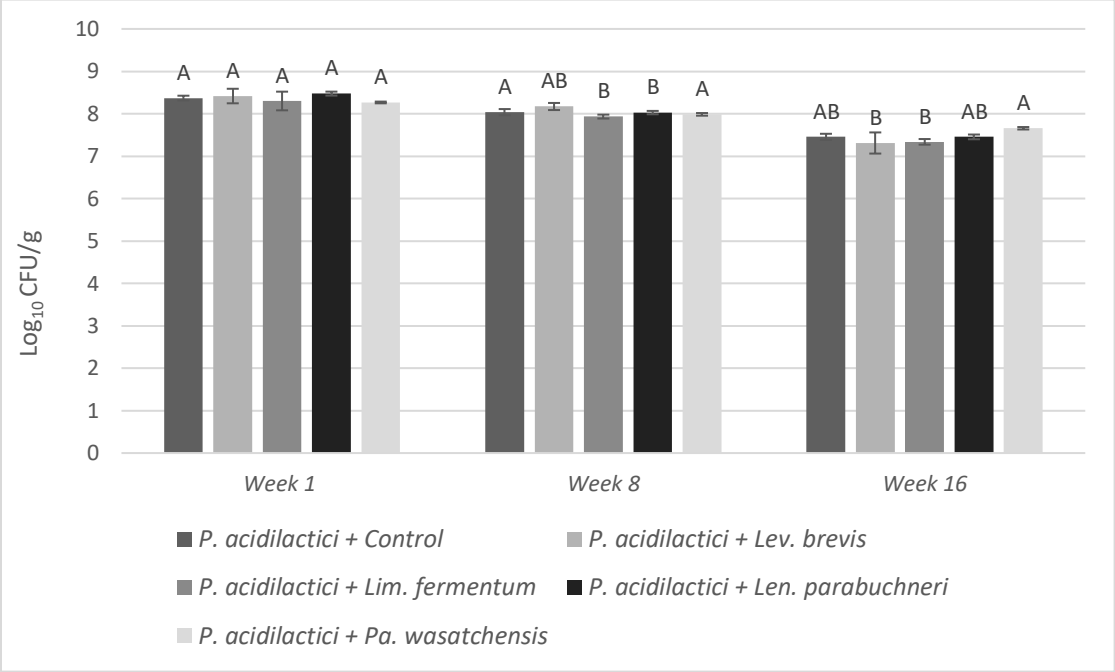

Figure 6. Growth of *Pediococcus acidilactici* 23F on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04 BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4.

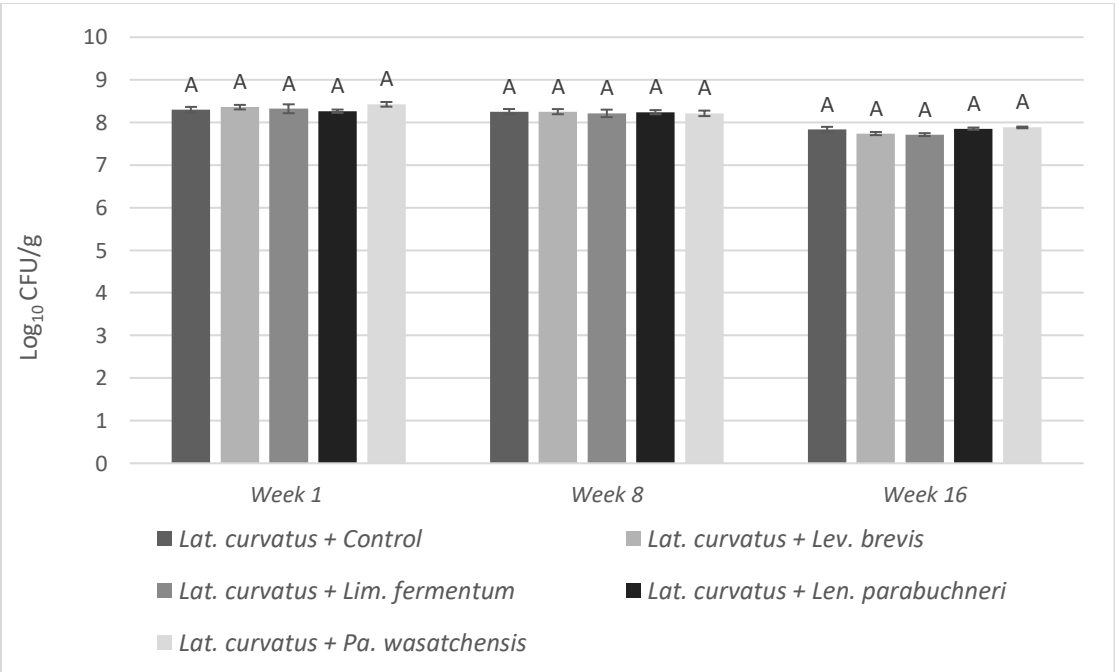

Figure 7. Growth of *Latilactobacillus curvatus* WSU1 on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis*

WDC04 BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4.

Gas producing Challenge culture levels

The control cheeses without challenge cultures showed no signs of gas producing bacteria, but since only serial dilutions of 10^{-2} and above were plated the symbol † is used to indicate that these groups are below the minimum detectable level of 10 CFU/g or 1 Log10 CFU/g. Because of this, all test cheeses were statistically significantly higher from the control at all time points (Figure 8). At week 1, the Control + *Pa. wasatchensis WDC04* were statistically significantly lower from all other sample groups. This remained the same at both week 8 and week 16. At week 8, the Control + *Lim. fermentum* 305-1 was statistically significantly lower than Control + *Len. parabuchneri* ($P = 0.049$). During the same week Control + *Lim. fermentum* 305-1 was statistically significantly higher than *Pa. wasatchensis* WDC04 ($P= 0.001$). By week 16, only Control + *Pa. wasatchensis* WDC04 remained statistically significant lower from the other samples containing heterofermentative NSLAB challenge cultures (Figure 8). The most likely reason for this is that *Pa. wasatchensis* WDC04 has a slower growth rate.

When *Lcb. rhamnosus* 20DK04 was used as a protective ALAB there were several significant differences between the challenge culture plate counts at week 1 and week 8 (Figure 9). During week 1, there were significantly lower levels of *Lim. fermentum* 305-1 and *Pa. wasatchensis* WDC04 when compared to samples containing *Lev. brevis* 277-1. This remained true when samples were tested at week 8, but by week 16 only *Pa. wasatchensis* WDC04 showed significantly less gas producing colonies when compared to the other samples.

Compared to the other protective ALAB, *Lcb. paracasei* 20DK06 showed the

largest reduction in gas producing colonies at week 1 for all challenge cultures except *Pa. wasatchensis* WDC04 (Figure 10). However, within the *Lcb. paracasei* plus challenge culture samples it wasn't until week 8 that any significant differences between challenge culture plate counts were observed ($P = <0.0001$). During week 8, *Lim. fermentum* 305-1 and *Len. parabuchneri* had significantly lower plate counts compared to *Lev. brevis* 277-1, while *Pa. wasatchensis* was significantly lower than all the groups (Figure 10). Week 16 showed less significant differences than week 8 with only *Len. parabuchneri* and *Pa. wasatchensis* WDC04 having lower plate counts compared to *Lev. brevis* 277-1 (Figure 10).

Week 1 had more significantly different groups than any other week when comparing challenge culture plate counts in the presence of the protective ALAB culture *P. acidilactici* 23F (Figure 11). Both *Len. parabuchneri* and *Lim. fermentum* 305-1 had similar plate count numbers, but only *Lim fermentum* 305-1 was significantly lower than *Lev. brevis* 277-1 ($P = 0.043$). *Paucilactobacillus wasatchensis* WDC04 plate counts were significantly lower than all other sample groups during week 1. During week 8, only two sample groups showed significantly differently lower plate counts, *Pa. wasatchensis* WDC04 and the Control group. By week 16, there were no differences between the four challenge cultures in cheeses containing *P. acidilactici* 23F.

When *Lat. curvatus* WSU1 was used as a protective ALAB, it showed similar results to *P. acidilactici* 23F (Figure 12). During week 1, *Pa. wasatchensis* WDC04 had significantly lower plate counts compared to *Lev. brevis* 277-1 and this trend continued through to week 8. However, week 16 had no significant differences between cheeses containing the four challenge cultures. (Figure 12).

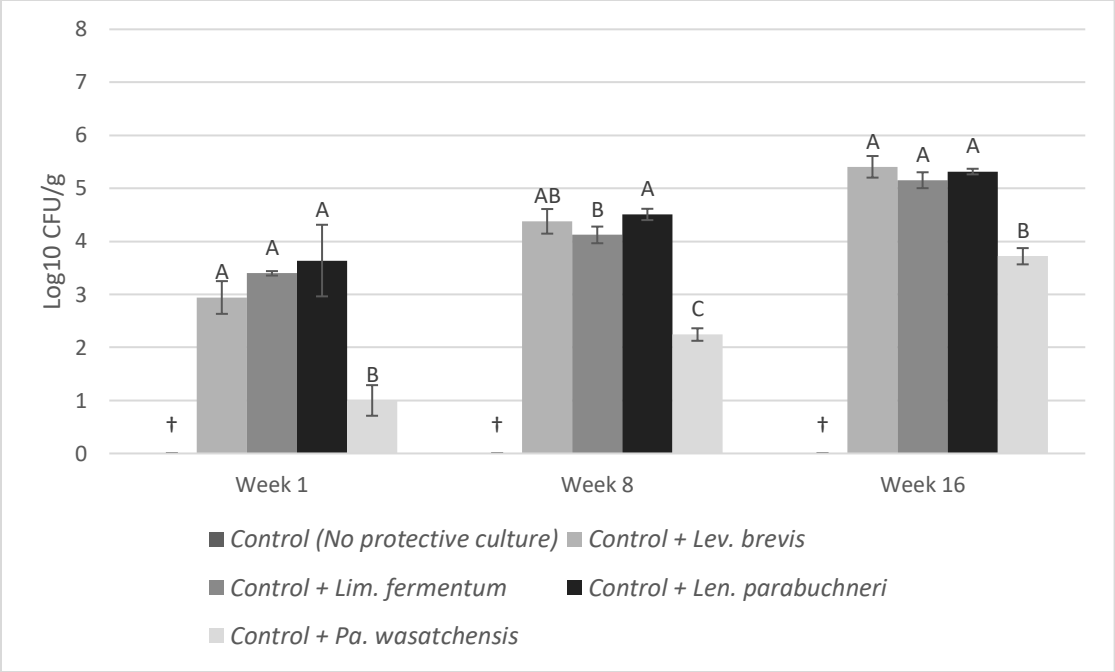

Figure 8. Growth of Challenge cultures (gas-producing NSLAB) (*Limosilactobacillus fermentum* 305-1, *Paucilactobacillus wasatchensis* WDC04, *Levilactobacillus brevis* 277-1, *Lentilactobacillus parabuchneri*, and Control = No gas producing NSLAB) in the absence of a protective adjunct culture on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04, BPW + vancomycin + ribose, groups with different letters show significant differences from each other within each time point, error bars=SE, n=4. † = below the minimum detectable level < 10 CFU/g

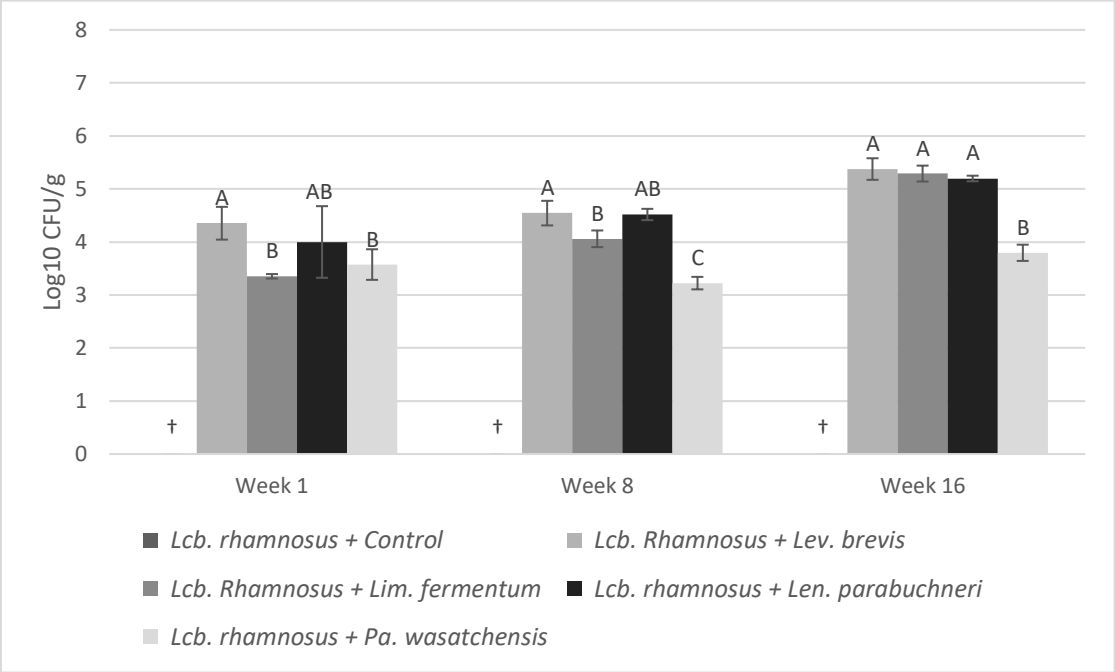

Figure 9. Growth of Challenge cultures (gas-producing NSLAB) (*Limosilactobacillus fermentum* 305-1, *Paucilactobacillus wasatchensis* WDC04, *Levilactobacillus brevis* 277-1, *Lentilactobacillus parabuchneri*, and Control = No gas producing NSLAB) in the presence of a protective adjunct culture *Lacticaseibacillus rhamnosus* 20DK04 on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04, BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4. † = below the minimum detectable level < 10 CFU/g

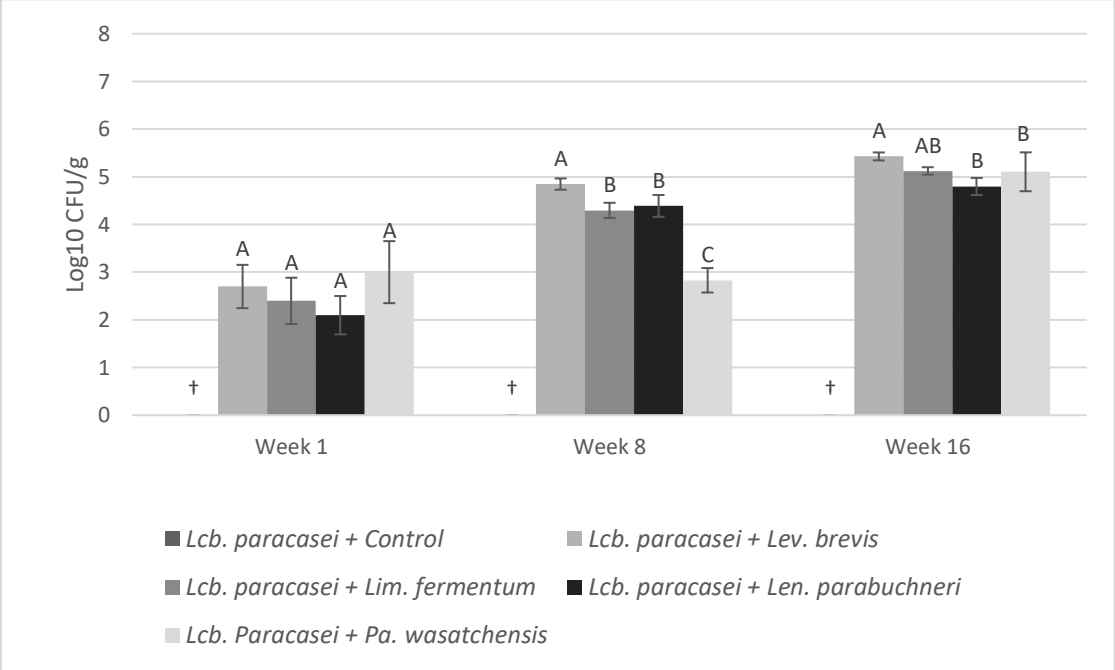

Figure 10. Growth of Challenge cultures (gas-producing NSLAB) (*Limosilactobacillus fermentum* 305-1, *Paucilactobacillus wasatchensis* WDC04, *Levilactobacillus brevis* 277-1, *Lentilactobacillus parabuchneri*, and Control = No gas producing NSLAB) in the presence of a protective adjunct culture *Lacticaseibacillus paracasei* 20DK06 on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis*, WDC04 BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4. † = below the minimum detectable level < 10 CFU/g

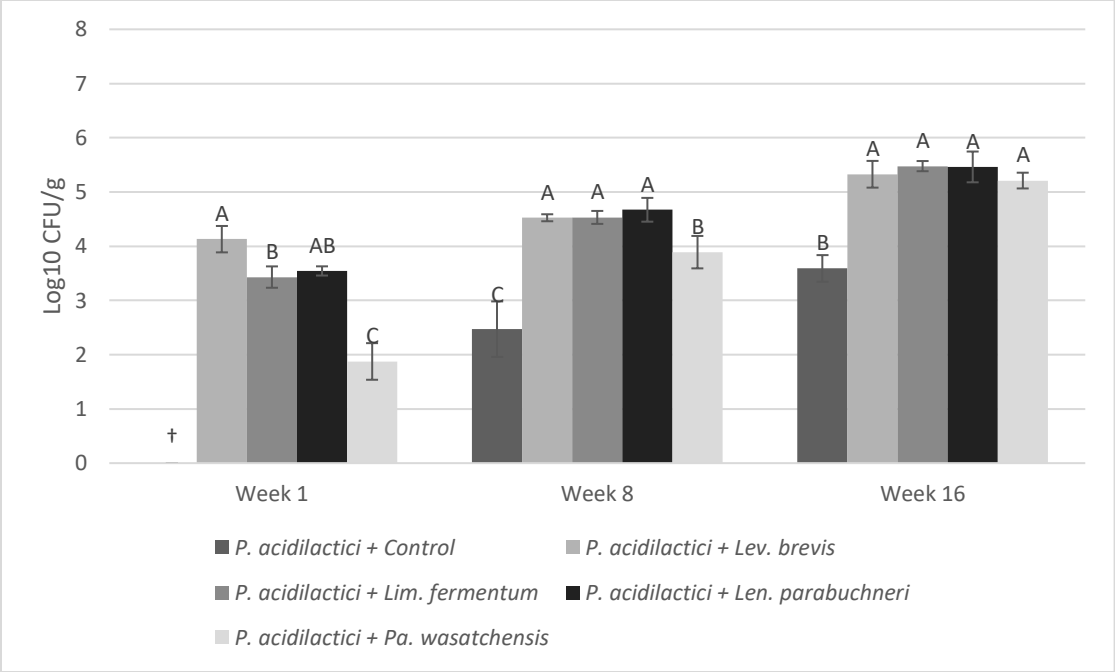

Figure 11. Growth of Challenge cultures (gas-producing NSLAB) (*Limosilactobacillus fermentum* 305-1, *Paucilactobacillus wasatchensis* WDC04, *Levilactobacillus brevis* 277-1, *Lentilactobacillus parabuchneri*, and Control = No gas producing NSLAB) in the presence of a protective adjunct culture *Pediococcus acidilactici* 23F on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04, BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4. † = below the minimum detectable level < 10 CFU/g

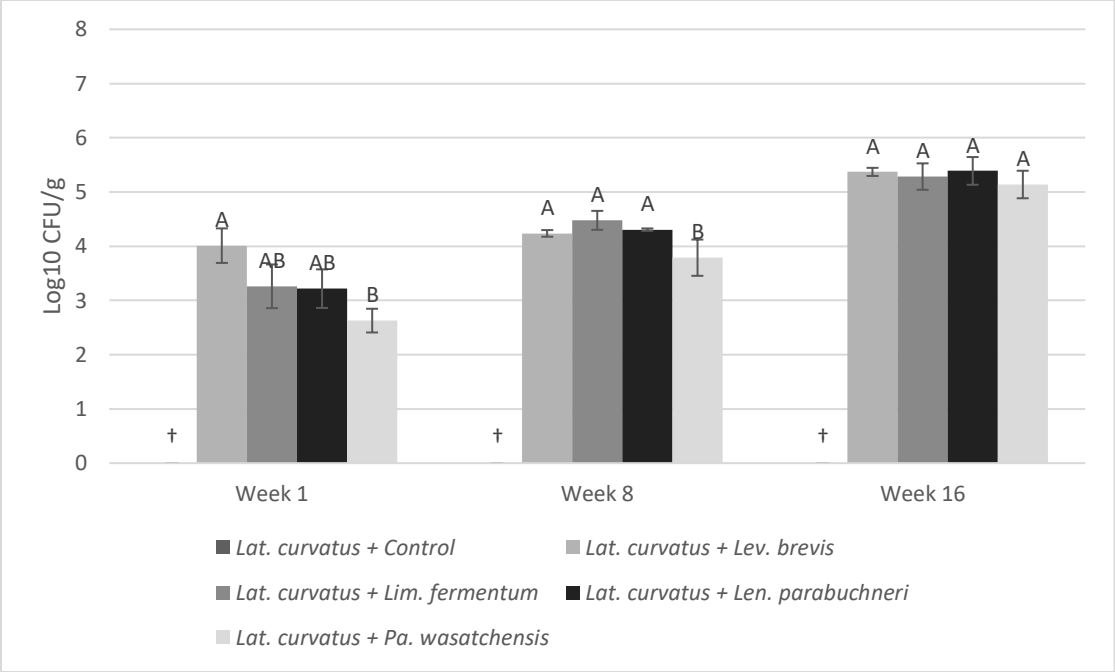

Figure 12. Growth of Challenge cultures (gas-producing NSLAB) (*Limosilactobacillus fermentum* 305-1, *Paucilactobacillus wasatchensis* WDC04, *Levilactobacillus brevis* 277-1, *Lentilactobacillus parabuchneri*, and Control = No gas producing NSLAB) in the presence of a protective adjunct culture *Latilactobacillus curvatus* WSU1 on 3M LAB Petrifilm with BPW + vancomycin added, or for samples containing *Paucilactobacillus wasatchensis* WDC04, BPW + vancomycin + ribose. Groups with different letters show significant differences from each other within each time point, error bars=SE, n=4. † = below the minimum detectable level < 10 CFU/g

Inhibition of Levilactobacillus brevis 277-1 *Gas Production*

Figure 13 shows the progression of gas production over time of *Lev. brevis* 277-1 in the presence of four different protective ALAB. This figure shows that *P. acidilactici* 23F and *Lat. curvatus* WSU1 slowed gas production over time. The gas production data was analyzed by calculating the number of days it took each sample set to produce a certain level of gas. Figure 14 shows the number of days it took *Lev. brevis* 277-1 to produce 30 mm of gas. There were no significant differences between protective ALAB when comparing the number of days, it took Lev. brevis 277-1 to produce this much gas. but it took 12 and 17 days longer on average for cheeses containing *P. acidilactici* 23F

and *Lat. curvatus* WSU1 to reach 30 mm of gas, respectively (Figure 14). The number of days it took *Lev. brevis* 277-1 to reach 60 mm of gas did show significant differences between protective ALAB when comparing cheeses containing *Lat. curvatus* WSU1 with *Lcb. paracasei* 20DK06. *Latilactobacillus curvatus* WSU1 containing cheeses took significantly longer to reach 60mm of gas (P = 0.042). The control cheeses were also statistically significantly different when compared to *Lat. curvatus* WSU1 containing cheeses (P = 0.039) (Figure 15). These significant differences are no longer seen in the graph showing days to 90 mm of gas (Figure 16). However, this is more likely a function of the duration of the study rather than there being no significant differences. This graph and any graph that has ‡ in the data label indicates that that data had samples that never reached that level of gas during the duration of the study and were given the maximum value 112 days for statistical analysis. The number of samples that reached that level of gas are also indicated by the fractions located in the bar for that sample set. These samples not reaching certain levels of gas by the end of the study does imply that these protective ALAB could be used in the making of cheddar cheese when the heterofermentative NSLAB is known and would help reduce gas production. In Figure 16 you can see that the samples in the control group or the group contained *Lev. brevis* 227-1 and no protective adjunct culture had 25 of 28 samples that reached 90 mm of gas, while the sample group that contained *P. acidilactici* F23 had 14 of 28 and *Lat. curvatus* WSU1 had 16 of 28 samples that reached 90 mm of gas. That is a 39% and 32% reduction in the number of subsamples that reached 90 mm of gas respectively.

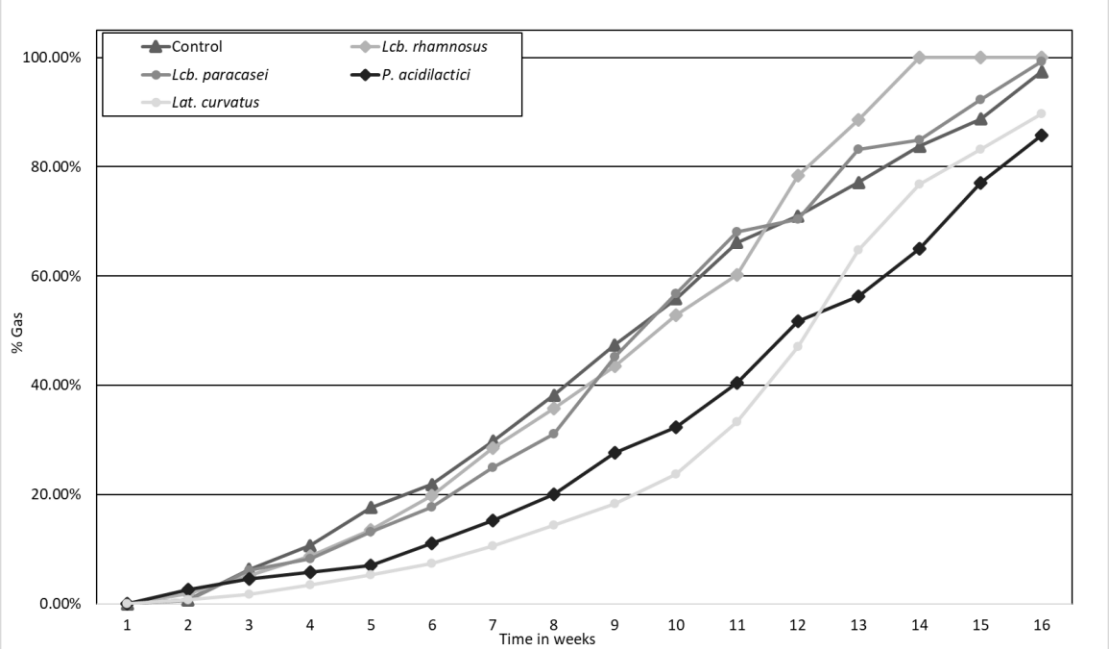

Figure 13. Gas production of *Levilactobacillus brevis* 277-1 in the presence of four different adjunct cultures over time. Percentages obtained by dividing the height in millimeters of gas measured in the packaging headspace above the cheese by 95 mm, which was the maximum height available in the head space.

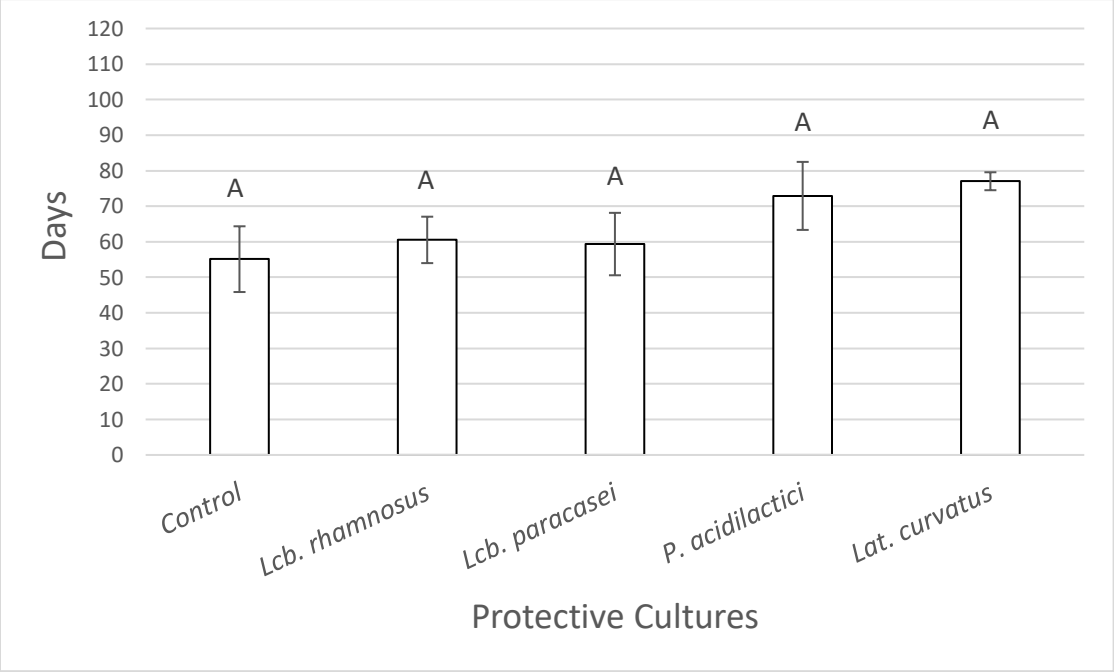

Figure 14. Number of days it took *Levilactobacillus brevis* 277-1 to produce 30 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different data labels show significant differences from each other.

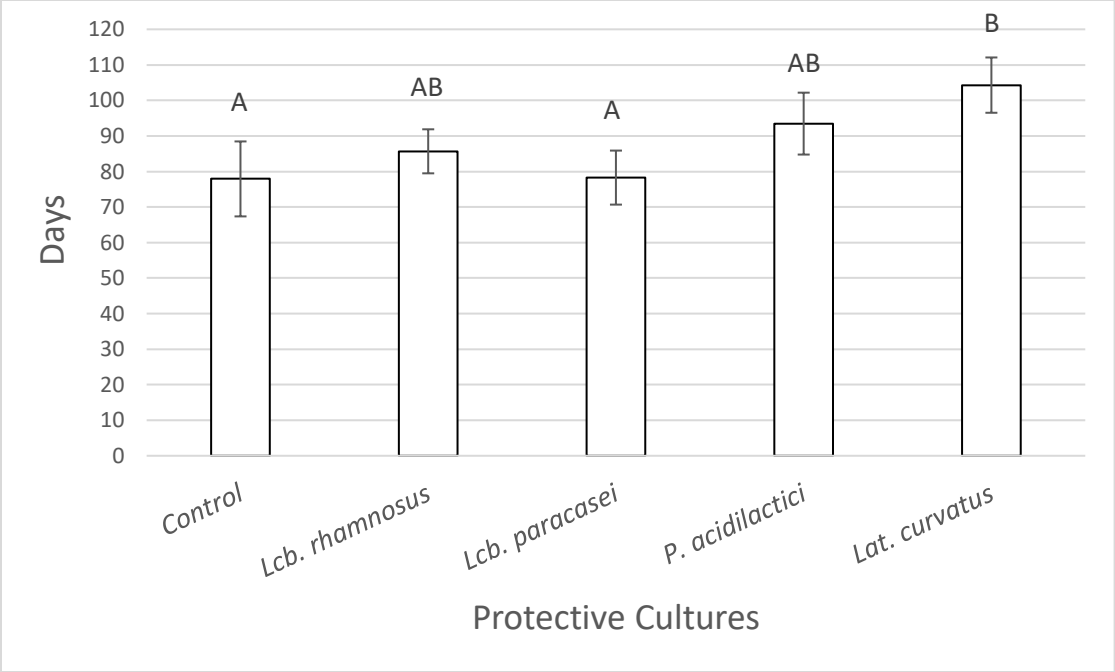

Figure 15. Number of days it took *Levilactobacillus brevis* 277-1 to produce 60 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different data labels show significant differences from each other.

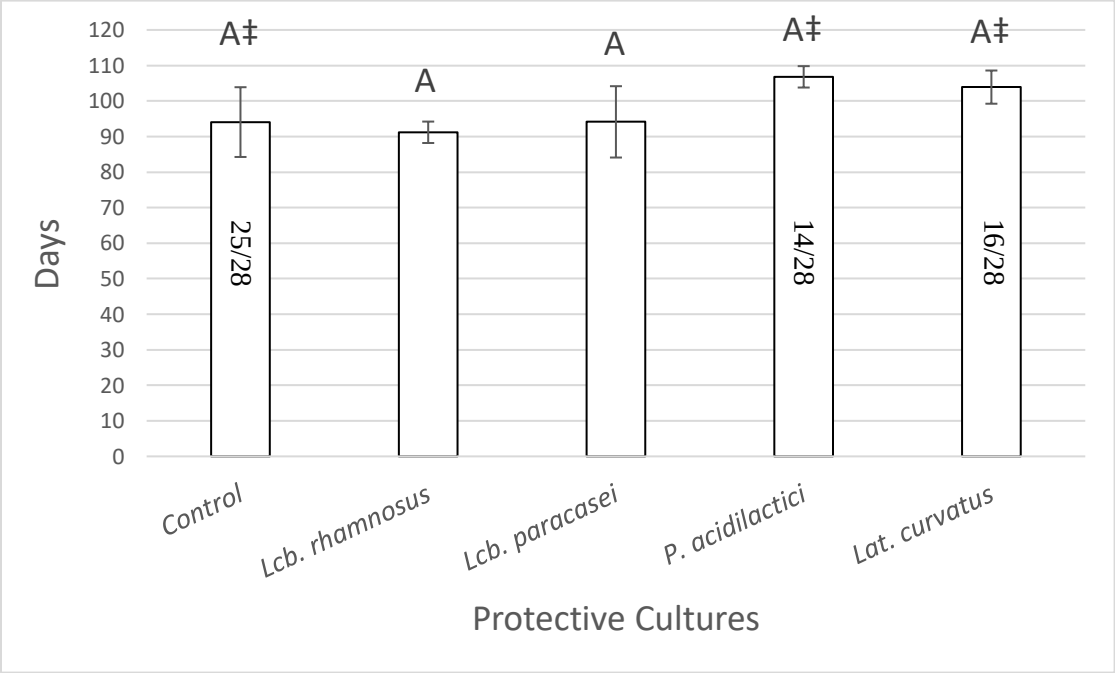

Figure 16. Number of days it took *Levilactobacillus brevis* 277-1 to produce 90 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different data levels show significant differences from each other, ‡ = contained samples that didn't reach 90 mm of gas and were given the maximum value 112 days for statistical analysis. The fractions on the bars indicate the number

of subsamples that reached 90mm of gas in those sample sets.

Inhibition of Limosilactobacillus fermentum 305-1 *gas production*

When *Lat. curvatus* WSU1 was used as a protective adjunct culture and challenged with *Lim. fermentum* 305-1, it decreased the amount of time before the first incidence of gas by three weeks (Figure 17) and also increased the total amount of gas produced by *Lim. fermentum* 305-1 when compared to other challenge protective cultures. The number of days it took *Lim. fermentum* 305-1 to produce enough gas to fill the head space to 2.5 mm showed no significant differences between sample groups. All sample groups that contained a protective adjunct culture had subsamples that never reached this height, while all samples from the control group reached at least 2.5 mm during the duration of this study (Figure 18).

Although our statistical analysis did not show any significance in the number of days, our data does show that *Lim. fermentum* 305-1 was affected by the different ALAB. When compared to the control group the sample groups that used *Lcb. paracasei* 20DK06 and *Lat. curvatus* WSU1 as protective cultures showed a 50% reduction in the number of subsamples that reached 2.5 mm. A similar trend can be seen with *Lcb. rhamnosus* 20DK04 and *Lat. curvatus* WSU1 having a 100% and 53% reduction when compared to the control group (Figure 18). Only *P. acidilactici* 23F and *Lat. curvatus* WSU1 sample sets had subsamples that reached at least 5 mm of gas. This could be due to these two protective ALAB producing substrates that *Lim. fermentum 305-1* was able to metabolize or *Lim. fermentum* 305-1 needed more time to produce gas.

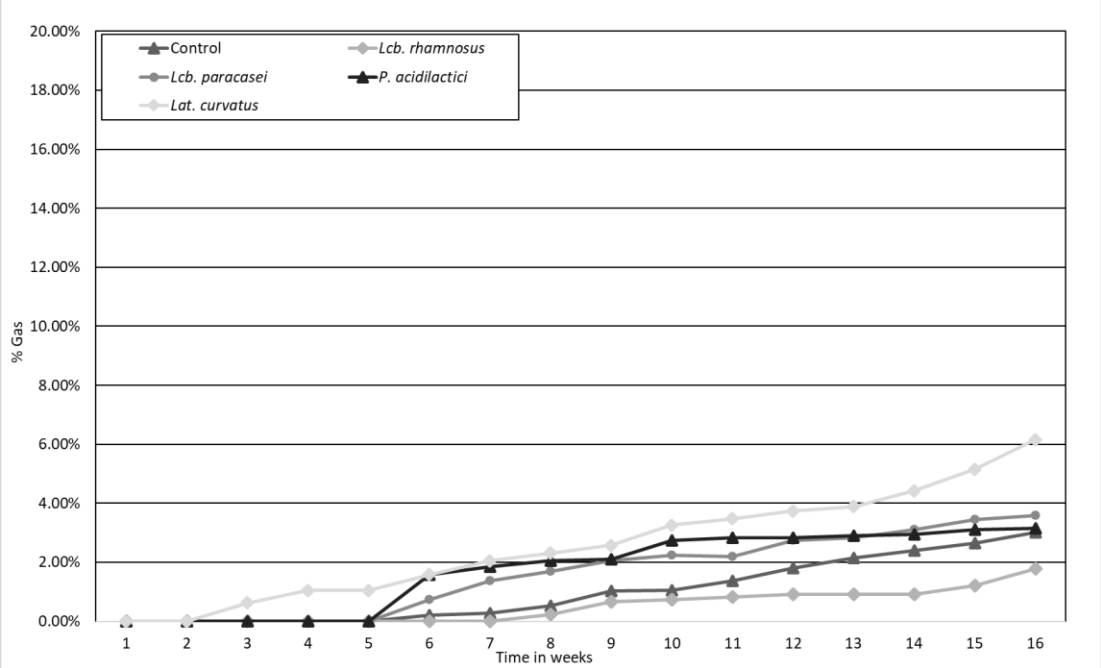

Figure 17. Gas production of *Limosilactobacillus fermentum* 305-1 in the presence of different adjunct culture over time. Percentages obtained by dividing the height in millimeters of gas measured in the headspace above the cheese by the maximum height available in the head space (95 mm).

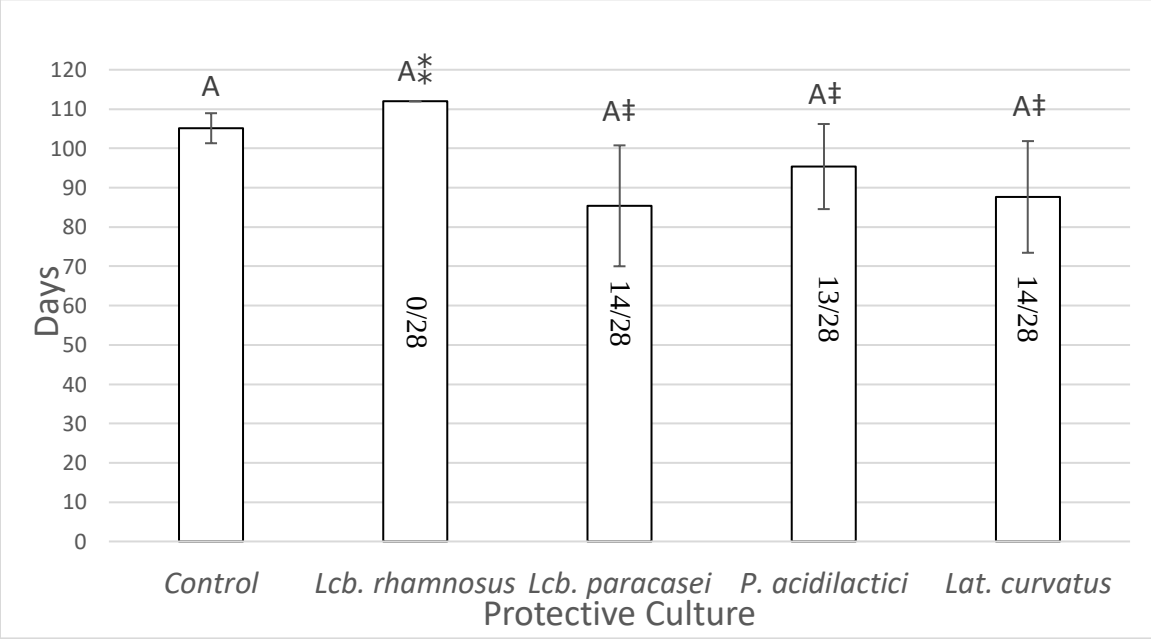

Figure 18. Number of days it took *Limosilactobacillus fermentum* 305-1 to produce 2.5 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different data levels show significant differences from each other, ‡ = contained samples that didn't reach 2.5 mm of gas and were given the maximum value 112 days for statistical analysis. ⁂ = no samples reached 2.5 mm,

and all were given the maximum value 112 for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 2.5mm of gas in those sample sets.

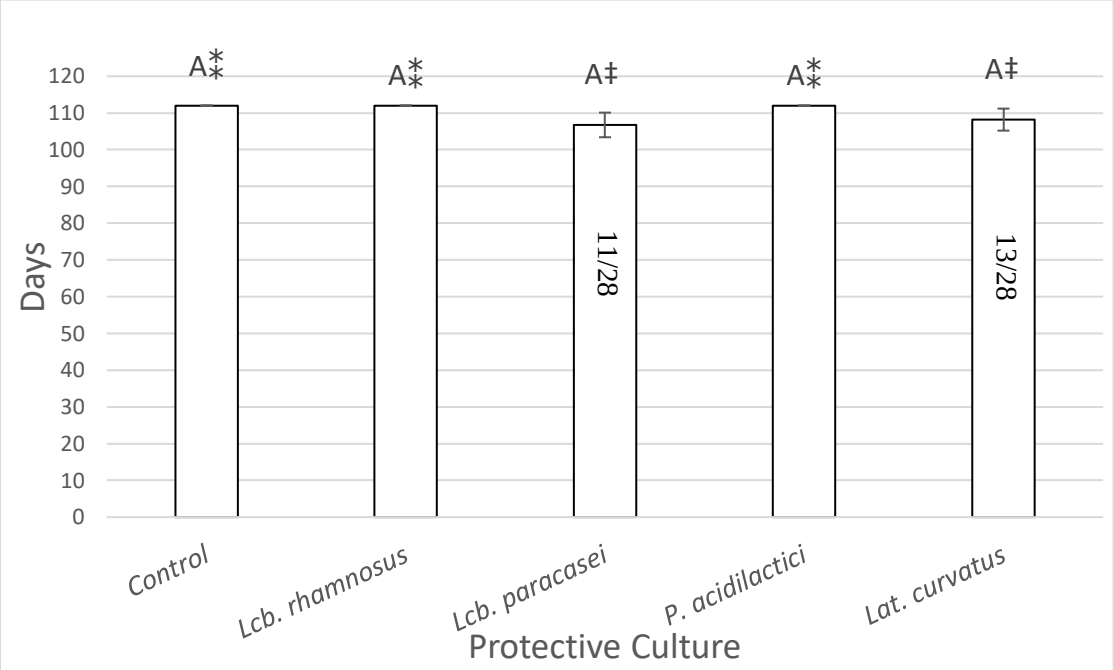

Figure 19. Number of days it took *Limosilactobacillus fermentum* 305-1 to produce 5 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different data levels show significant differences from each other, ‡ = contained samples that didn't reach 5 mm of gas and were given the maximum value 112 days for statistical analysis, ⁂ = no samples reached 5 mm, and all were given the maximum value 112 for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 5mm of gas in those sample sets.

Inhibition of Lentilactobacillus parabuchneri gas production

The progression of gas levels over time (Figure 20) showed an increase in gas production compared to the control for *Lcb. paracasei* 20DK06 + *Len. parabuchneri* cheeses. Cheeses containing the protective cultures *Lat. curvatus* WSU1, *Lcb. paracasei* 20DK06, and *P. acidilactici* F23 showed a decrease in time before the first incidence of gas was observed. The opposite was true for *Lcb. rhamnosus* 20DK04 + *Len. parabuchneri* cheeses which took 5 weeks before the first incidence of gas was

observed while it took the control + *Len. parabuchneri* 4 weeks before gas was observed. *Lcb. rhamnosus* 20DK04 along with the protective ALAB *Lat. curvatus* WSU1 and *P. acidilactici* 23F also reduced the overall amount of gas produced by *Len. parabuchneri* (Figure 20).

The number of days that it took *Len. parabuchneri* to produce 5 mm of gas in the presence of the different ALAB showed no significant differences (Figure 21). The control cheeses as well as the cheeses that contained *Lcb. rhamnosus* 20DK04 and *Lcb. paracasei* 20DK06, had large standard errors due to one subsample group producing gas at a faster rate than the rest of the subsample groups. There was no difference between protective adjunct levels between these sub samples so this is most likely due to *Len. parabuchneri* metabolizing other substrates in the cheese.

The number of days until there were 10 mm of gas in the head space also showed no significant differences between sample groups. All sample groups had sub samples that didn't produce 10 mm of gas but the cheese samples that used *P. acidilactici* 23F as a protective adjunct culture had no subsamples that reached 10 mm of gas which was a 50% reduction when compared to the control (Figure 22). This can also be seen in (Figure 23) showing the number of days to 20 mm of gas. This figure however shows that the sample groups that used *Lat. curvatus* WSU1, *P. acidilactici* 23F, and *Lcb. rhamnosus* 20DK04 as protective ALAB stopped *Len. parabuchneri* from ever producing 20 mm of gas. This was a 50% reduction in the number of subsamples that reached 20 mm of gas when comparing these groups to the control group that contained not protective adjunct culture. This shows promise for these protective ALAB to be used to stop the production of gas by *Len.*

parabuchneri after a certain amount of time. This could greatly reduce defects caused by late-stage gas production and also reduce bloating in the retail environment after repackaging.

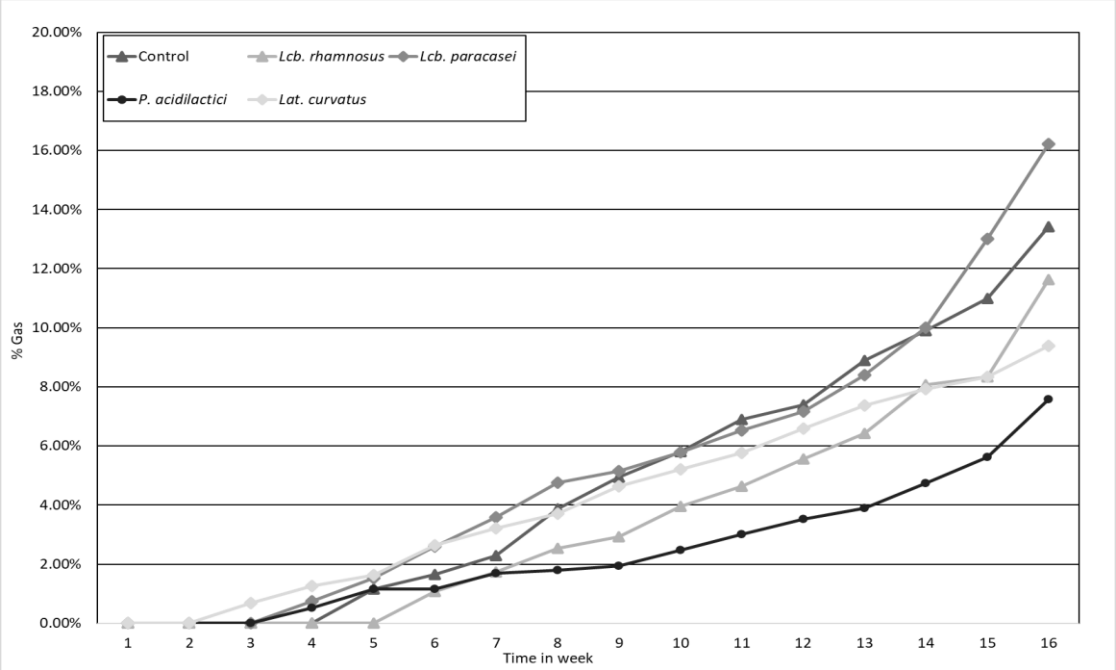

Figure 20. Gas production of *Lentilactobacillus parabuchneri* in the presence of different adjunct culture over time. Percentages obtained by dividing the height in millimeters of gas measured in the headspace above the cheese by the maximum height available in the head space (95 mm).

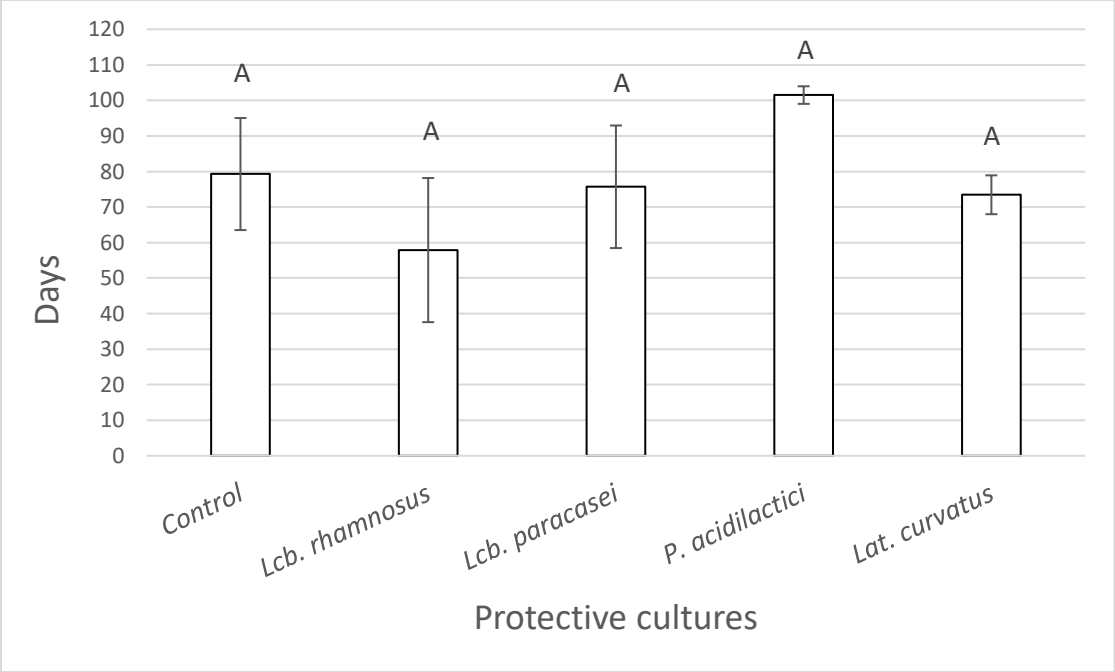

Figure 21. Number of days it took *Lentilactobacillus parabuchneri* to produce 5mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different letters show significant differences from each other.

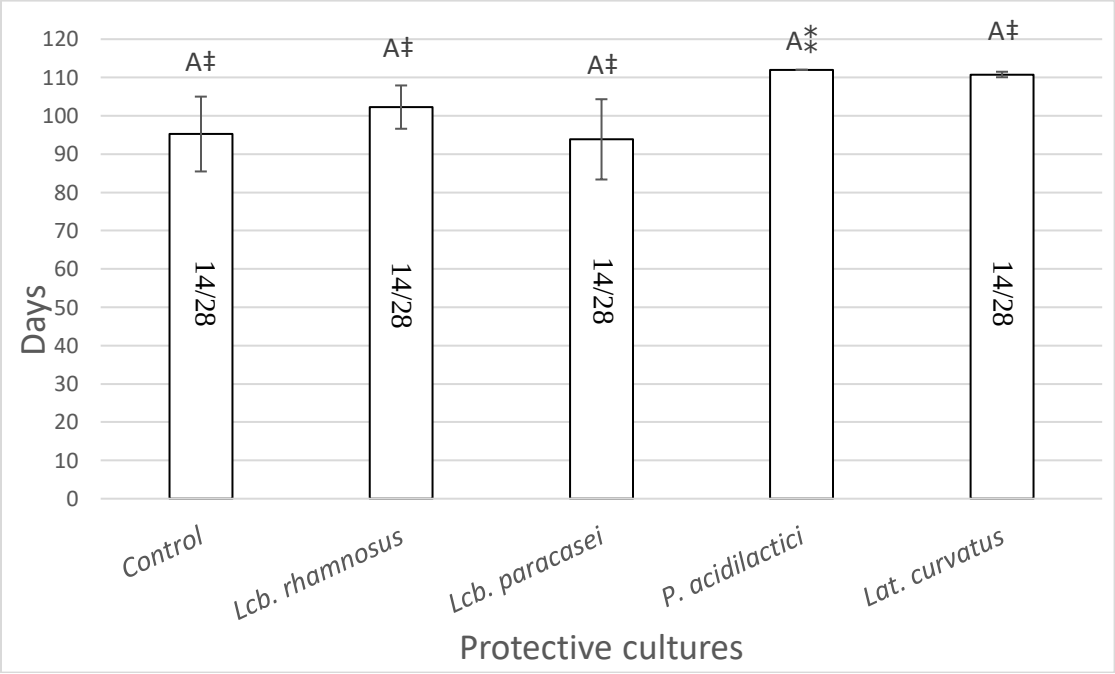

Figure 22. Number of days it took *Lentilactobacillus parabuchneri* to produce 10 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different letters show significant differences from each other, ‡ = contained samples that didn't reach 10 mm of gas and were given the maximum value 112 days for statistical analysis, ⁑ = no samples reached 10 mm, and all were

given the maximum value 112 for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 10mm of gas in those sample sets.

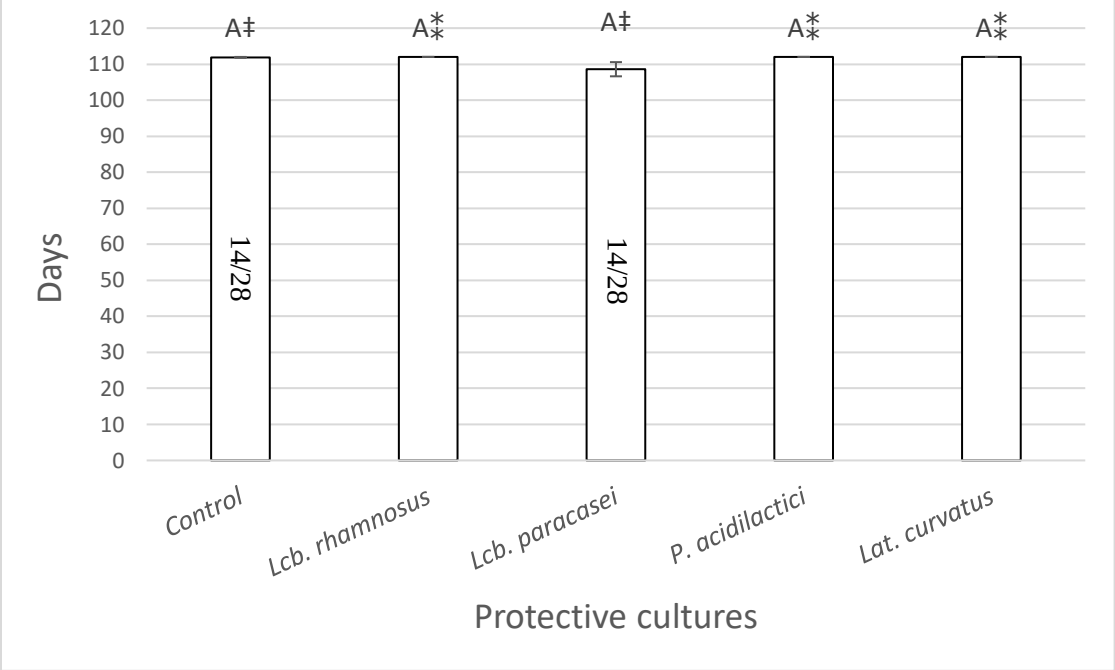

Figure 23. Number of days it took *Lentilactobacillus parabuchneri* to produce 20 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different letters show significant differences from each other, ‡ = contained samples that didn't reach 20 mm of gas and were given the maximum value 112 days for statistical analysis, ⁑ = no samples reached 20 mm, and all were given the maximum value 112 for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 20mm of gas in those sample sets.

Inhibition of Pa. wasatchensis WDC04 *gas production*

On average, *Pa. wasatchensis* WDC04 produced 15 percent more gas compared to control when *P. acidilactici* 23F was used as a protective adjunct. *Paucilactobacillus wasatchensis* WDC04 has the ability to metabolize many substrates including galactose, components of lysed starter cultures cells, gluconate, and amino acids (McMahon, Sorenson, et al. 2022). We hypothesis that *P. acidilactici* 23F produces a substrate through its metabolism that *Pa. wasatchensis* WDC04 is able to utilize, allowing it to produce higher levels of gas. When *Lcb. rhamnosus* 20DK04 and *Lcb. paracasei*

20DK06 were used as protective ALAB, *Pa. wasatchensis* WDC04 produced less gas than the control and all other cheeses (Figure 24).

The time that it took *Pa. wasatchensis* WDC04 to produce 10 mm of gas in the presence of each adjunct culture is seen in Figure 25. Control cheeses that lacked protective ALAB reached 10 mm of gas around day 95, while *P. acidilactici* 23F and *Lat. curvatus* WSU1 containing samples all reached 10 mm of gas around day 60 and 82, respectively. Only 64% of the subsamples that used *Lcb. rhamnosus* 20DK04 reached 10 mm of gas while none of the subsamples that contained *Lcb. paracasei* 20DK06 reached this level of gas during the duration of the study (Figure 25). Cheeses containing Lcb. *rhamnosus* and *Lcb. paracasei* were significantly different from the rest of the sample's groups. When control, *P. acidilactici* 23F, and *Lat. curvatus* WSU1 were analyzed using a T-test they were significantly different from each other and all other sample groups (Figure 25).

The number of days until 20 mm of gas showed fewer significant differences with fewer cheeses reaching this height in the head space above the cheese (Figure 26). Only 50% of the control cheese subsamples and 89% of the *Lat. curvatus* WSU1 containing cheese subsamples reached this level of gas. Again *P. acidilactici* 23F was significantly differently faster at producing gas from all other cheese groups and all the subsamples that used this protective adjunct culture reached 20 mm of gas (Figure 26). The data for the days to 30 mm of gas is very similar, with the only differences being that the control group and *P. acidilactici* 23F containing cheeses were not significantly different form each other, most likely due to the large Standard Error that *P. acidilactici* 23F cheeses exhibited, and that none of the subsamples that contained *Lat. curvatus* 23F reached this

level of gas. (Figure 27).

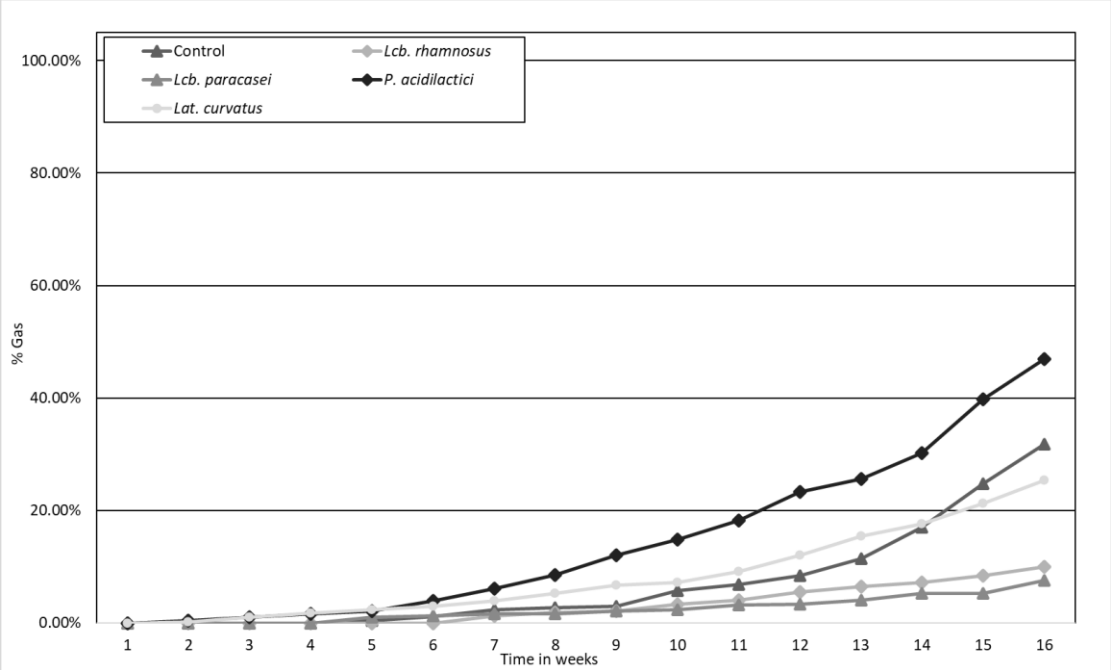

Figure 24. Gas production of *Paucilactobacillus wasatchensis* WDC04 in the presence of different adjunct culture over time. Percentages obtained by dividing the height in millimeters of gas measured in the headspace above the cheese by the maximum height available in the head space (95 mm), Error bars = SE, n=4.

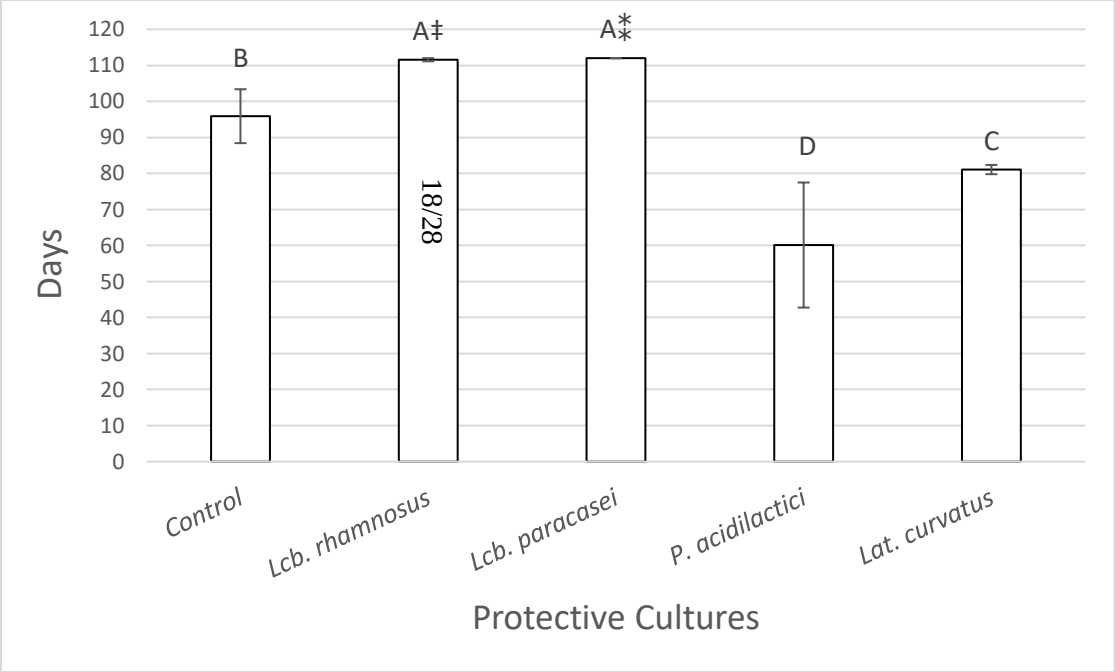

Figure 25. Number of days it took *Paucilactobacillus wasatchensis* WDC04 to produce 10 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different letters show significant differences from each other, ‡ = contained samples that didn't reach 10 mm of gas and were given the maximum value 112 days for statistical analysis, ⁂ = no samples reached 10 mm, and all were given the maximum value 112 days for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 10mm of gas in those sample sets.

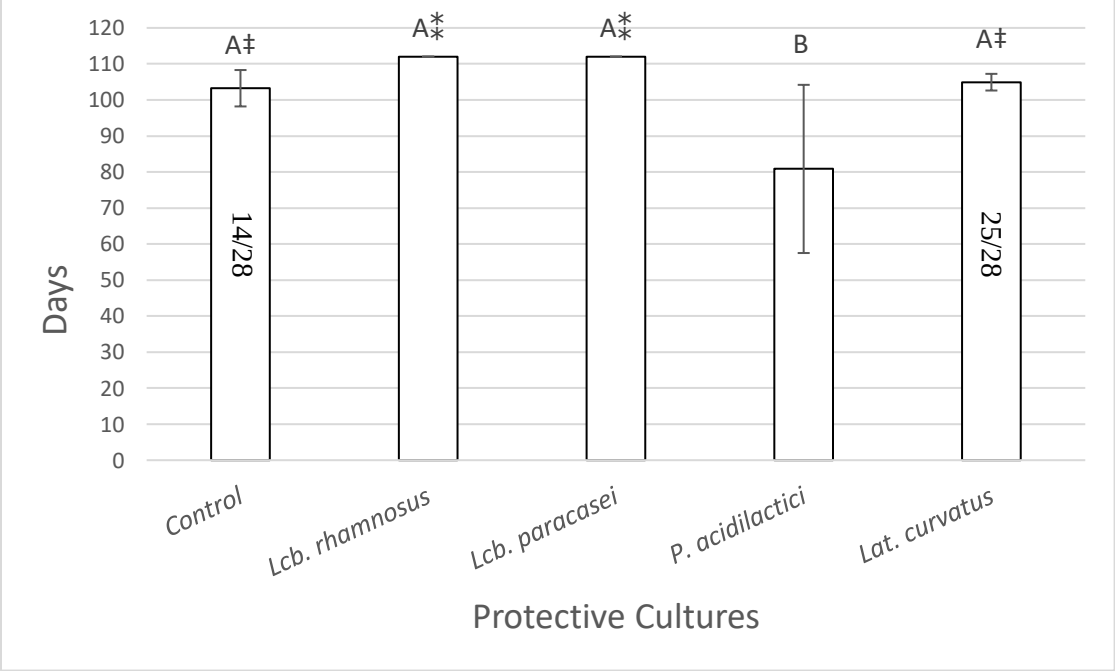

Figure 26. Number of days it took *Paucilactobacillus wasatchensis* WDC04 to produce 20 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different letters show significant differences from each other, ‡ = contained samples that didn't reach 20 mm of gas and were given the maximum value 112 days for statistical analysis, ⁑ = no samples reached 20 mm, and all were given the maximum value 112 days for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 20mm of gas in those sample sets.

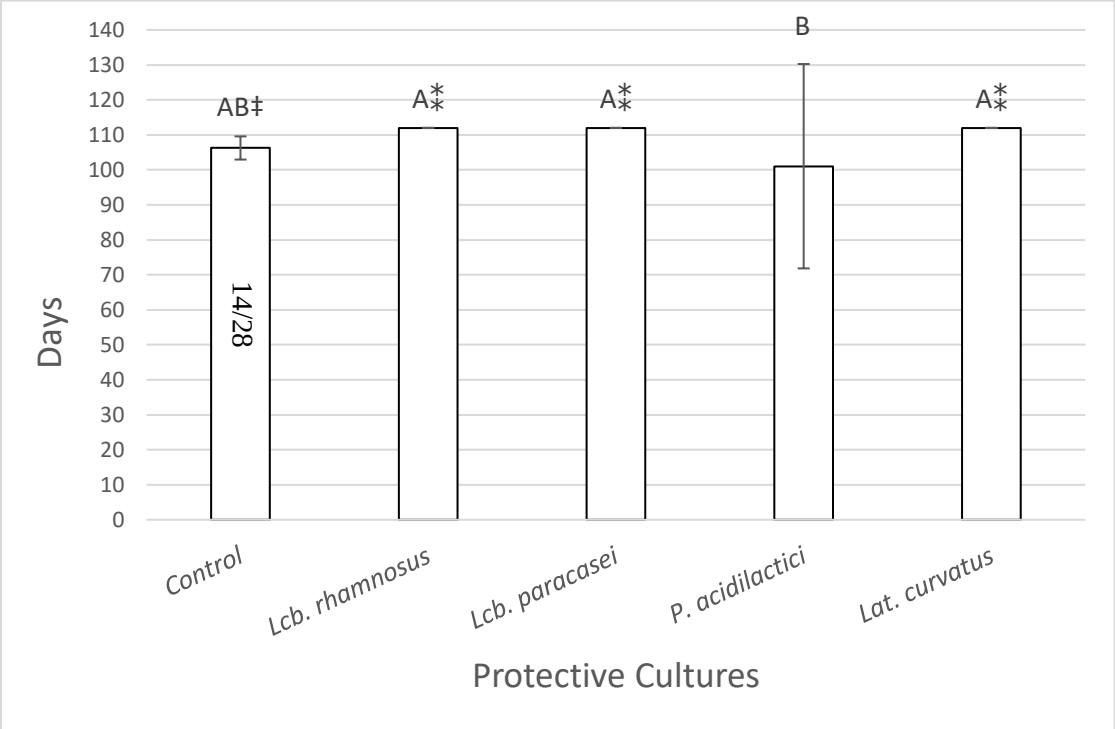

Figure 27. Number of days it took *Paucilactobacillus wasatchensis* WDC04 to produce 30 mm of gas in the presence of different protective adjunct cultures. Error bars = SE, n=4, Groups with different letters show significant differences from each other, ‡ = contained samples that didn't reach 30 mm of gas and were given the maximum value 112 days for statistical analysis, ⁑ = no samples reached 30 mm, and all were given the maximum value 112 days for statistical analysis. The fractions on the bars indicate the number of subsamples that reached 30mm of gas in those sample sets.

CONCLUSION

Adding protective ALAB to the cheese along with the starter cultures affects how fast the heterofermentative NSLAB grow and how much gas they produce. Each protective adjunct culture had different effects depending on the heterofermentative NSLAB that it was paired with. Inoculating the cheese with the standardized, individually grown, heterofermentative NSLAB the day after the initial cheese make by grinding it and repressing it proved to be an effective method to introduce the gas-producing bacteria into the cheese. This method produced a close-knit body and after around two weeks it was almost impossible to distinguish it from cheese that was not processed this way. Because of this, this method shows promise to be used in the future to perform challenge studies while cutting down on cheesemaking.

The moisture, salt, S/M, galactose, lactose, and pH of the cheese samples were all within the target range selected prior to the study. The protective adjunct culture and Heterofermentative NSLAB were monitored using a new plating method on Petrifilm that proved to be effective and all had similar plate counts. This method of plating proved to be extremely valuable and time saving because it selects for the protective and challenge cultures and inhibits the SLAB on the Petrifilm.

Evaluating the level of gas by measuring the height that the gas reached in the head space above the cheese also provided good results. We analyzed this data by determining the number of days it took each sample group to reach a certain height in gas. This showed good results when analyzing NSLAB that produced a large amount of gas like *Lev. brevis* 277-1. However, when samples produced small amounts of gas or did not reach a certain level of gas it made it hard to determine significance. From our data

analysis there were significant decreases in gas production when *Lat. curvatus* WSU1 was challenged with *Lev. brevis* 277-1, and when *Lcb. rhamnosus* 20DK04 and *Lcb. paracasei* were challenged with *Pa. wasatchensis* WDC04. There was an increase in gas production and the gas production rate when the protective adjunct culture *P. acidilactici* 23F was challenged with the *Pa. wasatchensis* WDC04.

The number of subsamples that reached certain levels of gas for each sample group was also examined. When *P. acidilactici* 23F and *Lat. curvatus* WSU1 were challenged with *Lev. brevis* 277-1, there was a 39% and 32% reduction in the number of subsamples that reached 90 mm of gas respectively. In the sample groups that were challenged with *Lim. fermentum* 305-1, the protective adjunct culture *Lcb. rhamnosus* 20DK04 stopped all subsamples from ever reaching 2.5 mm of gas, and *Lat. curvatus* WSU1 stopped 53% of the subsamples from reaching 2.5 mm of gas. A similar trend was seen again when the heterofermentative LAB *Len. parabuchneri* was challenged with the protective ALAB *Lat. curvatus* WSU1, *P. acidilactici* 23F, and *Lcb. rhamnosus* 20DK04. All three of these protective ALAB caused a 50% reduction in the number of subsamples that were able to reach 20 mm of gas when compared to the control or *Len. parabuchneri* only group. Finally, when *Pa. wasatchensis* WDC04 was challenged with the protective ALAB there were mixed results. When *Lcb. paracasei* 20DK06 and *Lcb. rhamnosus* 20DK04 were used to challenge the *Pa. wasatchensis* WDC04 they were able to stop all the subsamples from ever reaching 20 mm of gas which is a 50% reduction when compared to the control. On the contrary when *P. acidilactici* 23F caused all of the sub samples to reach 20 mm and 30 mm of gas which is a 50% increase when compared to control. This could be due to *P. acidilactici* 23F producing substrates that *Pa.*

wasatchensis WDC04 is able utilize.

Our hypothesis was that adding ALAB during cheese production will cause inhibition of heterofermentative non-starter lactic acid bacteria growth and gas production through competitive inhibition and other factors. This hypothesis was observed to be mostly correct and strain dependent. We saw reductions in gas production and the rate of gas production for many cheeses. There were also sample groups that had an increase or remained the same when compared to the control groups. This points towards the need of a cocktail of protective ALAB to combat gas if the heterofermentative NSLAB is unknown.

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APPENDIX

Cheddar - Protective Cultures

UtahState UNIVERSITY

Cheese Type:	**Full Fat Cheddar Cheese**
Manufacture Date:	9/1/20
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: no adjunct control
Cheesemaker	RAEES C.

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1367	3.68	3.07	.834	6.75

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 (Based on 1350 lb)
Start Filling Vat	-1:00		40	70		6.65	6.75	
Vat Filled, begin heating	-0:20	7:12	15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	7:45	5	88	88.7			**61 g** A3040 plus ___ g of protective culture.
Add Coagulant, Set Vat	7:47 0:00	7:56	30	88	88.8			**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators. 1:58
Vat Cut	~~7:58~~ 0:30 8:20	8:20	5	88	87.9			Cut 1 min @ 10 RPM ✗ Cut 1 min @ 11 RPM ✗ Cut 2 min @ 12 RPM ✗ Stir 30 s @ 12 RPM ✗ Cut 1 min @ 14 RPM
Start Rest ~~(5 minutes)~~	0:35	8:27	5	88	89.0			Stir 2 min @ 6 RPM ✗ Stir 3 min @ 8 RPM ✗
Start Fore work ~~(25 minutes)~~	0:40	8:37	10	88				Cut 1 min @ 14 RPM ✗ Stir 2 min @9 RPM ✗ Stir 3 min @ 10 RPM ✗ Stir 3 min @ 12 RPM ✗ Cut 1 min @ 14 RPM
Start Cook	0:50	8:44	30	88	89.3	START 6.55 END 9:26	6.70 6.65 6.58	Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. ✗ Stir 6 min @ 12 RPM, Cut 1 min ✗ ~~9:05~~ Stir 8 min @ 14 RPM, Cut 1 min ✗ Stir 8 min @ 15 RPM, Cut 1 min 9:13 Stir 8 min @ 16 RPM 9:21
Start stir-out ~~(35 minutes)~~	1:20	9:14	25	102.5	102.6	9:27	6.59 6.53	recut Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	9:40 1:45	9:40	15	102.5	~~102.6~~ 102.9	6.45	6.44	Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

9:57 6.37

Start forming cheese curd in to pack	2:00	9:57	10 5	101		6.35			Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:05	10:05	5	100	100	6.25	6.34		Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:10	10:11	20	98			6.26	10:15	Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:30	10:42	20	96	97.5	6.0	5.10	6.12	Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	11:02 2:50	10:51	25	94		5.8	5.77		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:15		5				5.58	140.2	Expect ~ 145 lb
Cheese milled	3:20	11:40	10	94		5.50	5.53		Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application	3:30	11:45	5	92				4.06#	Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 4.06# Salt
~~Sugar Addition~~									Optional. Add up to 0.5% galactose with salt
2nd Salt	3:35	11:50	5	91					
3rd Salt	3:40	11:55	5	90					
Fill curd into hoops	3:45		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:00	12:20	3 h						Press in horizontal press at 60 psi
Packaging	3:20 7:00	3:40	30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:30			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	22.10					
2	24.00 lb	22.15					
3	24.00 lb	22.15					
4	24.00 lb	22.10					
5	24.00 lb	22.10					
6	24.00 lb	15.80					

Notes: 37.6 + 35.9 + 32.80 + 33.85 = 140.2

Figure 28: Vat 1 Control cheese make sheet.

Cheddar - Protective Cultures

UtahState UNIVERSITY

Cheese Type:	**Full Fat Cheddar Cheese**
Manufacture Date:	9/1/2020
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: BC20DK0004 ADD 9.07 g TO 200mL MILK ADD 11.45 mL TO VAT
	Cheesemaker RHEES C.

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1414	3.68	3.07	0.83	6.72

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 (Based on 1350 lb)
Start Filling Vat	-1:00	10:40	40	70		6.65		
Vat Filled, begin heating	-0:20	10:54	15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	11:41	5	88				**61 g** A3040 plus 9.07 g of protective culture.
Add Coagulant, Set Vat	0:00	11:45	30	88				**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators.
Vat Cut	12:15 0:30	12:17 12:18	5	88				Cut 1 min @ 10 RPM Cut 1 min @ 11 RPM Cut 2 min @ 12 RPM Stir 30 s @ 12 RPM Cut 1 min @ 14 RPM
Start Rest (5 minutes)	12:22 0:35	12:25	5	88		SKIPPED		Stir 2 min @ 6 RPM Stir 3 min @ 8 RPM
Start Fore work (25 minutes)	12:30 0:40	12:30	10	88				Cut 1 min @ 14 RPM Stir 2 min @9 RPM Stir 3 min @ 10 RPM Stir 3 min @ 12 RPM
Start Cook	0:50	12:30 12:30	30	88	89.2	6.55		Cut 1 min @ 14 RPM Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. Stir 6 min @ 12 RPM, Cut 1 min Stir 8 min @ 14 RPM, Cut 1 min 12:39 Stir 8 min @ 15 RPM, Cut 1 min 12:47 Stir 8 min @ 16 RPM
Start stir-out (35 minutes)	1:20	1:05	25	102.5	103.6	6.59 108	6.59	Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:30 1:45	1:43	15	102.5		6.45	6.45	Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

1:58 6:40

Start forming cheese curd in to pack	2:00	1:58 2:09	5	101		6.35	~~6.39~~	6.34	Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:05	2:14	5	100	101.9	6.25	~~6.26~~	6.26	Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:10	2:20	20	98					Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:30	2:41	20	96	101.4	6.0	~~5.99~~	5.99	Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	2:50	3:01	25	94	99.5	5.8		5.71	Turn slabs to keep warm. (every 10 min)
Weigh Curd	2:15	3:20	5		99.5			5.49	Expect ~ 145 lb 146.4 lb
Cheese milled	3:20		10	94		5.50	5.49		Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application	3:30	3:38	5	92				4.25#	Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 4.247
Sugar Addition									Optional. Add up to 0.5% galactose with salt
2nd Salt	3:35	~~3:44~~	5	91					
3rd Salt	3:40	3:49	5	90					
Fill curd into hoops	3:45		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:00	4:05	3 h						Press in horizontal press at 60 psi
Packaging	7:05 7:00		30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:30			72					Place cheese in 38°F cooler

3:43

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	~~22.20~~					
2	24.00 lb	~~22.15~~					
3	24.00 lb	~~22.45~~					
4	24.00 lb	~~22.10~~					
5	24.00 lb	~~22.05~~					
6	24.00 lb	22.06					

Notes: 16.60 + 17.50 + 19.10 + 18.35 + 18.50 + 21.05 + 16.95 + 18.40

Figure 29: Vat 2, Adjunct culture: *Lacticaseibacillus rhamnosus 20DK04*, cheese make sheet.

Cheddar - Protective Cultures

UtahState UNIVERSITY

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	9/15/20
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	BL20 DK0004 V3
	Adjunct: ADD 9.07g to 200mL milk
	ADD 11.43
	Cheesemaker

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350 1450	3.75	3.1	0.83	6.65
Actual:	1481	3.78	3.12	0.825	6.71

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 (Based on 1350 lb)	
Start Filling Vat	-1:00		40	70	72.5	6.65		X	
Vat Filled, begin heating	-0:20	6:50	15	88					Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	7:30	5	88				65.08	65.8 g A3040 plus _____g of protective culture.
Add Coagulant, Set Vat	0:00	7:37	30	88					49.6 mL Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators. 7:42
807 Vat Cut	0:30	8:08	5	88				8:08	Cut 1 min @ 10 RPM X
								8:09	Cut 1 min @ 11 RPM X
								8:10	Cut 2 min @ 12 RPM X
								8:17	Stir 30 s @ 12 RPM X
								8:13	Cut 1 min @ 14 RPM X
Start Rest (5 minutes)	0:35	8:14	5	88					Stir 2 min @ 6 RPM X
								8:16	Stir 3 min @ 8 RPM X
Start Fore work (25 minutes)	0:40	8:19	10	88					Cut 1 min @ 14 RPM X
								8:20	Stir 2 min @9 RPM X
								8:22	Stir 3 min @ 10 RPM X
								8:25	Stir 3 min @ 12 RPM X
								8:28	Cut 1 min @ 14 RPM X
Start Cook	0:50	8:30	30	88		6.55	6.64		Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time.
								8:30	Stir 6 min @ 12 RPM, Cut 1 min X
								[illegible]	Stir 8 min @ 14 RPM, Cut 1 min X
								8:46	Stir 8 min @ 15 RPM, Cut 1 min X
							6.50	8:55	Stir 8 min @ 16 RPM X
Start stir-out (35 minutes)	1:20	9:03	35	102.5	102.5		6.46		Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	9:38 1:55	9:26	15	102.5		6.45	6.44		Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

8:30

Drain curd	2:10	9:36	10	102.5		6.4	6.36		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20	9:47	5	101		6.35	6.23		Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	9:59	5	100	99.5	6.25			Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30	10:05	20	98	99				Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	10:25	20	96	97	6.0	5.93		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	10:45	25	94	96	5.8	5.74		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35		5				5.48		Expect ~ 145 lb
Cheese milled	3:40	11:05	10	94		5.50			Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application		11.65	5	92				4.6#	Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 4.6
Sugar Addition	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt 11:17	3:55	11:22	5	91					
3rd Salt 11:23	4:00	11:29	5	90					
Fill curd into hoops 11:32	4:05		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	11:52	3 h						Press in horizontal press at 60 psi
2:52 Packaging	7:20	305	30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40	3:28		72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	22.4					
2	24.00 lb	22.2					
3	24.00 lb	22.3					
4	24.00 lb	22.2					
5	24.00 lb	22.0					
6	24.00 lb	21.4					

Notes: 36.15, 37.60, 38.30, 12.65, 33.40 = 158.10

Figure 30: Vat 3, Adjunct culture: *Lacticaseibacillus rhamnosus 20DK04*, cheese make sheet.

Cheddar - Protective Cultures

V11

UtahState UNIVERSITY

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	12/8/2016
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: NSU-1 650 mL
Cheesemaker	RAEES

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1450	4.12 3.96	3.36 4.14	0.816	6.64

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 (Based on 1350 lb)
Start Filling Vat	-1:00		40	70		6.65		
Vat Filled, begin heating	-0:20		15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	10:25	5	88				**61 g** A3040 plus X g of protective culture. 650 mL yogurt
Add Coagulant, Set Vat	10:30 0:00	10:30	30	88				**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators.
Vat Cut	11:00 0:30	10:52	5	88				X Cut 1 min @ 10 RPM X Cut 1 min @ 11 RPM X Cut 2 min @ 12 RPM X Stir 30 s @ 12 RPM X Cut 1 min @ 14 RPM
Start Rest (5 minutes)	10:57 0:35	10:58	5	88				X Stir 2 min @ 6 RPM 11 X Stir 3 min @ 8 RPM 11:03
Start Fore work (25 minutes)	11:03 0:40	11:03	10	88				X Cut 1 min @ 14 RPM X Stir 2 min @9 RPM 09 X Stir 3 min @ 10 RPM 06 X Stir 3 min @ 12 RPM 09
Start Cook	11:13 0:50	11:13	30	88		11:13 6.55	6.61	X Cut 1 min @ 14 RPM 12 Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. X Stir 6 min @ 12 RPM, Cut 1 min X Stir 8 min @ 14 RPM, Cut 1 min 20 X Stir 8 min @ 15 RPM, Cut 1 min 29 X Stir 8 min @ 16 RPM 38
Start stir-out (35 minutes)	11:46 1:20	11:46	35	102.5		11:47	6.48	Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:55	12:00	15	102.5		12:00 6.45	=6.46	Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10	12:15	10	102.5		12:15 6.4	6.4		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20	12:25	5	101		6.35			Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	12:30	5	100	98	6.25	6.36		Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30		20	98	97				Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	1:00	20	96	97	6.0	6.08		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	1:20	25	94	96	5.8	5.97		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35		5			1:30 1.40	5.87 5.80		Expect ~ 145 lb
Cheese milled	3:40		10	94		5.50			Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application			5	92					Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 5.0
~~Sugar Addition~~	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55		5	91					
3rd Salt	4:00		5	90					
Fill curd into hoops	4:05		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	2:30	3 h						Press in horizontal press at 60 psi
Packaging	7:20	5:30	30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	21.8					
2	24.00 lb	21.9					
3	24.00 lb	21.9					
4	24.00 lb	22.4					
5	24.00 lb	21.8					
6	24.00 lb	21.8					
		23.1					

Notes: ~~33.95~~ + 31.5 + 32.4 + ~~26.7~~ + 23.35 + 24.95

33.8 + 26.6 + 33.8 + 30.05 + 24.95 + 23.36

172.56 2.9 + #5

2.93 %

Figure 31: Vat 4, Adjunct culture: *Latilactobacillus curvatus WSU1*, repetition two cheese make sheet.

Cheddar - Protective Cultures

V5

UtahState UNIVERSITY

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	9/29/2010
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: BL20BK00006 (CH2) ADD 2.5g g TO 200mL MILK ADD 11.6 mL TO VAT
Cheesemaker	RHEES

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1376	3.85	3.19	.828	~~6.57~~ 6.74

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 (Based on 1350 lb)
Start Filling Vat	-1:00		40	70		6.65		
Vat Filled, begin heating	-0:20		15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	7:31	5	88				**61 g** A3040 plus 11.6 g of protective culture. 11.6 mL
Add Coagulant, Set Vat	0:00	7:36	30	88				**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators.
Vat Cut	0:30	~~8:08~~ ~~8:08~~	5	88				Cut 1 min @ 10 RPM X Cut 1 min @ 11 RPM X Cut 2 min @ 12 RPM X Stir 30 s @ 12 RPM X Cut 1 min @ 14 RPM X
Start Rest (5 minutes)	0:35	8:14	5	88				Stir 2 min @ 6 RPM X Stir 3 min @ 8 RPM X
Start Fore work (25 minutes)	41 0:40	8:18	10	88				Cut 1 min @ 14 RPM X Stir 2 min @9 RPM X Stir 3 min @ 10 RPM X Stir 3 min @ 12 RPM X Cut 1 min @ 14 RPM X
Start Cook	51 0:50	8:30	30	88		6.55	6.65 6.52	8:38 Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. Stir 6 min @ 12 RPM, Cut 1 min X Stir 8 min @ 14 RPM, Cut 1 min X Stir 8 min @ 15 RPM, Cut 1 min X 9oz Stir 8 min @ 16 RPM
Start stir-out (35 minutes)	~~1:24~~ 1:20	9:02	35	102.5	102.5		6.47 6.44	9:20 Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd. 9:30
Start Pump over	1:55	9:33	15	102.5		6.45	6.44	9:3 Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10	9:43	10	102.5		6.4	6.4		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20	9:54	5	101		6.35	6.44		Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	10:01	5	100	101	6.25	6.19		Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:32 2:30	10:05	20	98	100		6.04		Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	10:25	20	96	99	6.0	5.84		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	10:45	25	94	98	5.8	5.79		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35	11:05	5		97		5.57		Expect ~ 145 lb 157.4
Cheese milled	3:40	11:20	10	94		5.50	5.54	5.49	Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application		11:27	5	92					Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 4.5
Sugar Addition	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55	11:32	5	91					
3rd Salt	4:00	11:37	5	90					
Fill curd into hoops	4:05	11:42	15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	12:00	3 h						Press in horizontal press at 60 psi
3:06 Packaging	7:20	3:00	30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40	3:35		72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	21.99					
2	24.00 lb	22.1					
3	24.00 lb	22.2					
4	24.00 lb	22.0					
5	24.00 lb	22.0					
6	24.00 lb						

Notes: 26.55 + 24.15 + 26.25 + 26.55 + 25.70 + 28.20

Figure 32: Vat 5, Adjunct culture: *Lacticaseibacillus paracasei 20DK06*, cheese make sheet.

Cheddar - Protective Cultures							
Cheese Type:	Full Fat Cheddar Cheese						
Manufacture Date:	9/29/20						
Pasteurization:	164 F, 16 Sec						
Cultures:	Starter: A3040 1 g = 1 U/10L					Thaw a bag of culture in cold water prior to use. Keep cold.	
	Adjunct: No Adjunct (Control)						
Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH		
Target:	1350	3.75	3.1	0.83	6.65		
Actual:	1428	3.85	3.19	0.83	~~6.65~~ 6.74		

Y6

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Cheesemaker

Process Step:	Time Line:		Min to Next Step	Temp. In (F)		pH		Ingredient Added and Process Details (Based on 1350 lb)	
	Tar.	Act.	Tar.	Tar.	Act.	Tar.	Act.		
Start Filling Vat	-1:00		40	70		6.65			
Vat Filled, begin heating	-0:20		15	88					Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	10:45	5	88					**61 g** A3040 plus ____ g of protective culture.
Add Coagulant, Set Vat	0:00	10:50	30	88					**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators.
Vat Cut	11:20 0:30	11:20	5	88					Cut 1 min @ 10 RPM X
									Cut 1 min @ 11 RPM X
									Cut 2 min @ 12 RPM X
									Stir 30 s @ 12 RPM X
									Cut 1 min @ 14 RPM X
Start Rest (5 minutes)	0:35	11:26	5	88					Stir 2 min @ 6 RPM X
									Stir 3 min @ 8 RPM X
Start Fore work (25 minutes)	11:31 0:40	11:31	10	88					Cut 1 min @ 14 RPM X
									Stir 2 min @9 RPM X
									Stir 3 min @ 10 RPM X
									Stir 3 min @ 12 RPM X
									Cut 1 min @ 14 RPM X
Start Cook	11:41 0:50	11:42	30	88		6.55	6.67	11:41	Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time.
									Stir 6 min @ 12 RPM, Cut 1 min X
									Stir 8 min @ 14 RPM, Cut 1 min X
							6.55		Stir 8 min @ 15 RPM, Cut 1 min X
								11:52	Stir 8 min @ 16 RPM X
Start stir-out (35 minutes)	1:20	11:52	35	102.5					Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:55	12:40	15	102.5		6.45	6.43		Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10	12:55	10	102.5		6.4	6.74		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20	1:05	5	101		6.35			Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	1:09	5	100	99.5	6.25	6.34		Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30	1:15	20	98	98.5		6.25		Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	1:35	20	96	98	6.0	6.05		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	1:55	25	94	98	5.8	5.9		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35		5				5.55	#162.6	Expect ~ 145 lb
Cheese milled	3:40	2:40	10	94		5.50	5.50		Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application		2:45	5	92				4.7#	Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 4.7 #
Sugar Addition	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55	2:50	5	91					
3rd Salt	4:00		5	90					
Fill curd into hoops	4:05		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	3:15	3 h						Press in horizontal press at 60 psi
Packaging	7:20		30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb						
2	24.00 lb						
3	24.00 lb						
4	24.00 lb						
5	24.00 lb						
6	24.00 lb						

Notes: 26.50 + 25.25 + 26.50 + 24.90 + 16.20 + 29.55 = 13.70 = 162.6

Figure 33: Vat 6, Control repetition two cheese make sheet.

Cheddar - Protective Cultures

V7 (6)

UtahState UNIVERSITY

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	10/13/wu
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: 23F (pedio) 65mL
	Cheesemaker Rhees

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1377	3.82	3.22	0.84	6.61

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 (Based on 1350 lb)
Start Filling Vat	-1:00		40	70		6.65		
Vat Filled, begin heating	-0:20	6:30	15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	7:20	5	88	88.8			**61 g** A3040 plus 65mL g of protective culture.
Add Coagulant, Set Vat	0:00	7:25	30	88				**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators. 7:33
Vat Cut	7:55 0:30	7:55	5	88				Cut 1 min @ 10 RPM X Cut 1 min @ 11 RPM X Cut 2 min @ 12 RPM X Stir 30 s @ 12 RPM X Cut 1 min @ 14 RPM X
Start Rest (5 minutes)	0:35	8:02	5	88				Stir 2 min @ 6 RPM X 37:00 Stir 3 min @ 8 RPM X
Start Fore work (25 minutes)	0:40	8:06	10	88				0:40 Cut 1 min @ 14 RPM X 0:41 Stir 2 min @9 RPM X 0:43 Stir 3 min @ 10 RPM X 0:46 Stir 3 min @ 12 RPM X 0:49 Cut 1 min @ 14 RPM X
Start Cook	0:50 0:50	8:17	30	88	88.7	6.55	6.64	Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. :50 Stir 6 min @ 12 RPM, Cut 1 min X :57 Stir 8 min @ 14 RPM, Cut 1 min X 1:06 Stir 8 min @ 15 RPM, Cut 1 min X 1:15 Stir 8 min @ 16 RPM X
Start stir-out (35 minutes)	1:23 1:20	8:50	35	102.5		6.50	6.50	Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:55 1:55	9:12	15	102.5	102.7	6.45	6.45	Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10	9:21	10	102.5	102.5	6.4	6.4		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20	9:30	5	101	102	6.35			Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	9:35	5	100	102	6.25	6.22		Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30	9:45	20	98	102		6.18		Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	10:05	20	96	100	6.0	5.97		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	10:15	25	94	98	5.8	5.8		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35	10:50	5				5.56		Expect ~ 145 lb 152.6
Cheese milled	3:40		10	94		5.50			Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application		10:02	5	92				4.416	Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture.
Sugar Addition	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55	10:07	5	91					
3rd Salt	4:00	10:12	5	90					
Fill curd into hoops	4:05	11:17	15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	11:30	3 h						Press in horizontal press at 60 psi
Packaging 2:30	7:20	2:30	30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	22.5					
2	24.00 lb	22.1					
3	24.00 lb	22.1					
4	24.00 lb	22.1					
5	24.00 lb	22.1					
6	24.00 lb	22.1					

Notes: 41.15 + 35.90 + 37.45 + 38.10

Figure 34: Vat 7, Adjunct culture: *Pediococcus acidilactici 23F*, cheese make sheet.

Cheddar - Protective Cultures

V8

UtahState UNIVERSITY

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	10/13/2021
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: BL2 DK 000006 CH2 ADD 2.599 TO 200mL MILK ADD 11.6 mL TO VAT
Cheesemaker	RHEES

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1422	3.88	3.24	0.84	6.61

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 (Based on 1350 lb)	
Start Filling Vat	-1:00		40	70		6.65	6.61		
Vat Filled, begin heating	-0:20		15	88					Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	10:22	5	88					**61 g** A3040 plus 11.6 mL of protective culture.
Add Coagulant, Set Vat	0:00	10:28	30	88	88.9				**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators.
Vat Cut	10:58 0:30	10:58	5	88				:30 :31 :32 :34 :34	Cut 1 min @ 10 RPM X Cut 1 min @ 11 RPM X Cut 2 min @ 12 RPM X Stir 30 s @ 12 RPM X Cut 1 min @ 14 RPM X
Start Rest (5 minutes)	0:35 0:35	11:05	5	88				:35 :37	Stir 2 min @ 6 RPM X Stir 3 min @ 8 RPM X
Start Fore work (25 minutes)	0:40	11:10	10	88				:40 :41 :43 :46 :49	Cut 1 min @ 14 RPM X Stir 2 min @9 RPM X Stir 3 min @ 10 RPM X Stir 3 min @ 12 RPM X Cut 1 min @ 14 RPM
Start Cook	:50 0:50	11:20	30	88		11:57 6.55 12:10	6.52 6.47	:50 :57 1:05 1:14	Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. Stir 6 min @ 12 RPM, Cut 1 min X Stir 8 min @ 14 RPM, Cut 1 min X Stir 8 min @ 15 RPM, Cut 1 min / Stir 8 min @ 16 RPM X
Start stir-out (35 minutes)	1:22 1:20	11:37	35	102.5					Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:55	12:15	15	102.5	102.7	6.45	6.42		Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10 1:59	12:24	10	102.5	102.5	6.4	6.39		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20 2:02	12:32	5	101		6.35	6.33		Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25 2:08	12:18	5	100	100	6.25	6.30		Curd mat should be 3-4 inches deep aiong both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30 2:12	12:43	20	98	100	12:46 1253	6.24 6.19		Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50 2:33	12:02	20	96	100	6.0	6.04		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10 2:54	1:23	25	94	100	5.8	5.8		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35		5			86	5.69		Expect ~ 145 lb 161.35
Cheese milled	3:40		10	94		5.50	5.46		Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application		1:55	5	92				4.67#	Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture.
~~Sugar Addition~~	~~3:50~~								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55	2:00	5	91					
3rd Salt	4:00	2:05	5	90					
Fill curd into hoops	4:05		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	2:30	3 h						Press in horizontal press at 60 psi
Packaging	5:30 7:20		30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	22.1					
2	24.00 lb	22.1					
3	24.00 lb	22.2					
4	24.00 lb	22.3					
5	24.00 lb	22.2					
6	24.00 lb	23.0					

Notes: 42.1 + 42.95 + 41.0 + 35.3 = 161.35

Figure 35: Vat 8, Adjunct culture: *Lacticaseibacillus paracasei 20DK06*, repetition two cheese make sheet.

Cheddar - Protective Cultures

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	11/3/20
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: 650 mL of WSN 1
Cheesemaker	Rhees

V9

UtahState UNIVERSITY

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH	
Target:	1350	3.75	3.1	0.83	6.65	6.70
Actual:	1455	3.47	3.21	0.81	6.66	

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 (Based on 1350 lb)
Start Filling Vat	-1:00		40	70		6.65		
Vat Filled, begin heating	-0:20		15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	7:12	5	88				**61 g** A3040 plus _____g of protective culture. 650 mL
Add Coagulant, Set Vat 7:17	0:00	7:17	30	88				**46 mL** Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators. 7:25
Vat Cut 7:47	0:30 :30	switched to plant clock 7:53	5	88				Cut 1 min @ 10 RPM X Cut 1 min @ 11 RPM X Cut 2 min @ 12 RPM X Stir 30 s @ 12 RPM X Cut 1 min @ 14 RPM X
Start Rest (5 minutes)	0:35 :35	7:58	5	88				Stir 2 min @ 6 RPM X :37 Stir 3 min @ 8 RPM X
Start Fore work (25 minutes)	0:40 :40	8:04	10	88				:40 Cut 1 min @ 14 RPM X :41 Stir 2 min @9 RPM X :43 Stir 3 min @ 10 RPM X :46 Stir 3 min @ 12 RPM X
Start Cook	0:50 :50	8:14	30	88		6.55		:49 Cut 1 min @ 14 RPM X Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. X :50 Stir 6 min @ 12 RPM, Cut 1 min X :57 Stir 8 min @ 14 RPM, Cut 1 min X 1:06 Stir 8 min @ 15 RPM, Cut 1 min X 1:15 Stir 8 min @ 16 RPM X
Start stir-out (35 minutes)	1:20 1:23	8:48 ~~8:42~~	35	102.5			6.55 6.48	8:42 8:56 Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:55 1:40	9:04	15	102.5		6.45	6.45	Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10	9:15	10	102.5	102.3	6.4	6.41		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20		5	101		6.35			Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	9:32	5	100		6.25	6.29	9:32	Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30	9:38	20	98	102.4		6.16	9:48	Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	9:58	20	96	100	6.0	6.0		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	10:18	25	94	99	5.8	5.79		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35		5		98		5.70	10:38	Expect ~ 145 lb
Cheese milled	3:40		10	94		5.50	5.50	10:48	Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application			5	92					Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture.
Sugar Addition	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55		5	91					
3rd Salt	4:00		5	90					
Fill curd into hoops	4:05		15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	1:30	3 h						Press in horizontal press at 60 psi
Packaging	7:20		30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	22.3					
2	24.00 lb	22.7					
3	24.00 lb	22.1					
4	24.00 lb	22.6					
5	24.00 lb	22.2					
6	24.00 lb	22.0					

Notes: 39.20 + 41.65 + 40.90 + 41.85 = 163.6

Figure 36: Vat 9, Adjunct culture: *Latilactobacillus curvatus WSU1*, repetition two cheese make sheet.

Cheddar - Protective Cultures

V10

UtahState UNIVERSITY

Cheese Type:	Full Fat Cheddar Cheese
Manufacture Date:	12/8/20
Pasteurization:	164 F, 16 Sec
Cultures:	Starter: A3040 1 g = 1 U/10L — Thaw a bag of culture in cold water prior to use. Keep cold.
	Adjunct: 23F pedio 65 mL
Cheesemaker	Rhees

Milk Info.	lbs. of Milk	% Fat	% Prot.	P/F	pH
Target:	1350	3.75	3.1	0.83	6.65
Actual:	1436	3.36	4.12	0.816	6.64

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 (Based on 1350 lb)
Start Filling Vat	-1:00		40	70		6.65		
Vat Filled, begin heating	-0:20		15	88				Adjust milk temp. to 88 F while agitating at 12 RPM
Add Starter, begin ripening	-0:05	7:20	5	88				61 g A3040 plus of protective culture. 65 mL
Add Coagulant, Set Vat	0:00	7:28	30	88				46 mL Double strength Maxiren chymosin Dilute 20:1 in chlorine free, cold water. Stir for 8 min then stop agitators. 7:36
Vat Cut	7:58 0:30	7:58	5	88				Cut 1 min @ 10 RPM Cut 1 min @ 11 RPM Cut 2 min @ 12 RPM Stir 30 s @ 12 RPM Cut 1 min @ 14 RPM
Start Rest (5 minutes)	0:35	8:04	5	88				Stir 2 min @ 6 RPM Stir 3 min @ 8 RPM
Start Fore work (25 minutes)	8:09 0:40	8:09	10	88				Cut 1 min @ 14 RPM Stir 2 min @9 RPM Stir 3 min @ 10 RPM Stir 3 min @ 12 RPM Cut 1 min @ 14 RPM
Start Cook	8:19 0:50	8:20	30	88		8:20 6.58 6.55 8:52 6.47		Heat Slowly at beginning (1 F/2.5-3 min) for the first 4 degrees, then proportionally faster during the remaining time. Stir 6 min @ 12 RPM, Cut 1 min Stir 8 min @ 14 RPM, Cut 1 min Stir 8 min @ 15 RPM, Cut 1 min Stir 8 min @ 16 RPM 5!
Start stir-out (35 minutes)	8:53 1:20	8:53	35	102.5				Stir continuously to prevent clumping and assist syneresis (water expulsion) @ 16 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:55	9:05	15	102.5		6.45	6.44	Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.

Drain curd	2:10	9:12	10	102.5		6.4	6.4		Start draining curd after it fills drain table
Start forming cheese curd in to pack	2:20	~~9:30~~	5	101		6.35			Continue to stir on drain table after flow of whey drops off. Allow 5 passes of the agitator to reduce moisture. Form cheese into mat.
Cheese in Pack	2:25	9:30	5	100		6.25	6.28		Curd mat should be 3-4 inches deep along both sides of the drain table with a 8 inch trough down the middle.
Cheese cut & turned for the first time	2:30	9:40	20	98	99.4	9:40 9:50	6.17 6.09		Cut curd mat into slabs about 6 inches wide, then flip over so that rough side is down. Flip after 10 min
Cheese slabs stacked 2 high	2:50	10:00	20	96	99	6.0	5.89		Cut slabs (cross cut) then flip over so that top is now bottom. Flip after 10 min
Cheese slabs stacked 3 high	3:10	10:20	25	94	99	5.8	5.7		Turn slabs to keep warm. (every 10 min)
Weigh Curd	3:35		5						Expect ~ 145 lb
Cheese milled	3:40	10:45	10	94		5.50	5.51		Cut cheese slabs long ways, and cut using the cheese mill.
1st Salt Application		10:49	5	92					Salt is added at a rate of 2.90 lb/100 lb of curd. (~4.2 lbs of salt) Add 1/3 of the salt evenly over the curd while stirring. Let stir for 5 min. Draining free moisture. 4.35
~~Sugar Addition~~	3:50								Optional. Add up to 0.5% galactose with salt
2nd Salt	3:55	~~10:54~~	5	91					
3rd Salt	4:00	~~10:59~~	5	90					
Fill curd into hoops	4:05	11:04	15	88					Place 24 lb of salted cheese in sanitized lined hoops.
Press	4:20	11:20	3 h						Press in horizontal press at 60 psi
Packaging	7:20	2:20	30	72					Remove cheese from press, place in barrier bags and seal in vacuum chamber. Label each bag and box.
Cool	7:40			72					Place cheese in 38°F cooler

Block Weight and proximate analysis

Sample/Block	Pre-Press Weight	Press Weight	% Moist.	% Fat	% Salt	pH	Date
1	24.00 lb	22.1					
2	24.00 lb	22.0					
3	24.00 lb	21.9					
4	24.00 lb	22.0					
5	24.00 lb	22.0					
6	24.00 lb	22.1					
		24.0					

Notes: 28.85 + 32.70 + 29.40 + 29.55 + 24.30 + 25.40

150.17 (170.2)

3.55%

Figure 37: Vat 10, Adjunct culture: *Pediococcus acidilactici 23F*, repetition two cheese make sheet.