Evaluation of antimicrobials against multi-strain cocktails of *Salmonella, Escherichia coli* O157:H7 and *Listeria monocytogenes* using a kinetic growth inhibition assay

C. B. Bottini and P. M. Muriana

STORY IN BRIEF

Salmonella serovars and *Escherichia coli* O157:H7 have typically been problem foodborne pathogens in raw meat and poultry products while *Listeria monocytogenes* has been the main problem pathogen for ready-to-eat **(RTE)** meat products. Many individual studies have examined the use of various antimicrobials to inhibit pathogens on these types of products as either pre-processing interventions or for prevention of growth during shelf life. The objective of this study was to evaluate various concentrations of antimicrobials in head-to-head trials against multi-strain cocktails of *Salmonella, E. coli* O157:H7*,* and *L. monocytogenes*. The antimicrobials that were tested included, acetic acid, Aftec 3000, AvGard XP, citric acid, Cytoguard, lactic acid, lauric arginate, peroxyacetic acid, Purac XTend FX25, and Syntrx 3300. Mixed strain "cocktails" were prepared comprising either 6 strains of *Salmonella,* 4 strains of *E. coli* O157:H7*,* and 5 strains of *L. monocytogenes*. Each was cultured separately in TSB broth and mixed prior to application in microplate kinetic growth assays using a Tecan microplate reader to obtain growth curves at different concentrations of the antimicrobials. Measurements were taken as optical density (OD_{595nm}) readings every 30 minutes for up to 48 hours. Data identify the levels of antimicrobial that give slight and complete inhibition of the various pathogen cocktail mixtures. These can be used as the basis for subsequent studies as well as food applications.

Key Words: antimicrobials, *E. coli* O157:H7*, Listeria*, meat, *Salmonella*

INTRODUCTION

Salmonella, Escherichia coli O157:H7 and *Listeria monocytogenes* are considered important pathogens responsible for causing the most food-related human illnesses worldwide. In the United States, diseases related to foodborne pathogens are an important and emotional public health problem; it is estimated that annually 48 million people get sick of food-related illness that cause 3,000 deaths and 128,000 hospitalizations (CDC., 2013).

Salmonella is considered an important foodborne pathogen commonly associated with gastrointestinal infections in humans from raw meats, poultry, eggs, and sometimes found in processed foods such as cake mixes and peanut butter. The illness, salmonellosis, may affect individuals or large groups and the symptoms include: diarrhea, fever, abdominal pain, nausea, vomiting and loss of appetite. The bacteria alter the cells of the villi in the intestinal wall, increasing water secretion and decreasing absorption, resulting in diarrhea.

Escherichia coli O157:H7 is considered one of the most dangerous pathogens that can cause gastrointestinal illness after consuming contaminated foods because it only requires the consumption of as few as 10 bacterial cells to result in illness. *Escherichia coli* O157:H7 has been responsible for foodborne outbreaks with raw ground beef and contaminated produce, notably lettuce and spinach. The common symptoms caused by this pathogen are bloody diarrhea

known as Hemorrhagic Colitis Syndrome (Kay et al., 1994). In some cases the infection can result in a more severe syndrome known as Hemolytic Uremic Syndrome which may lead to kidney failure or death (Suo et al., 2010).

Listeria monocytogenes is a Gram-positive, facultative, intracellular foodborne pathogenic bacterium that is often present on raw meat, yet found on RTE meats as a post-processing contaminant. It is a microorganism responsible for causing an infection called listeriosis that may cause meningitis or sepsis, resulting in high mortality rates (Suo et al., 2010). Newborns are the largest group affected, following by elderly and immunocompromised patients. The microorganism is widely distributed in many foods, such as raw produce, poultry, dairy products, and is an unwanted resident of meat processing plants. According to Kushwaha and Muriana (2010) the pathogen has been a recurring problem in meat processing facilities especially because *L. monocytogenes* is capable of strong adherence to equipment and/or surfaces in meat processing facilities, resulting in the formation of tenacious biofilms.

Currently, these pathogens have been problematic to the raw and processed meat industry, causing foodborne illness and causing product recalls. The USDA-FSIS has recently developed an incentivized method to promote safety in processed meats by their final rule for control of *Listeria* in RTE meats which elaborates three processing categories: Alternative 3 process (sanitation alone), Alternative 2 (use of either post-process lethality *or* antimicrobials to prevent growth of *Listeria* during shelf life), or Alternative 1 (post-process lethality step *and* ingredients to prevent growth during shelf life). In many instances, the use of antimicrobials can be used in both Alternative 1 and 2 to ensure safe food products.

The objective was to evaluate the effect of antimicrobials head-to-head against the same mixtures of pathogens and determine the level of antimicrobial that gives slight and complete inhibition of the various pathogen cocktail mixtures. These can then be used as the basis for subsequent studies as well as food applications.

MATERIALS AND METHODS

Kinetic (microplate) growth assays were used to evaluate which antimicrobial concentration would be effective to inhibit, suppress, and kill these organisms. The antimicrobials were prepared at a predetermined concentration in media (TSB broth). Antimicrobial solutions were filter sterilized using a 0.45µm sterile syringe filter. Each strain was prepared separately using TSB broth and a mixed strain cocktail, was prepared comprising either 6 strains of *Salmonella,* 4 strains of *E. coli* O157:H7, or 5 strains of *L. monocytogenes* and used to inoculate antimicrobials in microplate growth assays that were measured with a Tecan microplate reader. The microplate format allowed the evaluation of different concentrations of the antimicrobial in successive compartments in the 96-well flat bottom microplates. Measurement parameters were optical density (OD_{595nm}) readings every 30 min at 30^oC for up to 48 hours.

All the antimicrobials were tested at various concentrations in head-to-head trials against multi-strain cocktails of *Salmonella, E. coli* O157:H7*,* and *L. monocytogenes*. Although many of these antimicrobials have been shown to be effective in various peer reviewed publications, they have often been examined on different products, against different challenge strains, and at different concentrations.

Lactic acid is a weak-organic acid and by tradition is widely used to control pathogens in foods. The antimicrobial activity occurs through diffusion of lactate molecules into microbial cells where they dissociate into the toxic anionic forms depending on pH (Ibrahim et al., 2008).

Citric acid is another organic acid used primarily in the food and beverage industry, mainly as an acidulant. In the beverage industry citric acid is used to provide taste and complement fruit and berry flavor, but it also works similar to lactic acid by dissociating to anionic forms when the pH is above its pKa. It is the main organic acid produced by fermentation consumed throughout the world (Berovic and Legisa, 2007).

Acetic acid is a preservative commonly known as vinegar. It has effectiveness as an antimicrobial due to its ability to lower the pH and cause instability of bacterial cell membranes. The effectiveness of acetic acid against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium, is by reducing bacterial populations on meat surfaces (Stivariusa et al., 2002).

Lauric arginate (LAE) is a derivate of the naturally occurring chemicals and has been approved for use as an antimicrobial intervention on RTE meats. According to Taormina and Dorsa (2009), their study using the 'sprayed lethality in container' (SLIC) method showed that a 5% solution of LAE obtained a 5-log reduction of *L. monocytogenes* on hams, concluding that LAE is an effective intervention.

Purac XTend FX25 is an antimicrobial produced by Purac and is effective against Gram-positive and -negative bacteria, yeasts, and moulds. It can be used in a wide range of food applications, with little impact on taste and color when used in foods. The antimicrobial includes the ingredient, polylysine, which is an essential amino acid and is made by natural fermentation.

The following antimicrobials were also tested: Peroxyacetic acid, Syntrx 3300, Cytoguard + PAA (5% Cytoguard + 0.15% Perasan), Aftec 3000, and AvGard XP.

RESULTS AND DISCUSSION

Select strains of *Salmonella, E. coli* O157:H7 and *L. monocytogenes* for this experiment showed differences in inhibitory activity with various antimicrobials. The objective is to determine the minimum concentration that provides maximum inhibition, as well as that which provides minimum inhibition. For the antimicrobials that have been tested (acetic acid, Aftec 3000, AvGard XP, citric acid, Cytoguard, lactic acid, lauric arginate, peroxyacetic acid, Purac XTend FX25, and Syntrx 3300) the average (%) of Minimum Inhibitory Concentration (MIC) differs for all microorganisms (Table 1).

Table 1. Average (%) of Minimum Inhibitory Concentrations of various mixed-strain cocktails of foodborne pathogens

	Salmonella	*E.coli* O157:H7	*L.monocytogenes*
Antimicrobials	**MIC**	**MIC**	**MIC**
Acetic acid	4.00	4.00	4.00
Aftec 3000	1.25	1.25	1.25
AvGard XP	0.75	0.75	0.75
Citric acid	0.50	0.50	0.50
Cytoguard	2.50	2.50	1.25
Lactic acid	0.50	0.50	0.25
Lauric arginate	0.008	0.008	0.004
Peroxyacetic acid	0.063	0.063	0.125
Purac XTend FX25	0.50	0.25	0.016
Syntrx 3300	2.00	2.00	2.00

MIC = Minimum Inhibitory Concentration

The effects of the antimicrobials tested (acetic acid, Aftec, AvGard, citric acid, Cytoguard, lactic acid, lauric arginate, peroxyacetic acid, Purac, and Syntrx 3300) were found to be different for each microorganism, hence the MIC was dependent on the interaction between pathogen and antimicrobial (Table 1). We determined that the MIC would be the concentration that resulted in near complete suppression of growth as observed in the kinetic growth curve assays. Data identifies the levels of antimicrobial that gives slight or complete inhibition of the various pathogen cocktail mixtures allowing us to identify an approximate use level in foods.

Since *Salmonella* and *E. coli* O157:H7 have been a problem as foodborne pathogens in raw meat and poultry products and *L. monocytogenes* has been the main problem pathogen for RTE meat products, these data will be used to determine levels of use of these antimicrobials in order to prevent growth during shelf life and improve the safety of meat products for consumers.

Future work will include tests with more antimicrobials currently used in the food industry as well as implementing these inhibitory ranges of antimicrobial in applied studies with meat products. Results from these studies will also be used to design mixed assays to determine if synergistic activities result from low level mixtures of these antimicrobials. The effective use of

antimicrobials in meat and poultry processing will largely be determined by cost effective applications. Therefore, identifying synergistic activities provided by minimum concentrations can help maximize the cost-use benefit to processors.

LITERATURE CITED

Berovic, M., and M. Legisa. 2007. Citric acid production. Biotechnol. Annu. Rev. 13:303-343.

Centers for Disease Control and Prevention. 2013. Estimating Foodborne Illness: An Overview. Available from: http://www.cdc.gov/foodborneburden/estimates-overview.html. Accessed 2014 February 28.

Ibrahim, S. A., H. Yang, and C. W. Ceo. 2008. Antimicrobial activity of lactic acid and copper on growth of *Salmonella* and *Escherichia coli* O157:H7 in laboratory medium and carrot juice. Food Chem. 109(1):137-143.

Kay, B. A., P. M. Griffin, N. A. Strockbine, and J. G. Wells. 1994. Too fast food: Bloody diarrhea and death from *Escherichia coli* O157:H7. Clin. Microbiol. Newsletter. 16(3):17-19.

Kushwaha, K., and P. M. Muriana. 2010. Analysis of tissue invasiveness of adherent strains of *Listeria monocytogenes* by in vivo mouse assay. Int. J. Food Microbiol. 141(1-2):104-109.

Stivariusa, M. R., F. W. Pohlmanb, K. S. McElyeab, and J. K. Appleb. 2002. The effects of acetic acid, gluconic acid and trisodium citrate treatment of beef trimmings on microbial, color and odor characteristics of ground beef through simulated retail display. Meat Sci. (60):245–252.

Suo, B., Y. He, G. Paoli, A. Gehring, S-I. Tu, and X. Shi. 2010. Development of an oligonucleotide-based microarray to detect multiple foodborne pathogens. Mol. Cell. Probe. 24(2):77-86.

Taormina, P. J., and W. J. Dorsa. 2009. Inactivation of *Listeria monocytogenes* on hams shortly after vacuum packaging by spray application of lauric arginate. J. Food Protect. 72(12): 2517–2523.

ACKNOWLEDGEMENTS