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Antimicrobial activities of lauric arginate and cinnamon oil combination against foodborne pathogens: Improvement by ethylenediaminetetraacetate and possible mechanisms

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ABSTRACT

The objective of this work was to study if ethylenediaminetetraacetate (EDTA) could enhance the antimicrobial activity of lauric arginate (LAE) and cinnamon oil (CO) combination, and the possible mechanisms. With 500 mg/L of EDTA, 5 mg/L of LAE and 200 mg/L of CO showed an increased log reduction of *Escherichia coli* O157: H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* by ca. 4, 5, and 1 log CFU/mL, respectively. EDTA at 500 mg/L significantly increased the permeability of outer membrane of *E. coli* O157: H7 based on a crystal violet assay. Scanning electron microscopy (SEM) showed that 600 mg/L CO damaged the cell membrane of *S.* Enteritidis, while 40 mg/L LAE did not. Atomic force microscopy demonstrated that LAE caused the aggregation of DNA molecules. It was hypothesized that EDTA increased the permeability of the outer membrane of Gram-negative bacteria to facilitate the penetration of LAE and CO enabling enhanced antimicrobial activity. Compared to the treatment with LAE or CO alone, severe damage of *L. monocytogenes* membrane occurred with LAE and CO in combination based on SEM, increase in loss of intracellular nucleic acids, and increase of extracellular ATP level, suggesting LAE and CO acted synergistically on *L. monocytogenes* cell membranes.

1. Introduction

Lauric arginate (ethyl-N^{α}-lauroyl-L-arginine ethylester monohydrochloride; LAE) is a generally-recognized-as-safe preservative approved by the United States Food and Drug Administration in 2005, and the dosage limit in food products is 200 mg/L (USDA., 2005). It is a cationic surfactant derived from lauric acid, L-arginine and ethanol (Ruckman, Rocabayera, Borzelleca, & Sandusky, 2004). LAE has been shown to be non-toxic to human because it is metabolized rapidly *in vivo* to lauric acid and arginine, which are naturally occurring dietary components (Hawkins, Rocabayera, Ruckman, Segret, & Shaw, 2009). LAE has a broad antimicrobial spectrum and the minimum inhibitory concentration of LAE against *Listeria monocytogenes* Scott A is as low as 11.8 mg/L in tryptic soy broth (TSB) at 32 °C (Ma, Davidson, & Zhong, 2013). However, a much higher amount (>200 mg/L) of LAE is needed in complex food matrices due to its interaction with negatively charged food components (Bonnaud, Weiss, & McClements, 2010; Ma et al., 2013). Because a high concentration (>50 mg/L) of LAE can lead to a bitter taste (Zheng, 2014), strategies are needed to lower the LAE level used in food products.

Combinations of natural antimicrobials with synergistic antimicrobial effects are a possible way to lower the concentration of each antimicrobial needed in the food matrix (Ma et al., 2013; Noll, Prichard, Khaykin, Sinko, & Chikindas, 2012; Techathuvanan, Reyes, David, & Davidson, 2014). Essential oils (EOs) have gained a lot of attention for possible use as natural antimicrobial preservatives in recent years (Chen, Zhang, & Zhong, 2015; Ma et al., 2016; Pan, Chen, Davidson, & Zhong, 2014; Zhang, Ma, Critzer, Davidson, & Zhong, 2015). EOs have a broad spectrum of antimicrobial activity but, due to binding with hydrophobic components, are needed at high concentrations in complex food products to enable sufficient inhibition of foodborne pathogens. For example, 3000 mg/L eugenol in 2% reduced fat milk only reduced the amount of *L. monocytogenes* by less than 1 log CFU/mL at 32 °C after 24 h (Ma et al., 2013). Lowering the amount of EOs used in the food products is also desired since high concentrations of EOs affect sensory quality of food products. Previously, a synergistic effect against the

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Gram-positive bacteria *L. monocytogenes* was found when combining LAE and cinnamon leaf oil or eugenol, while the combination was antagonistic against Gram-negative *Escherichia coli* O157:H7 and *Salmonella* Enteritidis (Ma et al., 2013).

The major difference between Gram-negative and Gram-positive bacteria is that the lipopolysaccharide (LPS) outer membrane of Gram-negative bacteria can protect against the penetration of antimicrobial compounds, especially hydrophobic compounds, while Gram-positive bacteria do not have an outer membrane (Bladen & Mergenhagen, 1964). Ethylenediaminetetraacetic acid (EDTA) can chelate divalent cations that are critical to the ordered structure of LPS outer membrane of Gram-negative bacteria (Nikaido, 2003; Ruiz, Kahne, & Silhavy, 2009), which increases the permeability of the outer membrane (Vaara, 1992). Studies have shown that EDTA can enhance activities of nisin, lysozyme, and monolaurin (Branen & Davidson, 2004). Therefore, we hypothesize that EDTA could overcome the antagonistic effect of LAE-EO combination against Gram-negative bacteria.

The objective of the present study was to test antimicrobial activities of the combinations of LAE, cinnamon oil (CO), and EDTA against both Gram-positive and Gram-negative bacteria and better understand the underlying mechanisms of these interactions. Possible mechanisms for the enhancement of activity by EDTA and/or interactions among antimicrobials investigated included disruption of the cell membrane, loss of intracellular nucleic acids, and loss of ATP, as well as observation of cell morphology. Interaction of antimicrobials and bacterial DNA was also investigated.

2. Materials and methods

2.1. Materials

LAE with a brand name of Mirenat®-TT was provided by Vedeqsa Inc. (New York, NY). The commercial product contained 15.5 g/100 g LAE. CO and EDTA were purchased from Sigma–Aldrich Corp. (St. Louis, MO).

2.2. Bacterial culture

L. monocytogenes Scott A, *E. coli* O157:H7 ATCC 43895, and *S.* Enteritidis were used in the present study. All strains were from Department of Food Science and Technology at University of Tennessee in Knoxville. Strains were stored in sterile 20 g/100 g glycerol at −20 °C and transferred at least 2 times in TSB for *E. coli* O157:H7 and *S.* Enteritidis or in TSB supplemented with yeast extract (TSBYE) for *L. monocytogenes* before testing. Unless stated otherwise, *L. monocytogenes* Scott A was incubated at 32 °C, while *E. coli* O157:H7 ATCC43895 and *S.* Enteritidis were incubated at 37 °C.

2.3. Microbial growth kinetics in tryptic soy broth

Growth curves of bacteria were determined in 96-well microtiter plates using a spectrophotometric plate reader (Synergy HT MultiMode Microplate Reader, BioTek, Winooski, VT). Culture with ca. 10^7 CFU/mL bacteria and an antimicrobial solution were added at 120 μL each into each well. The optical density (OD) at 600 nm was automatically recorded at an interval of 30 min during incubation at 37 °C (for *S.* Enteritis and *E. coli* O157:H7) or 35 °C (for *L. monocytogenes*) for up to 12 h. Stock solutions with 500 mg/L LAE or 4 g/100 mL EDTA were prepared in water and adjusted to pH 6.8 using 1.0 M NaOH or HCl. The stock solution of CO was prepared by dissolving 5 g/100 mL CO in 90 mL/100 mL aqueous ethanol. The same ethanol concentration as in CO sample was used as an ethanol control, while wells without antimicrobial were treated as positive controls. Concentrations of antimicrobials used in inhibiting *S.* Enteritidis and *E. coli* O157:H7 were 5 mg/L LAE, 500 mg/L EDTA, and 200 mg/L CO, while those used against *L. monocytogenes* were 2.5 mg/L LAE, 100 mg/L EDTA, and 100 mg/L CO. Experiments were performed in triplicate.

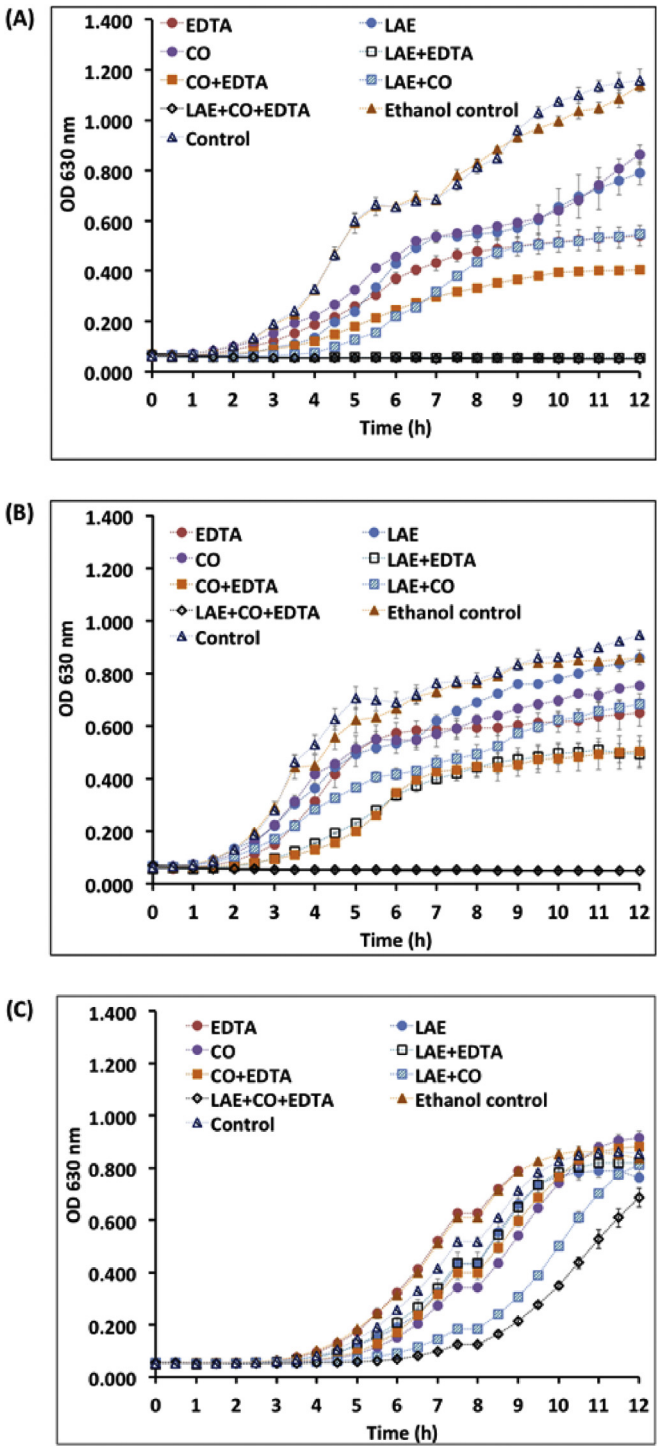

Fig. 1. Growth curves of (A) *Escherichia coli* O157:H7 ATCC 43895, and (B) *Salmonella* Enteritidis at 37 °C and (C) *Listeria monocytogenes* Scott A at 35 °C in tryptic soy broth. Treatments for *E. coli* O157:H7 and *S.* Enteritidis contained 5 mg/L lauric arginate (LAE), 500 mg/L EDTA, and 200 mg/L cinnamon oil (CO) alone or in combinations. Treatments for *L. monocytogenes* contained 2.5 mg/L lauric arginate (LAE), 100 mg/L EDTA, and 100 mg/L CO alone or in combination. Errors are standard deviations (n = 3).