Evaluation of the efficacy of commercial protective cultures to inhibit mold and yeast in cottage cheese

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ABSTRACT

Biopreservation is defined as using microbes, their constituents, or both to control spoilage while satisfying consumer demand for clean-label products. The study objective was to investigate the efficacy of bacterial cultures in biopreserving cottage cheese against post-processing fungal contamination. Cottage cheese curd and dressing were sourced from a manufacturer in New York State. Dressing was inoculated with 3 different commercial protective cultures—PC1 (mix of Lacticaseibacillus spp. and Lactiplantibacillus spp.), PC2 (Lacticeaseibacillus rhamnosus), and PC3 (Lactic. rhamnosus)—following the manufacturer recommended dosage and then mixed with curd. A control with no protective culture was included. Nine species of yeast (Candida zeylanoides, Clavispora lusitaniae, Debaryomyces Hansenii, Debaryomyces prosopidis, Kluyveromyces marxianus, Meyerozyma guilliermondii, Pichia fermentans, Rhodotorula mucilaginosa, and Torulaspora delbrueckii) and 11 species of mold (Aspergillus cibarius, Aureobasidium pullulans, Penicillium chrysogenum, Penicillium citrinum, Penicillium commune, Penicillium decumbens, Penicillium roqueforti, Mucor genevensis, Mucor racemosus, Phoma dimorpha, and Trichoderma amanonicum) were included in the study. Fungi strains were previously isolated from dairy processing environments and were inoculated onto the cheese surface at a rate of 20 cfu/g. Cheese was stored at 6 ± 2°C. Yeast levels were enumerated at 0, 7, 14, and 21 d postinoculation. Mold growth was visually observed on a weekly basis through d 42 of storage and imaged. Overall, the protective cultures were limited in their ability to delay the outgrowth of 3 strains: D. hansenii, Tor. delbrueckii, and Mey. guilliermondii. The efficacy of these protective cultures against molds in cottage cheese was more promising, with all protective cultures showing the ability to delay spoilage of at least 1 mold strain. Both PC1 and PC2 were able to delay Pen. chrysogenum outgrowth, and PC1 also delayed Pen. commune, Pen. decumbens, and Pen. roqueforti to different extents compared with the controls. This study demonstrates that commercial lactic acid bacteria cultures vary in their performance to delay mold and yeast outgrowth, and thus each protective culture should be evaluated against the specific strains of fungi of concern within each specific dairy facility.

Key words: cottage cheese, bioprotection, spoilage

INTRODUCTION

In 2017, average annual consumption of cottage cheese in the United States was estimated at 675 million pounds (~307 million kg; Statistica, 2020). Cottage cheese is a soft, fresh cheese formed by mixing dry curd with a creaming mixture (FDA, 2020). For regular cottage cheese with 4% fat, moisture content is around 80% with pH values as high as 5.2 (USDA, 2001; Jesus et al., 2016). These attributes make cottage cheese highly susceptible to microbial spoilage (Lucey, 2011).

Fungi are ubiquitous in nature. Fresh cheeses are nutrient rich, and fungal contamination can occur at different points of the value chain, including at the farm level, at the processing plant, and once it has reached consumers. Fungal strains commonly associated with fresh cheese spoilage include Candida spp., Debaryomyces spp., Pichia spp., Penicillium spp., Aspergillus spp., and Mucor spp. (Sperber and Doyle, 2009; Fernandez et al., 2017; Garnier et al., 2017b; Ho et al., 2016).

To control potential spoilage, the food industry has adopted hurdle technology, which involves creating barriers (hurdles) to microbial growth, including heat treatment, modified atmospheric packaging, food-grade preservatives, and biopreservation (Fernandez et al., 2017). These are being explored and used in the dairy industry as pasteurization contamination represents a continued challenge. However, even with the use of hurdle technology, technologies may create ad-
ditional challenges in regard to consumer acceptability. For example, the ability of carbon dioxide to dissolve in water and fat has been shown to result in packaging collapse and negatively affect consumer acceptability of modified atmospheric packaging (Ho et al., 2016). Some fungal strains are able to degrade sorbate, creating a “kerosene-like” flavor and decreasing the effectiveness of sorbate as a preservative (Pitt and Hocking, 2009; Fernandez et al., 2017). Exploration into other technologies is necessary to prevent spoilage while maintaining consumer acceptance levels.

Increased consumer demand for clean-label products has necessitated the exploration of natural alternatives to protect against fungal spoilage. Biopreservation is gaining interest as a way to naturally preserve food, enhance food safety, and extend shelf life using agents of animal, plant, or bacterial origin. Bacteriocins, propionic bacteria, and lactic acid bacteria (LAB) are examples of biopreservatives of bacterial origin (Garnier et al., 2017a). Lactic acid bacteria are non-spore-forming, facultative, aerobic, gram-positive bacteria that have a long history of being used in food fermentations. Due to this history and their Generally Recognized as Safe status by the US Food and Drug Administration, there is great interest in expanding the application of LAB as a method of biopreservation (Crowley et al., 2013).

The antifungal activity of LAB can be attributed to a range of mechanisms. First, the production of a broad range of acids such as lactic acid, phenyllactic, and 4-hydroxyphenyllactic reduces the medium pH (Cheong et al., 2014; Galvez et al., 2014). Another proposed mechanism of action is the secretion of metabolites such as reuterin by Lactobacillus spp., which may disrupt DNA synthesis (Galvez et al., 2014). Dipeptides, diacetyl, bacteriocins, and fatty acids exhibiting antifungal activity can also contribute to the antifungal properties of LAB (Crowley et al., 2013; Aunsbjerg et al., 2015). Effective inhibition of yeast and mold thought to be competition for essential resources, such as manganese, by LAB has also been demonstrated (Siedler et al., 2020).

Several commercially available LAB cultures are marketed for their antifungal efficacy, but little published data exist for cheese manufacturers to gauge how these blends compare in antifungal spectrum and their potential for shelf-life improvement in fresh cheeses. In this study, we investigated the efficacy of LAB cultures in biopreservation against spoilage fungi in cottage cheese. The study objectives were to evaluate the efficacy of 3 commercially available LAB cultures advertised as providing protection against fungal spoilage in fresh cheese and to provide a benchmark for cottage cheese producers to determine which cultures may be most effective for their products. We hypothesized that commercial LAB cultures vary in performance of mold and yeast inhibition at both genus and species level, and thus each may be ideal against specific strains depending on the food matrix.

MATERIALS AND METHODS

Lactic Acid Bacteria and Fungi Strains

Three commercial bioprotective cultures—PC1 (mix of Lactcaseibacillus spp. and Lactiplantibacillus spp.), PC2 (Lactcaseibacillus rhamnosus), and PC3 (Lact. rhamnosus)—were used throughout the study to challenge fungal spoilage organisms in cottage cheese. The manufacturer and brand name of the protective cultures were not disclosed for proprietary purposes, but further information is available upon written request to the authors. However, cultures were confirmed as a mix of Lactcaseibacillus spp. and Lactiplantibacillus spp., Lact. rhamnosus, and Lact. rhamnosus, respectively, through 16s rDNA sequencing (Makki et al., 2020). All protective cultures were kept at −80 ± 2°C until use.

Fungal spoilage strains used throughout the study were previously isolated from dairy processing plants and were sourced from the Cornell University Food Safety Laboratory isolate collection and the Alcaine Research Group isolate collection (Ithaca, NY). Nine species of yeast (Candida zeylanoides, Clavispora lusitaniae, Debaryomyces hansenii, Debaryomyces prosopidis, Kluyveromyces marxianus, Mycrozyma guilliermondii, Pichia fermentans, Rhodotorula mucilaginosa, and Torulaspora delbrueckii) and 11 species of mold spanning 6 genera (Aspergillus cibarius, Aureobasidium pullulans, Penicillium chrysogenum, Penicillium citrinum, Penicillium commune, Penicillium decumbens, Penicillium roqueforti, Mucor genevensis, Mucor racemosus, Phoma dimorpha, and Trichoderma amazonicum) were used throughout the study. Fungal stocks were kept frozen at −80 ± 2°C. Table 1 shows genus and species identification, type of microorganism, and isolation source for spoilage organisms used throughout the study.

Preparation of Yeast Inoculum

For each respective yeast strain, frozen yeast stocks were allowed to thaw on ice and propagated on the surface of potato dextrose agar plates (PDA; Hardy Diagnostics, Santa Maria, CA) followed by incubation at 25°C for 48 h. A single colony was isolated from each plate and inoculated in 5 mL of potato dextrose broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India), followed by incubation at 25°C for 16 h. Cultures were transferred into sterile 2-mL cryovials (Simport, Beloeil, QC, Canada) and supplemented with glycerol.
at 50% (vol/vol) to form yeast stock suspensions. Yeast stocks were stored at −80 ± 2°C until use. To enumerate yeast stocks, each stock was serially diluted in PBS and plated on PDA, followed by incubation at 25°C for 48 h. Yeast colonies were enumerated using a Q-Count colony counter (Advanced Instruments, Norwood, MA).

Preparation of Mold Spore Suspensions

Frozen isolates of each mold strain were allowed to thaw on ice and spotted onto the surface of malt extract agar plates (Difco, Franklin Lakes, NJ), followed by incubation at 25°C for 30 d until spore formation. Plates were flooded with PBS containing 0.1% Tween 80 (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan), and then gently scraped using a sterile cell spreader to release spores and mycelia. Mycelia were filtered from the mold suspensions by passing through 4 layers of sterile cheesecloth. Spore formation was confirmed microscopically (Reichert microscope, Reichert Technologies, Depew, NY). In sterile 50-mL conical tubes (VWR, Radnor, PA), spore suspensions were supplemented with glycerol at 50% (vol/vol). Spore suspensions were stored at −80 ± 2°C until use.

For each mold strain, spore suspension concentrations were determined by microscopy with a Neubauer’s improved counting chamber hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Spore counts were confirmed by plating in duplicate on dichloran rose bengal chloramphenicol agar (Becton, Dickinson and Co., Sparks, MD), followed by incubation at 25°C for 5 d.

Cottage Cheese Sourcing and Inoculation with Protective Cultures

Separate dry curd and cream dressing of freshly made, preservative-free, regular cottage cheese (4% fat) were sourced from a cheese manufacturer in upstate New York. Immediately before inoculation, each commercial protective culture was resuspended in UHT processed fat-free milk (Parmalat, Buffalo, NY) and used to inoculate cream dressing. For each treatment, cream dressing was inoculated with the commercial protective cultures (PC1, PC2, and PC3) to achieve the manufacturer recommended dosage in the cottage cheese. Curd was added to cream dressing and mixed well to ensure homogeneity. A positive control with no protective culture was included. All steps were carried out under aseptic conditions. Cheese was stored under refrigeration conditions (6 ± 2°C) overnight.

Efficacy of Bioprotective Cultures Against Yeast on Cottage Cheese

Following overnight refrigeration of creamed cottage cheese, four 6-well plates (3.5 cm in diameter; Falcon, Corning, NY) were prepared for each yeast strain, where a single plate was assigned for each of the 3 treatments (PC1, PC2, PC3) and the positive control.

### Table 1. Genus and species identification, type, location, and isolation source for spoilage organisms used throughout the study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type</th>
<th>ID</th>
<th>Isolate source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida zeylanoides</td>
<td>Yeast</td>
<td>B90031</td>
<td>Cheese</td>
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<tr>
<td>Clavispora lusitaniae</td>
<td>Yeast</td>
<td>B90007</td>
<td>Raw milk</td>
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<tr>
<td>Debaryomyces hansenii</td>
<td>Yeast</td>
<td>B90013</td>
<td>Cheese</td>
</tr>
<tr>
<td>Debaryomyces prosopidis</td>
<td>Yeast</td>
<td>B90028</td>
<td>Cheese</td>
</tr>
<tr>
<td>Kluyveromyces marxianus</td>
<td>Yeast</td>
<td>B90008</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Meyerozyma guilliermondii</td>
<td>Yeast</td>
<td>E20377</td>
<td>Yogurt</td>
</tr>
<tr>
<td>Pichia fermentans</td>
<td>Yeast</td>
<td>B90001</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Rhodotorula mucilaginosa</td>
<td>Yeast</td>
<td>E20331</td>
<td>Dairy processing environment</td>
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<td>Torulaspora delbrueckii</td>
<td>Yeast</td>
<td>E20412</td>
<td>Yogurt</td>
</tr>
<tr>
<td>Aspergillus citarius</td>
<td>Mold</td>
<td>E20323</td>
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<td>Aureobasidium pullulans</td>
<td>Mold</td>
<td>E20290</td>
<td>Yogurt</td>
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<td>Mold</td>
<td>TD0021</td>
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<td>E20369</td>
<td>Yogurt</td>
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<td>Trichoderma amazonicum</td>
<td>Mold</td>
<td>E20387</td>
<td>Yogurt</td>
</tr>
</tbody>
</table>

1All strains were from the Food Safety Laboratory at Cornell University (Ithaca, NY) except for *Mucor genevensis*, which was from the Alcaine Research Group collection at Cornell University.

2References identification of isolate in Food Microbe Tracker. Additional information can be found at www.foodmicrobetracker.com.
In each well, 5 ± 0.5 g of cottage cheese was added aseptically. In each well, yeast strains were inoculated on the surface of cheese samples with a target rate of 20 cfu/g. Throughout the study, plates were stored at refrigeration temperature (6 ± 2°C).

Yeast enumeration was conducted at d 0, 7, 14, and 21 postinoculation. For sampling, the contents of one well were aseptically transferred to a stomacher bag (Whirl-Pak, Nasco, Fort Atkinson, WI) and 45 mL of PBS was added to realize a 1:9 ratio followed by homogenization at 230 rpm for 60 s using a Seward Stomacher 400 Circulator blender (Seward Ltd., Worthing, UK). Samples were serially diluted and plated in duplicate on PDA supplemented with 25 mg of chloramphenicol/L of medium (Sigma-Aldrich, St. Louis, MO). Plates were incubated at 25°C for 5 d. Figure 1 shows a diagram outlining yeast inoculation and enumeration in cottage cheese.

To assess mold outgrowth on cottage cheese, plates were visually observed at d 0, 7, 14, 21, 28, 35, and 42 postinoculation and imaged using a PowerShot SX530 160-MP HS digital camera (Canon, Tokyo, Japan). After 42 d, fungal spoilage occurred in the negative control cheeses. The following scale was used to report results: (−) no visible mold growth, (±*) cheeses transitioning into matte appearance in some replicates, (±) matte appearance with no colored mold growth in all replicates, (++*) cheeses transitioning into colored mold growth in some replicates, and (+++) mold growth with change in color across replicates. Figure 2 shows a schematic diagram of mold inoculation and visual examination in cottage cheese stored at 6 ± 2°C.

**Cheese Physical Properties**

Moisture content and pH values were measured for 2 samples per treatment using a microwave oven (CEM Inc., Matthews, NC) and edge pH meter (Hanna Instruments, Smithfield, RI), respectively.

**Statistical Analysis**

Experiments were performed in triplicate for both yeast and mold studies. Significant differences ($P < 0.05$) between the counts of each yeast strain were determined by comparing yeast counts (log cfu/g) for each protective culture against the control cheese with no protective culture using a 1-way ANOVA with Bonferroni correction performed individually at time points 7, 14, and 21 d postinoculation. For these studies, a biological difference was defined as having both a significant difference ($P < 0.05$) and at least a 1 log
RESULTS AND DISCUSSION

The average pH values of the control cheese and the PC1-, PC2-, and PC3-inoculated cheeses were 4.97 ± 0.09, 4.75 ± 0.16, 4.95 ± 0.04, and 4.90 ± 0.06, respectively. Average moisture levels of the control cheese and the PC1-, PC2-, and PC3-inoculated cheeses were 78.32 ± 1.32, 78.51 ± 1.44, 78.50 ± 1.43, and 79.10 ± 1.28%, respectively. For the yeast challenge study, negative controls of each treatment showed no growth through 21 d of incubation at 6 ± 2°C. In the context of this study, the yeast count that results in spoilage was selected as >5 log cfu/g. At this level, consumers can detect flavor changes, visible defects, and textural changes (Zantar et al., 2014). For yeast counts below the detection limit, a value of 1.3 log cfu/g was used to express counts.

Overall, the protective cultures were mostly ineffective at controlling the growth of yeast in cottage cheese (Figure 3). Of the 9 strains used in experiments, 6 strains (Cla. lusitaniae, Can. zeylanoides, D. prosopidis, Klu. marxianus, Pic. fermentans, and R. mucilaginosa) all readily grew in cottage cheeses, with no significant difference in outgrowth between the control and the treatments with protective cultures (Figure 3). Two strains, Can. zeylanoides and D. prosopidis, rapidly surpassed the spoilage limit of 5 log cfu/g by d 7, whereas the other 4 strains reached the spoilage limit by d 14.

Only 1 of the protective cultures in this study, PC1, had an effect on the outgrowth of D. hansenii, Mey. guilliermondii, and Tor. delbrueckii compared with the control (Figure 3). In the case of D. hansenii, at d 14, PC1 had significantly slowed the outgrowth of this strain compared with the control and other protective cultures (Figure 3) to below the spoilage limit. However, by d 21 D. hansenii was able to overcome the inhibitory effect of PC1, surpassing the spoilage limit and reaching a final level that was not significantly different from that of the control. In regard to Mey. guilliermondii, cheeses treated with PC1 resulted in significantly lower counts compared with the control cheese and the PC2 and PC3 treatments on d 14 and 21 (P < 0.0001; Figure 3F), although Mey. guilliermondii counts still exceeded spoilage limits by d 14. Our results indicate that PC1 does inhibit Mey. guilliermondii and may delay time to spoilage if the load of Mey. guilliermondii in a cottage cheese facility is lower than the inoculation level used in this study, although more research is needed. Torulaspora delbrueckii was the only yeast strain that was significantly inhibited by a protective culture, again PC1, compared with the control and that was kept from exceeding the spoilage limit over the 21-d study. Recent studies on Lactic. rhamnosus and Lacticaseibacillus paracasei have demonstrated the ability of several strains to inhibit the outgrowth of both D. hansenii and Tor. delbrueckii in fermented milk through competition for manganese (Siedler et al., 2020). In this study, manganese levels in the cottage cheese were not measured, so it is unclear whether such trace mineral competition accounts for the ability of PC1 to affect the growth of these spoilage organisms.
in cottage cheese; however, PC2 and PC3, which are *Lactic. rhamnosus* strains, did not show any inhibitory effect. In a similar challenge study that we performed with these protective cultures in queso fresco, all 3 cultures were able to inhibit *D. hansenii* outgrowth, and 2 were able inhibit *Tor. delbrueckii* (Makki et al., 2020), but it is not uncommon to see LAB, including *Lactic. rhamnosus* strains, perform differently in different dairy substrates (Leyva Salas et al., 2018, 2019). The difference in matrix attributes, such as pH (which was around 4.9 for the cottage cheese vs. 6.3 for the queso fresco in our study) as well as moisture (which was around 78% for the cottage cheese vs. 60% in the queso fresco), potentially affects the activity of both the protective cultures and the challenge fungi, although more research is need to understand in what manner (Makki et al., 2020).

In this study, the antifungal activity of protective cultures against mold in cottage cheese was evaluated by comparing visible mold growth on control cheese with no protective culture and on cheeses with protective cultures inoculated with the same mold strain. Examination was performed on a weekly basis over 42 d of refrigerated storage (6 ± 2°C). Day 42 was selected as the cutoff point of the study because beyond that negative control cheeses, thus not inoculated with mold, started showing visual growth. Table 2 shows the results of mold growth over 42 d on cottage cheese at 6 ± 2°C. For 3 mold species (*Asp. cibarius*, *Tri. amazonicum*, and *Pen. citrinum*), no visual growth was observed throughout d 42 on any treatment, including positive control with no protective culture (Table 2). The viability of the stocks was confirmed (data not shown),...
and although the isolates were from dairy products and the dairy environment, these results suggest that they do not grow well in cottage cheese.

None of the protective cultures inhibited the outgrowth of strains of *Aur. pullulans*, *Muc. genevensis*, or *Muc. racemosus* compared with the control (Table 2). Figure 4 shows *Muc. racemosus* outgrowth on cottage cheese at d 7 and 14 after incubation at 6 ± 2°C. At d 14, all cheeses inoculated with protective cultures as well as control cheese showed “cat hair” growth characteristic for *Mucor* spp. on their surface. This aligns with our queso fresco study, where none of the protective cultures delayed visible outgrowth of the *Mucor* spp. strains compared with the control (Makki et al., 2020).

A study screening antifungal LAB in both yogurt and cheese systems also found only slight inhibition of *Muc. racemosus* by *Lactic. rhamnosus* strains (Leyva Salas et al., 2018). Another study screening LAB strains, including *Lactic. rhamnosus*, did show evidence of inhibition of *Muc. racemosus* in an overlay assay; however, its performance in a dairy matrix was not evaluated (Fernandez et al., 2017).

The 4 *Penicillium* spp. strains that grew on cottage cheese were all inhibited differently by each protective culture (Table 2). Visible outgrowth of *Pen. decumbens* was inhibited over the 42-d challenge study by PC1, whereas the other 2 protective controls did not delay outgrowth compared with the control. A similar pattern was observed with *Pen. chrysogenum*, which was inhibited over the study by PC1, and but in this case PC2 was also able to delay visible outgrowth of the mold by 1 wk over the control to d 21; PC3 did not inhibit the outgrowth of *Pen. chrysogenum*. Figure 5 shows *Pen. chrysogenum* growth on cottage cheese at d 21 and 42 of storage at 6 ± 2°C. Fernandez et al. (2017) demonstrated the ability of *Lactic. rhamnosus* strain A238, both alone and when used with *Bifidobacterium animalis* ssp. lactis A026, to inhibit *Pen. chrysogenum* outgrowth on cottage cheese by at least 21 d under refrigerated conditions. In our queso fresco study, 2 of the protective cultures were able to inhibit the outgrowth of *Pen. chrysogenum* over the 42-d study (Makki et al., 2020).

Both *Pen. roqueforti* and *Pen. commune* grew well in cottage cheese, with only PC1 able to delay visible outgrowth by 1 wk compared with the control (Table 2). Partial inhibition of the level of *Pen. commune* by PC1 continued through d 42. In contrast, another study by Cheong et al. (2014) screening the antifungal activity of 800 LAB isolates in cottage cheese found 12 isolates that exhibited antifungal activity against *Pen. commune*, although all were strains of *Lactiplantibacillus*.

In our queso fresco study, visible outgrowth of *Pen. roqueforti* and *Pen. commune* was delayed by vary-
ing degrees by all 3 protective cultures (Makki et al., 2020). A study evaluating the effect of commercially available protective cultures to improve Greek yogurt quality also found Pen. commune readily inhibited over 60 d, even with culture levels used at rates below the manufacturer recommendations (Buehler et al., 2018).

Interestingly, all 3 protective cultures demonstrated some level of efficacy against Pho. dimorpha (Table 2). All 3 were able to delay visible outgrowth by 21 wk, to d 28, and PC1 was able to delay outgrowth for an additional 2 wk until d 42. All 3 cultures were also effective at delaying Pho. dimorpha outgrowth in queso fresco (Makki et al., 2020). This suggests potential efficacy of Lactobacillus spp. in protecting against spoilage caused by Pho. dimorpha in several cheese matrices under refrigerated conditions.

Several other studies have also examined the interaction of LAB and molds in dairy products and found it to be quite varied. As with yeast, this competition may be due to direct competition for trace nutrients. For
example, outgrowth of strains of *Penicillium brevicompactum*, *Penicillium crustosum*, and *Penicillium solitum* was shown to be inhibited by *Lactic. rhamnosus* strains but was restored when provided with excess manganese. Another study found that diacetyl production by *Lactic. paracasei* was able to inhibit the outgrowth of *Pen. solitum* (Aunsbjerg et al., 2015). A study by Sedaghat et al. (2016) demonstrated the ability of Lactip. plantarum PIN, Lactip. plantarum CAG23, Lactic. casei D31, Lactip. plantarum NBRC107151, and Lactip. pentosus H39 to delay mycelial growth of *Aspergillus flavus* and *Aspergillus parasiticus* on cheese surfaces under different storage conditions. A study by Leyva Salas et al. (2018) also demonstrated the variability in inhibition of other mold species not included in this study, such as *Penicillium bialwiezense* and *Galactomyces geotrichum*, by LAB including *Lactic. rhamnosus* strains that are commonly found in commercial protective cultures, and the variability across dairy matrices such as cheese and yogurt. This highlights the importance of understanding both the resident spoilage organisms within a dairy facility and the application of protective cultures in different dairy products.

In this study, protective cultures were incorporated into the cottage cheese dressing before being mixed with the cheese curd and subsequently packaged. Adding protective cultures together with cheese starter culture could theoretically allow the protective culture more time to multiply and hence enhance the antifungal activity against selected mold and yeast strains. Conversely, these cultures could potentially compete with starter culture over nutrients, or either culture could secrete metabolites compromising medium quality for optimum growth. It is likely that adding protective cultures to cottage cheese at a different processing step would result in a different inhibitory scheme against the fungal challenge strains, and thus cheese processors should validate the step at which protective culture is added to maximize antifungal activity.

In summary, the protective cultures evaluated in this study were mostly ineffective at controlling the growth of yeast in cottage cheese. Only 1 of the protective cultures, PC1, was able to delay the outgrowth of 3 strains, and only *Tor. delbrueckii* was kept below the detectable spoilage level over the 21-d period. The outgrowth of the other 6 yeast strains was unimpeded by any of the protective cultures in this study. The efficacy of these protective cultures against molds in cottage cheese was more promising, with all protective cultures showing the ability to delay visible spoilage of at least 1 mold strain in the study. In 5 of the 8 mold strains that grew in cottage cheese, PC1 was able to delay outgrowth compared with the control. This study was not an exhaustive study on all commercially available protective cultures, and new LAB strains are being constantly identified and used to replace or augment strains in currently available products. It is highly recommended that cottage cheese producers thoroughly evaluate any protective culture against the spoilage species and strains of interest before adoption to ensure their products get the extended shelf life they expect.

Overall, the findings show that commercial LAB cultures vary in performance against various yeast and molds, and thus each protective culture may exhibit a broad range of antifungal activity against different fungal strains taking into consideration food matrix factors. Cheese processors considering protective LAB cultures as a clean-label alternative should investigate the efficacy of these cultures against fungal strains of concern within their production system.

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