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Inhibitory effects of lactic and malic organic acids on autoinducer type 2 (AI-2) quorum sensing of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium

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ABSTRACT

Several organic acid based antimicrobials are reported to reduce bacterial populations but studies showing inhibition of autoinducer-2 (AI-2) activity or quorum sensing are limited. The effect of lactic and malic acids on autoinducer activity of selected strains of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium is tested in this study. The strains were screened for AI-2 like activity on spinach and cantaloupe homogenates using autoinducer sensing *Vibrio harveyi* biosensor strains. The ED 14 strain of *E. coli* O157:H7 and the SD 10 strain of *Salmonella* showed highest AI-2 like activity of 55 and 53 Relative Light Units respectively. These two strains were used to evaluate the AI-2 inhibitory activities of lactic and malic acids at 1–4% concentrations (alone or in combinations). Lactic acid at 4.0% had the highest inhibition of 80% on ED 14 *E. coli* strain while the combination treatment of lactic acid + malic acid at 4.0% each had the highest inhibition of 80% on SD 10 *Salmonella* strain. Results from the study indicate that the quorum sensing ability of the *E. coli* O157:H7 and *Salmonella* Typhimurium strains can be effectively inhibited by antimicrobial organic acids.

1. Introduction

Quorum sensing is a cell–cell signaling process used by certain bacteria to coordinate virulence gene expression and survival. During quorum sensing, certain bacteria are known to modulate the cellular functions through signaling compounds known as auto inducers (Silagyi, Kim, Lo, & Wei, 2009). Both gram-positive and gram-negative bacteria use this cell density dependent system as a response to environmental stresses such as lack of nutrients, inhibitory effects of temperature and host defense responses (Waters & Bassler, 2005). The autoinducer (AI) molecules affect the bacterial expression of various genes involved in virulence, toxicity, sporulation, plasmid transformation, antibiotic production and biofilm formation (Bainton et al., 1992; Bassler, 1999; Davies et al., 1998; Kendall & Sperandio, 2014; Luo & Farrand, 2001; Oger & Farrand, 2002; Sperandio, Torres, Girón, & Kaper, 2001). Certain bacteria exhibit quorum-sensing behavior as a regulatory process to ensure the presence of sufficient cell density before a specific gene product is made. This process allows them to multiply exponentially and initiate to express a certain phenotype such as biofilm production. Excess concentrations of autoinducer compounds beyond a threshold within the cells are known to activate (some repress) a regulatory protein that binds to specific DNA sequence regions and activates transcription mechanism resulting in the production of biofilm (Bassler, 1999; Moriera et al., 2006). Quorum sensing bacteria such as *Escherichia coli* O157:H7 and *Salmonella* Typhimurium are known to respond to two types of auto-inducers called acylated homoserine lactones (AI-1) and furanosyl borate diester (AI-2) (Lu, Hume, & Pillai, 2005; Reading et al., 2007). Marine bacterium *Vibrio harveyi* is commonly utilized as a bioluminescent reporter strain to detect these autoinducer molecules (Fig. 1). Inhibition of autoinducer molecules or their activity may affect quorum-sensing behavior or biofilm formation by certain

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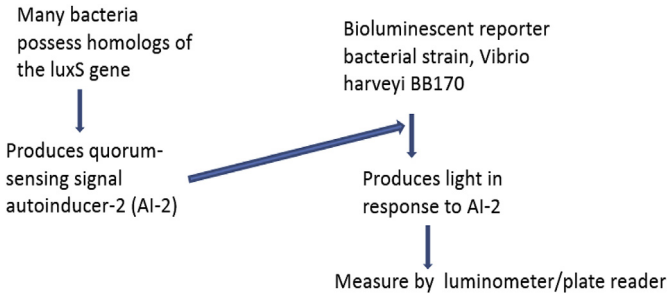

Fig. 1. Simplified scheme of the principle for detection of quorum sensing activity.

bacteria. Various natural compounds have been studied to demonstrate their role as AI-2 inhibitors. For example, fatty acids such as stearic acid, palmitic acid, oleic acid, and linoleic acids isolated from poultry meat (have shown to inhibit AI-2 activity (Widmer et al., 2007). Other examples of natural compounds that are reported to inhibit bacterial quorum sensing are vanilla extracts (Choo, Rukayadi, & Hwang, 2006) and traditional Chinese medicinal plant extracts (Koh and Tham, 2011). Pure compounds like *p*-coumaric acid have also been found to provide inhibitory effect against cell–cell communication in bacteria (Bodini, Manfredini, Epp, Valentini, & Santori, 2009). Interestingly, the antimicrobial effects of several organic acids against bacteria have been studied, but the studies on their inhibitory effects on biofilm formation and autoinducer-2 activities are limited.

Considering the importance of quorum sensing in biofilm formation by foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* Typhimurium that often contaminate minimally processed produce, it would be important to inhibit the autoinducer molecules of these strains so that their survival or persistence can be hindered on these foods. In this study the effect of lactic and malic acids on autoinducer-2 (quorum sensing molecule) activity of *E. coli* O157:H7 strains on spinach and *Salmonella* Typhimurium strains on cantaloupes was demonstrated. Reporter strain, *V. harveyi* (BB170), was used to detect quorum sensing based on its ability to produce bioluminescence in response to AI-2 activity.

2. Materials and methods

We used the *V. harveyi* reporter strain to detect the autoinducer-2 (AI-2) activity of *E. coli* O157:H7 and *Salmonella* Typhimurium strains inoculated in minimally processed produce samples (spinach and cantaloupe). The methodology of culture preparation, inoculation and autoinducer measurements using the reporter strain are indicated below.

2.1. Culture preparation of E. coli O157:H7, Salmonella Typhimurium and V. harveyi

The procedure for this method was obtained from previous research using *V. harveyi* for detecting quorum sensing (Kim et al., 2009). The reporter strain, *V. harveyi* BB170, produces light in response to AI-2 (due to luxS gene) and is useful in biological assays for detecting and quantifying AI-2 production by bacterial cultures (Taga & Xavier, 2011). Agar slants with pure cultures of the food pathogenic bacteria *E. coli* O157:H7 and Salmonella Typhimurium were obtained from the University of Georgia, Center for Food Safety, Griffin, GA. The bacterial strains were; *E. coli* O157:H7 green fluorescent protein (GFP)-labeled ED 14 (CV267); ED15 (6980-2); ED16 (6982-2); MD58 (CV261), MD46 (F4546); MD47 (K4492), *Salmonella* Typhimurium -GFP-labeled SD 10 and SD 11. Working cultures from the frozen stocks (−70 °C) were prepared by transferring aseptically to 10 mL of brain heart infusion broth media (BHI) (Becton Dickinson Microbiology Systems, Sparks, MD, U.S.A.) and incubated at 37 °C for 24 h with shaking (200-rpm) in a New Brunswick Scientific (Edison NJ, U.S.A.) agitating-incubator. Second-day inoculum of each strain was prepared by transferring 10 μl of first-day culture into 10 mL of fresh BHI and incubated for 24 h in a shaker maintained at 37 °C and this was used to inoculate (10^9 log CFU/mL) spinach and cantaloupe for the study. The *V. harveyi* BB170 (ATCC BBA-1117) was used as a reporter strain to detect AI-2 molecule while *V. harveyi* BB152 (ATCC BBA-1119) that produces AI-1 and AI-2, was used as a positive control. The *Vibrio* strains were grown in the auto-inducer bioassay (AB) medium. The AB medium was prepared as follows: A solution consisting of NaCl (17.5 g/L), $MgSO_4$ (12.3 g/L), and vitamin-free cas-amino acids (2 g/L) was dissolved in 1 L of water with final pH 7.5 and sterilized by autoclaving (15 min, 121 °C). When the solution was cooled, sterile 1 M potassium phosphate solution (pH 7.0, 10 mL/L), 50% glycerol (20 mL/L) and filter-sterilized 0.1 M L-arginine (10 mL/L) were added.

2.2. Preparation of antimicrobial solutions

Separate stock solutions of organic acids were prepared in 10 mL of sterile water by dissolving malic acid powder and lactic acid to a concentration of 10%. The stock solutions were diluted to prepare 1.0, 2.0, 3.0, and 4.0% of both organic acid test solutions one day before the study (Massey, Hettiarachchy, Martin, & Ricke, 2013). The solutions were vacuum filtered through Whatman no. 4 filter paper to remove insoluble particles that may interfere with the spraying. Deionized water was measured (similar to the weight of the organic acid solution) and the pH was adjusted to that of the test solutions with 1 N hydrochloric acid (HCl) to prepare the control solutions for the organic acids.

2.3. Spinach/cantaloupe homogenate preparation for auto-inducer assay

Spinach and cantaloupe were purchased fresh from a local grocery store on the day of the experiment. Spinach leaves and cantaloupe rinds were rinsed with water, and submerged in sodium hypochlorite solution (6.25 mL/L) for 3 min to reduce the microbial background. The leaves/rinds were submerged again in sterile water for 3 min, removed and left under the biological safety cabinet for 2 h to dry. After drying, the leaves/rinds were placed in separate sterile bags and weighed. Sterile water was added at a volume of twice the weight of the sample to each bag and stomached using a lab-scale masticator (Neutec Group Inc., Farmingdale, NY) for 3 min to prepare homogenates. Ninety microliter samples of the homogenates (spinach/cantaloupe) were dispensed into separate 96-well plates (Becton Dickinson and Co. Franklin lakes, NJ) before adding 10 μl of the second day culture of *E. coli* O157:H7 or *Salmonella* Typhimurium. The 96-well plates were incubated for 24 h at 25 °C. After incubation the homogenates with the pathogen cultures were transferred to sterile 1.5 mL Eppendorf tubes and centrifuged at 3,000*g* for 5 min at 25 °C to separate the bacterial cells. The cell-free supernatants (CFS) were collected and stored at −20 °C for autoinducer activity assay.

2.4. Auto-inducer activity assay

Autoinducer (AI-2) detection assay in minimally processed produce was conducted as described previously (Silagyi et al., 2009). The overnight cultures of the *Vibrio* reporter and positive control strains were separately diluted (1:5000) in fresh AB medium and the diluted cells (90 μl) were dispensed into a 96-well plate. For the auto-inducer activity assay the CFS of spinach/