EVALUATION OF ANTIMICROBIALS AND SALT REPLACERS FOR USE IN LOW-SODIUM DAIRY PRODUCTS

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ABSTRACT

Salt replacers and antimicrobial agents may be required to maintain consumer acceptability and safety of low-sodium dairy products. To determine which antimicrobials could potentially be effective in these products, this study evaluated the efficacy of eight commercial antimicrobials alone and in combination with six commercial salt replacers. Milk and low-sodium cheese agar systems were used as the growth medium for screening. Antimicrobials with and without salt replacers were added to the agar systems. Then cocktails of *Listeria monocytogenes*, *Salmonella* or *Escherichia coli* O157:H7 were spread plated on the supplemented agar and observed for growth after 24 and 48 h at 35C. Fermentates C and D, lauric arginate (LAE), and lactoperoxidase inhibited growth of all pathogens on milk agar. No pathogen growth was observed in the cheese agar containing lactoperoxidase. Salt replacers decreased efficacy of the fermentates and LAE against *Salmonella* and *E. coli* O157:H7.

PRACTICAL APPLICATIONS

Pressure has been put on the food industry to reduced sodium levels in food. Because reduction of salt from dairy products could change the microbial stability and flavor, alternative antimicrobials and salt replacers may be required. Many commercial antimicrobial systems and sodium replacer exist, but testing each potential combination in a specific food matrix is impractical. Through the use of model milk and cheese systems, eight antimicrobials and six salt replacers were screened for efficacy against selected pathogens. Four of the eight antimicrobials screened inhibited pathogen growth in the model systems. Findings also indicated that salt replacers can negatively impact antimicrobial efficacy. While variation in a product matrix can interfere with antimicrobial efficacy, these findings can be used to help guide selection of antimicrobials and salt replacers for use in dairy products.

INTRODUCTION

Salt (sodium chloride) is the primary source of sodium in the human diet and is added to foods for many functional reasons including flavor enhancement, texture and microbial stability. While sodium is an essential nutrient, excess consumption can lead to high blood pressure, which can increase risk of cardiovascular disease and stroke (He *et al.* 2009). The 2010 Dietary Guidelines for Americans recommend that daily sodium intake be reduced to 2,300 mg or less; however, fewer than 15% of Americans meet this recommendation. Average sodium intakes for Americans aged 2 years and older is 3,400 mg/day (United States Department of Agriculture [USDA] and United States Department of Health and Human Services [HHS] 2010). Because approximately 75% of dietary salt comes from

processed and restaurant foods, it will be challenging for consumers to achieve recommended intake levels using current consumption trends without the food industry reducing sodium levels in the foods they produce (Appel and Anderson 2010; Bibbins-Domingo *et al.* 2010; Institute of Medicine [IOM] 2010; United States Department of Agriculture [USDA] and United States Department of Health and Human Services [HHS] 2010). Some of the major sources of sodium in the U.S. diet include: yeast breads, chicken/chicken dishes, pizza, cold cut meats, pasta, condiments and cheese. Cheese ranked in the top 12 product categories that contribute over 100 mg of sodium per person per day in the U.S.A., indicating that efforts to reduce sodium in dairy products such as cheese are needed (National Cancer Institute 2010).

Dairy products contain important nutrients including: protein, calcium, potassium, magnesium, vitamin D and vitamin A; however, some dairy products like cheese are high in sodium (Johnson *et al.* 2009). The salt content of cheeses ranges from 0.5–0.7% in acid curd cheeses to as high as 4–6% in pickled cheeses (Guinee 2004). Salt acts as a critical hurdle to control growth of pathogens and spoilage microorganisms; if salt levels are reduced in foods like cheese the addition of alternative preservatives may be needed to ensure safety and a reasonable shelf life (Taormina 2010).

Studies on growth and survival of microorganisms in cheeses containing various concentrations of salt are limited. Total bacterial counts, yeast, mold and coliforms increased in a feta-type cheese as the concentration of NaCl was reduced from 4 to 1% (Aly 1995). In cheddar cheese, a reduction in sodium resulted in a longer survival time for *Salmonella* and a decreased log reduction for *Listeria monocytogenes* (Shrestha *et al.* 2011a,b). In cheese with a salt content of 1.8% wt/wt, *Escherichia coli* O157:H7 became undetectable after 7 days; however, in another cheese with a salt content of 0.52% *E. coli* O157:H7 was able to survive for up to 20 days (Katic and Stojanovic 2003; Lekkas *et al.* 2006).

While current research seems to indicate that sodium reduction in cheese may reduce microbial stability, research addressing strategies on how to compensate for this decrease is lacking. Numerous commercial antimicrobial systems and sodium replacer exist; however, testing each potential combination in a specific food matrix can be costly and time consuming. Therefore, the objectives of this study were to: (1) evaluate eight commercially available antimicrobials for their effectiveness against *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 in milk and low-sodium cheese agar systems; and (2) determine if sodium replacers used to compensate for flavor changes in reduced sodium products will impact antimicrobial efficacy.

MATERIALS AND METHODS

Bacterial Strains

Five strains of each *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* were used in the study. *E. coli* O157:H7 strains NFPA 4212, NFPA 4213, NFPA 4216, NFPA 4217 and NFPA 4219 were obtained from the culture collection of The National Food Laboratory (The NFL, Livermore, CA). Two of the *L. monocytogenes* strains, NFPA 6301 and NFPA 6306, were also obtained from The NFL, while the other three strains, NRRL B-33000, NRRL B-33096 and NRRL B-33113, were received from the National Center for Agricultural Utilization Research (Peoria, IL). *Salmonella enterica* strains, *S. javiana* FSL S5-406, *S. enteriditis* FSL S5-415, *S.* Heidelberg FSL S5-448, *S.* oranienburg FSL S5-642 and *S. typhimurium* FSL W1-030 were obtained from the International Life Sciences Institute, North America (Cornell University, Ithaca, NY). All strains were stored in cryogenic vials (Fisher Scientific, Hanover Park, IL) at −70C for long-term preservation. Prior to use, cultures were streaked on selective media and incubated at 35C for 24 h. *Salmonella* strains were streaked on xylose lysine deoxycholate agar (Neogen, Lansing, MI), while modified oxford agar (Difco, Sparks, MD) was used for *L. monocytogenes* and MacConkey agar with sorbitol (Neogen) for *E. coli* O157:H7.

Inocula Preparation

Each strain was individually transferred into 10 mL of tryptic soy broth (Neogen) and grown at 35C for 18 h. After incubation, equal volumes of each strain were pooled within a species to form a cocktail. Each cocktail was centrifuged ($1,800 \times g \times 15$ min), washed twice with 0.1% peptone water and resuspended in 0.1% peptone water. Direct microscope count was done with a Petroff-Hausser counting chamber (Hausser Scientific, Horsham, PA) to determine initial stock concentration. Cocktails were diluted to the target concentration using 0.1% peptone water. Initial counts were confirmed by plating on tryptic soy agar (TSA, Neogen) and incubating at 35C for 24 h.

Antimicrobials and Sodium Replacers

Eight antimicrobials and six sodium replacement agents received from several suppliers were used in the study (Tables 1 and 2). Antimicrobials and sodium replacement agents were stored based on manufacturer's recommendation until used in the study.

Antimicrobial	Product name, manufacturer	Product description
Fermentate A	MicroGARD 100, Danisco (New Century, KS, USA)	Cultured grade a nonfat dry milk powder, propionic acid, acetate
Fermentate B	MicroGARD 430, Danisco	Cultured grade a nonfat dry milk powder, maltodextrin, propionic acid, acetate
Nisin	Nisaplin, Danisco	Nisin (minimum 1,000 IU/mg), NaCl
Fermentate C	NovaGARD CB1, Danisco	Maltodextrin, cultured dextrose, sodium diacetate, NaCl, lysozyme, nisin
Lauric arginate	Protect-M, Purac (Gorinchem, The Netherlands)	Lauric arginate (10% wt/wt)
Fermentate D	PuraQ Verdad RV75, Purac	Cultured sugar, organic acids, residual sugars
Lactoperoxidase	SEA-i F75, Bienca (Ghent, Belgium)	Glucose, glucose oxidase, whey, lactoperoxidase, lactose, casein
Fermentate E	VMY1P, Purac	Cultured whey

Preparation and Testing of Antimicrobials in Milk and Cheese Agar

Efficacy of the antimicrobials was determined using an agar dilution method (Oussalah *et al.* 2007). Milk agar was prepared by dissolving 8 g of nonfat dry milk and 15 g of agar in 1 L of deionized water followed by sterilization in an autoclave. For the low-sodium cheese agar, immediately after autoclaving 1 L of deionized water with 15 g of agar, 100 g of low-sodium (0.86% wt/wt) low-moisture part-skim mozzarella cheese was melted and mixed into the agar. Both the milk and the cheese agars were held at 50C prior to the addition of antimicrobials. Tested antimicrobial concentrations (Tables 3 and 4) were determined using manufacturer's recommendations and preliminary efficacy results in TSA (data not shown). After the addition of antimicrobials, agars were poured into sterile Petri plates and allowed to solidify. *Salmonella*, *L. monocytogenes* or *E. coli* O157:H7 cocktails were spread plated onto milk and low-sodium cheese agar at concentrations of 10^1, 10^2 and 10^4 cfu/mL. Uninoculated milk agar and low-sodium cheese agar plates served as negative controls while inoculated plates without antimicrobial added served as positive controls. All test and control plates were incubated at 35C for 48 h. Plates were observed after 24 and 48 h for the presence or absence of growth. For all plates, absence of growth indicated the antimicrobial had an inhibitory effect.

Sodium Replacement and Antimicrobial Agent Interactions

To determine if sodium replacers affected antimicrobial efficacy, four antimicrobials exhibiting efficacy in milk agar were tested in combination with six sodium replacers. Based on the previous milk agar screening results, fermentate C, lauric arginate (LAE), fermentate D and lactoperoxidase were tested at a concentration of 1.0, 0.2, 0.2 and 0.25%, respectively. Sodium replacement concentrations were based on manufacturers' recommendations. Potassium chloride (KCl) and KCl blends A and B were tested at 1.0 and 2.0%, and calcium lactate, potassium lactate and fermentate F were tested at 0.01 and 0.02%. Each antimicrobial and sodium replacer were added to tempered milk agar. The agar was poured into sterile Petri plates and allowed to

Sodium replacement agent	Product name, manufacturer	Product description
Potassium chloride	Potassium chloride, Cargill (Minneapolis, MN, USA)	Potassium chloride
Calcium lactate	Puracal PP/USP, Purac	Calcium lactate
Potassium lactate	Puracal Hi Pure P Plus, Purac	Potassium lactate
Fermentate F	PuraQ Verdad NV10, Purac	Cultured corn sugar
Potassium chloride blend A	SaltWise 0029, Cargill	Potassium chloride; trehalose; autolyzed yeast extract; silicon dioxide
Potassium chloride blend B	SaltWise 1029, Cargill	Potassium chloride; trehalose; autolyzed yeast extract; silicon dioxide

TABLE 3. GROWTH OF *SALMONELLA*, *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* AT CONCENTRATIONS OF 10^4 CFU/ML ON MILK AGAR SUPPLEMENTED WITH VARIOUS ANTIMICROBIALS

Antimicrobial	Concentration %	Agar pH	Salmonella	E. coli O157:H7	L. monocytogenes
Fermentate A	0.5	6.72	24	24	NT
	1.0	6.71	48	24	NT
Fermentate B	0.5	5.93	24	24	24
	1.0	5.61	48	24	24
Nisin	0.03	6.74	NT	NT	24
	0.10	6.66	NT	NT	24
Fermentate C	1.0	5.49	NG	NG	NG
	2.0	5.27	NG	NG	NG
Lauric arginate	0.1	6.41	48	NG	NG
	0.2	6.42	NG	NG	NG
Fermentate D	0.2	4.78	NG	NG	NG
	0.4	4.70	NG	NG	NG
Lactoperoxidase	0.25	5.39	NG	NG	NG
	0.50	4.97	NG	NG	NG
Fermentate E	0.5	5.67	48	48	48
	1.0	5.38	NG	NG	NG

Time of observed organism growth (h). NG, no growth; NT, not tested per manufacturer's recommendations.

solidify. Plates were then spread plated with cocktails of *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* at a concentration of 10^4 cfu/mL. Uninoculated milk agar plates served as negative controls while inoculated milk agar plates without an antimicrobial or sodium replacement agent added served as positive controls. All test and control plates were incubated at 35C for 48 h. Plates were observed after 24 and 48 h for the presence or absence of growth.

RESULTS AND DISCUSSION

Efficacy of Antimicrobials in Milk Agar

To evaluate the effect of inoculum concentration on antimicrobial efficacy, three pathogen concentrations were tested. In the milk agar, outgrowth of the pathogens was not influenced by the initial concentration and therefore only results for the 10^4 cfu/mL concentration are reported and discussed.

Five of the antimicrobials evaluated in this study were fermentates (A, B, C, D and E). The observed efficacy of the fermentates in milk agar varied, with fermentates A and B and fermentates C and D showing no inhibition and complete inhibition of the test organisms, respectively (Table 3). Fermentate E showed partial inhibition, delaying growth at 0.5% and completely inhibiting growth at 1.0% for all test organisms. Fermentates are products of the *in situ* lactic acid bacteria-controlled acidification (Montville and Chikindas 2007). Fermentation products, such as organic acids, enzymes, diacetyl, bacteriocins and bacteriocin-like products, have been widely used as preservatives in the dairy, meat and poultry

TABLE 4. GROWTH OF *SALMONELLA*, *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* ON LOW-SODIUM CHEESE AGAR SUPPLEMENTED WITH VARIOUS ANTIMICROBIALS

Antimicrobial (concentration %)	Agar pH	Inoculum level (cfu/mL)	Salmonella	E. coli O157:H7	L. monocytogenes
Fermentate C (1.0)	5.39	10	NG	48	48
		10^2	48	48	48
		10^4	48	48	48
Lauric arginate (0.2)	4.96	10	NG	NG	NG
		10^2	48	NG	NG
		10^4	48	48	48
Fermentate D (0.2)	4.84	10	NG	NG	48
		10^2	48	48	48
		10^4	48	48	48
Lactoperoxidase (0.25)	4.96	10	NG	NG	NG
		10^2	NG	NG	NG
		10^4	48	48	NG

Time of observed organism growth (h). NG, no growth.

industries. Fermentation by-products are unique to the bacteria and the fermentation conditions, so the observed differences in antimicrobial efficacy were not unexpected.

According to the manufacturer, fermentates A and B are produced through the fermentation of grade A skim milk by *Propionibacterium shermanii* (Dave *et al.* 2003). Antimicrobial components of these fermentates are diacetyl, and lactic, acetic and propionic acids. Diacetyl has a broad antimicrobial activity and can inhibit the growth of *E. coli*, *Salmonella*, *Staphylococcus aureus* and *L. monocytogenes* when utilized at concentrations ranging between 200 and 1,000 ppm (Kang and Fung 1999; Lanciotti *et al.* 2003). The concentration of diacetyl in fermentates A and B is not known, but research showed that in brain heart infusion (BHI) broth, a nutrient-rich medium, there was no inhibition of gram-negative or gram-positive organisms at a concentration of 344 ppm. It was hypothesized that the lack of effectiveness in BHI was likely due to the nutrient composition of the medium and the relatively high incubation temperature; diacetyl is more effective at 10 and 20C than at 37C (Jay 1982). An insufficient concentration of diacetyl, the test parameters or a combination of the two factors could have contributed to the observed lack of efficacy in this study. The test conditions also could have affected the antimicrobial activity of the other components: lactic, acetic and propionic acids. The antimicrobial mechanism of organic acids has been described elsewhere (Ricke 2003). Organic acids are most effective when used in a system where the pH is below the acids pK_a because they are undissociated. Lactic, acetic and propionic acids have pK_a values of 3.79, 4.75 and 4.87, respectively. The pH of milk agar after the addition of the fermentates was much higher than each of the respective pK_a values, likely reducing the efficacy of the organic acids (Table 3). Reducing the pH of the milk agar may have improved efficacy; however, the focus of these experiments was to determine potential efficacy in dairy products with a pH similar to milk. Therefore, no pH adjustments were made prior to the addition of the antimicrobial and observed differences in agar pH were due to the addition of the antimicrobial alone.

Less is known about the composition of fermentates D and E. Both contain unspecified organic acid(s) and metabolites resulting from fermentation. Milk agar containing fermentate D had a pH of approximately 4.7. This reduction likely improved the antimicrobial activity of the organic acids and other antimicrobials in the fermentate, resulting in inhibition of all test microorganisms regardless of the antimicrobial concentration. The pH of milk agar containing fermentate E was approximately 5.6 and 5.3 at a concentration of 0.5 and 1.0%, respectively. Because growth of all test microorganisms was observed at the lower concentration, but not at the higher concentration, the efficacy of this fermentate is concentration dependent.

The major antimicrobial agents contained in fermentate C are lysozyme, nisin and sodium diacetate. Lysozyme works by attacking the peptidoglycan of the cell wall, causing degradation and lysis (Davidson and Taylor 2007). Lysozyme is typically more effective at lower temperatures where cell growth is slowed (Hughey and Johnson 1987). Lysozyme is also most active against gram-positive bacteria because of the accessibility to the peptidoglycan of the cell wall. Nisin also attacks the bacterial cell wall creating pores in the cellular membrane which causes cytoplasmic leakage which eventually leads to cell lysis (Dave *et al.* 2003). The antimicrobial activity of nisin is dependent upon the incubation temperature and pH of the product; in general, nisin is most effective against gram-positive organisms, at lower incubation temperatures and in acidic environments (Montville and Chikindas 2007). While both lysozyme and nisin are more effective against gram-positive organisms, we observed inhibition of gram-negative microorganisms in milk agar. Fermentate C also contains sodium diacetate which has been shown to inhibit gram-positive and gram-negative microorganisms. Even with a pK_a of 4.75, sodium diacetate was shown to be inhibitory in BHI broth (pH 5.25) at 35C to *L. monocytogenes*, *Salmonella* and *E. coli* (Shelef and Addala 1993; Davidson and Taylor 2007). In this study, the combination of lysozyme, nisin and sodium diacetate found in fermentate C was effective against all pathogens tested despite favorable growth conditions (pH and temperature).

While nisin applied as part of a fermentate effectively inhibited the test microorganisms in milk agar, no inhibition of *L. monocytogenes* was observed when nisin was used alone. Previous research showed nisin to be ineffective in preventing the growth of *Listeria* in a dairy-based system (Zapico *et al.* 1998; Staszewski and Jagus 2008). In both studies, nisin initially decreased *Listeria* counts but regrowth of the bacteria was observed. The lack of effectiveness in these studies was hypothesized to be caused by nonspecific binding of nisin to components in the dairy systems, too low a concentration of nisin or intrinsic or induced bacterial resistance. In this study, varying concentrations of both nisin and *L. monocytogenes* were tested; however, growth was observed in 24 h regardless of the test parameters. While bacterial resistance to nisin is possible, these findings along with the previous research indicate the dairy matrix interferes with the inhibition of *Listeria*.

In milk agar, LAE completely inhibited the growth of *L. monocytogenes* and *E. coli* O157:H7 at both antimicrobial test concentrations. However, *Salmonella* was completely and only partially inhibited by LAE at 0.2 and 0.1%, respectively. LAE is a food-grade cationic surfactant that exhibits broad spectrum antimicrobial activity and its mode of inactivation has been described elsewhere (Rodriguez *et al.*

2004; Bonnaud *et al.* 2010). In this research, LAE efficacy was determined in a solid milk matrix with no fat; previous research in broth and 2% milk systems also showed that *L. monocytogenes* and *E. coli* O157:H7 were more sensitive to LAE than *Salmonella* (Quimin *et al.* 2013).

In milk agar, lactoperoxidase inhibited the growth of the three organisms tested. The lactoperoxidase system is highly inhibitory to gram-negative pathogens and has exhibited antimicrobial activity against gram-positive organisms (Davidson and Taylor 2007; Arques *et al.* 2008a,b). While published data on the efficacy of this particular lactoperoxidase-based product are lacking, similar findings have been observed with other lactoperoxidase system screening in milk (Kamau *et al.* 1990; Zarei *et al.* 2010). The activity of lactoperoxidase is dependent upon the presence of hydrogen peroxide (H_2O_2) and either thiocyanate or a halide. In addition to lactoperoxidase, the antimicrobial tested also contained glucose and glucose oxidase, which utilizes glucose and oxygen to produce H_2O_2. In this study, it was presumed that milk agar supported the action of the lactoperoxidase by contributing thiocyanate (found in milk), in addition to the H_2O_2 produced by the glucose oxidase, for antimicrobial activity.

Efficacy of Antimicrobials in Low-Sodium Cheese Agar

Fermentates C and D, LAE, and lactoperoxidase were further tested in low-sodium cheese agar to assess their potential efficacy in a more complex food system. Reduction in efficacy was observed with all antimicrobials in the cheese agar (Table 4). The fermentates showed the greatest reductions in efficacy. For fermentate C, complete inhibition of *Salmonella* was only observed at an inoculum concentration of 10 cfu/mL, all other test conditions yielded growth after 48 h. *Salmonella* and *E. coli* O157:H7 at a concentration of 10 cfu/mL were completely inhibited by fermentate D; however, all other test conditions yielded growth after 48 h. Though the antimicrobial activity of fermentates relies heavily on pH value, the pH did not vary greatly between the milk and cheese agars; therefore, differences in antimicrobial activity were most likely due to other factors. The increased agar complexity likely contributed to the reduction in antimicrobial efficacy of the fermentates. Matrix components could have reacted or blocked the binding sites of fermentate components such as lysozyme and nisin (Hughey and Johnson 1987).

While more inhibition of the test microorganisms was observed with LAE and lactoperoxidase than with the fermentates, cheese agar still decreased efficacy compared to milk agar. LAE completely inhibited the growth of *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 at an inoculum level of 10 cfu/mL. At an inoculum level of 10^2 cfu/mL, LAE

provided complete inhibition of *E. coli* O157:H7 and *L. monocytogenes*, but growth of *Salmonella* was detected after 48 h. Growth of all three organisms was detected after 48 h at an inoculum level of 10^4 cfu/mL. The reduction in efficacy between the cheese and milk agar was not unexpected since interactions and efficacy reduction of LAE in complex food matrixes have been previously observed (Asker *et al.* 2009; Bonnaud *et al.* 2010; Soni *et al.* 2010; Sharma *et al.* 2013a,b). Compared to the other antimicrobials, lactoperoxidase was relatively unaffected by the cheese matrix. Lactoperoxidase completely inhibited the growth of *Salmonella* and *E. coli* O157:H7 at inoculum levels of 10 and 10^2 cfu/mL. At 10^4 cfu/mL, growth of both organisms was observed after 48 h of incubation. *L. monocytogenes* was completely inhibited by lactoperoxidase at every inoculum level tested.

Sodium Replacement and Antimicrobial Agent Interactions

To compensate for flavor changes in low-sodium dairy products, a replacement agent will need to be added to maintain consumer acceptability. To determine if the sodium replacement agents will affect antimicrobial activity, interactions were investigated in the milk agar. The milk agar was used instead of the low-sodium cheese agar so that only the interaction between the sodium replacement agent and the antimicrobial could be studied without the added effect of a cheese matrix. Results showed that lactoperoxidase was unaffected by the tested sodium replacement agents (Table 5). However, reductions in antimicrobial efficacy of the fermentates and LAE were observed for *Salmonella* and *E. coli* O157:H7. Interestingly, no growth of *L. monocytogenes* was observed regardless of sodium replacement agent, indicating that *L. monocytogenes* is more sensitive to the antimicrobials or the mechanisms of inhibition are different between the gram-positive and gram-negative microorganisms. For fermentate C, reductions in efficacy were only observed when combined with KCl, and KCl blends A and B at 2.0%. When the concentration was reduced to 1.0% no growth was observed. Sodium replacement agents containing KCl also reduced the efficacy of fermentate D at a concentration of 2.0%; however, reducing the concentration to 1.0% resulted in inhibition of *E. coli* O157:H7, but only delayed growth of *Salmonella*. The antimicrobial effectiveness of NaCl and KCl alone or in combination with other antimicrobials has shown that KCl can replace NaCl without altering microbial safety (Boziaris *et al.* 2007; Bidlas and Lambert 2008). However, previous research looked only at nisin or did not evaluate the replacement effects on *Salmonella* or *E. coli* O157:H7. In this research, it is difficult to predict how KCl might have reduced the efficacy of the fermentates given that the spe-

TABLE 5. GROWTH OF *SALMONELLA, ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* ON MILK AGAR SUPPLEMENTED WITH SODIUM REPLACERS AND ANTIMICROBIALS

Antimicrobial (concentration)	Organism (10^4 cfu/mL)	Time of observed organism growth (h) (concentration)											
		Calcium lactate		Potassium lactate		Fermentate F		KCl		KCl blend A		KCl blend B	
		0.01%	0.02%	0.01%	0.02%	0.01%	0.02%	1.0%	2.0%	1.0%	2.0%	1.0%	2.0%
Fermentate C (1.0%)	Salmonella	NG	NG	NG	NG	NG	NG	NG	24 h	NG	24 h	NG	24 h
	E. coli O157:H7	NG	NG	NG	NG	NG	NG	NG	24 h	NG	24 h	NG	24 h
	Listeria	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Lauric arginate (0.2%)	Salmonella	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h
	E. coli O157:H7	24 h	24 h	24 h	24 h	24 h	24 h	24 h	48 h	24 h	48 h	24 h	48 h
	Listeria	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Fermentate D (0.2%)	Salmonella	48 h	48 h	48 h	48 h	48 h	48 h	48 h	24 h	48 h	24 h	48 h	24 h
	E. coli O157:H7	NG	48 h	NG	NG	NG	48 h	NG	24 h	NG	24 h	NG	24 h
	Listeria	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Lactoperoxidase (0.25%)	Salmonella	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
	E. coli O157:H7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
	Listeria	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

NG, no growth.

cific composition of the product is not fully known. Further investigation on the replacement effects of KCl on *Salmonella* and *E. coli* O157:H7 is needed.

At the highest concentration tested, all of the sodium replacement agents reduced the efficacy of LAE against *Salmonella* and *E. coli* O157:H7. Potency of LAE can be reduced in the presence of anionic biopolymers (Bonnaud *et al*. 2010). Interactions with the anionic components of the sodium replacement agents and LAE likely led to reduced efficacy. When concentrations of the sodium replacers were reduced by 50% no growth occurred with KCl; however, all other salt replacers resulted in growth after 24 h.

CONCLUSIONS

Selecting antimicrobials for use in food is challenging due to the complex composition of food matrices. This research screened numerous antimicrobials in model milk and low-sodium cheese systems to determine which antimicrobials have the most potential for success in low-sodium dairy products. Findings showed that fermentates A, B and F and nisin had poor efficacy in the milk agar, which was likely due to pH. Fermentates C and D, LAE, and lactoperoxidase all had decreased activity in the low-sodium cheese agar, indicating the matrix itself affected the efficacy. This research also showed that sodium replacement agents can negatively affect antimicrobial efficacy as was seen with fermentates C and D and LAE. KCl, one of the most common salt substitutes, showed substantial interference with antimicrobial activity against *Salmonella* and *E. coli* O157:H7. Manufacturers can use these findings to help select antimicrobials for use in dairy products. Findings also indicate manufacturers should carefully examine the impact of sodium replacement agents not only on consumer acceptability, but also on microbial stability. The addition of sodium replacement agents could render existing antimicrobials ineffective and limit potential alternatives.

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