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

Ahmad Almasoud¹, Navam Hettiarachchy^{1*}, Srinivas Rayaprolu¹, Dinesh Babu², Young Min Kwon³ and Andy Mauromoustakos⁴

¹Department of Food Science, University of Arkansas, Fayetteville, AR 72704, Department of

²Department of Toxicology, University of Louisiana, Monroe, LA 71209, USA

³Poultry Science, University of Arkansas, Fayetteville, AR 72701

⁴Department of Agricultural Statistics, University of Arkansas, Fayetteville, AR 72701

*Author for correspondence: Navam S. Hettiarachchy

Department of Food Science & Institute of Food Engineering,

University of Arkansas,

2650 N. Young Avenue,

Fayetteville,

AR 72704

Phone: (479) 575-4779

Fax: (479) 575-6936

Email: nhettiar@uark.edu

Short version of title (Effect of organic acids on quorum sensing)

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 *E. 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 *V. 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 to 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.

Key words: Quorum sensing, auto inducers, pathogenic bacteria, produce, organic acids.

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 autoinducers (Silagyi *et al.*, 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 and 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; Luo and Farrand 2001; Sperandio *et al.*, 2001; Oger and Farrand 2002; Kendall and Sperandio 2014). 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 *E. 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 *et al.*, 2005; Reading *et al.*, 2007). Marine bacterium *Vibrio harveyi* is commonly utilized as a bioluminescent reporter strain to detect these autoinducer molecules (Figure-1). Inhibition of autoinducer molecules or their activity may affect quorum-sensing behavior or biofilm formation by certain 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 *et al.*, 2006) and traditional Chinese medicinal plant extracts (Koh and Tham 2001). Pure compounds like *p*-coumaric acid have also been found to provide inhibitory effect against cell-cell communication in bacteria (Bodini *et al.*, 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, *Vibrio harveyi* (BB170), was used to detect quorum sensing based on its ability to produce bioluminescence in response to AI-2 activity.

Materials and Methods

We used the *Vibrio 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.

Culture preparation of *E. coli* O157:H7, *Salmonella* Typhimurium and *Vibrio harveyi*.

84 The procedure for this method was obtained from previous research using *Vibrio harveyi*
85 for detecting quorum sensing (Kim *et al.*, 2009). The reporter strain, *Vibrio harveyi* BB170,
86 produces light in response to AI-2 (due to luxS gene) and is useful in biological assays for
87 detecting and quantifying AI-2 production by bacterial cultures (Taga, and Xavier, 2005). Agar
88 slants with pure cultures of the food pathogenic bacteria *E. coli* O157:H7 and *Salmonella*
89 *Typhimurium* were obtained from the University of Georgia, Center for Food Safety, Griffin,
90 GA. The bacterial strains were; *E. coli* O157:H7 green fluorescent protein (GFP)-labeled ED 14
91 (CV267); ED15 (6980-2); ED16 (6982-2); MD58 (CV261), MD46 (F4546); MD47 (K4492),
92 *Salmonella Typhimurium* -GFP-labeled SD 10 and SD 11. Working cultures from the frozen
93 stocks (-70 °C) were prepared by transferring aseptically to 10mL of brain heart infusion broth
94 media (BHI) (Becton Dickinson Microbiology Systems, Sparks, MD, U.S.A.) and incubated at
95 37 °C for 24h with shaking (200-rpm) in a New Brunswick Scientific (Edison NJ, U.S.A.)
96 agitating-incubator. Second-day inoculum of each strain was prepared by transferring 10µl of
97 first-day culture into 10mL of fresh BHI and incubated for 24h in a shaker maintained at 37 °C
98 and this was used to inoculate (10^9 log CFU/mL) spinach and cantaloupe for the study. The
99 *Vibrio harveyi* BB170 (ATCC BBA-1117) was used as a reporter strain to detect AI-2 molecule
100 while *V. harveyi* BB152 (ATCC BBA-1119) that produces AI-1 and AI-2, was used as a positive
101 control. The *Vibrio* strains were grown in the auto-inducer bioassay (AB) medium. The AB
102 medium was prepared as follows: A solution consisting of NaCl (17.5g/L), MgSO₄ (12.3g/L),
103 and vitamin-free cas-amino acids (2g/L) was dissolved in 1L of water with final pH 7.5 and
104 sterilized by autoclaving (15min, 121 °C). When the solution was cooled, sterile 1 M potassium
105 phosphate solution (pH 7.0, 10mL/L), 50% glycerol (20mL/L) and filter-sterilized 0.1 M L-
106 arginine (10mL/L) were added.

Preparation of antimicrobial solutions.

Separate stock solutions of organic acids were prepared in 10mL 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 *et al.*, 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 1N hydrochloric acid (HCl) to prepare the control solutions for the organic acids.

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 (6.25mL/liter) for 3min 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 2h 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 3min 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 μ L of the second day culture of *E. coli* O157:H7 or *Salmonella* Typhimurium. The 96-well plates were incubated for 24h at 25 °C. After incubation

the homogenates with the pathogen cultures were transferred to sterile 1.5mL Eppendorf tubes and centrifuged at 3,000g for 5min at 25 °C to separate the bacterial cells. The cell-free supernatants (CFS) were collected and stored at –20 °C for autoinducer activity assay.

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 (90µl) were dispensed into a 96-well plate. For the auto-inducer activity assay the CFS of spinach/cantaloupe homogenates that were earlier exposed to *E. coli* O157:H7 or *Salmonella* Typhimurium strains, potentially containing AI-2 molecules, were added to the reporter strain cultures previously dispensed into a 96-well plate. The wells with *V. harveyi* BB152 strain were used as positive control. The plates were incubated for 3h at 30 °C with shaking and luminescence measurements were made using a plate reader at 490nm. This assay measured the uninhibited or the highest relative AI-2 activity among the stains of *E. coli* O157:H7 and *Salmonella* Typhimurium.

Assay to measure the effect of organic acids on the AI-2 activity

The inhibitory effects of malic (MA) and lactic (LA) acids on AI-2 activity by *E. coli* O157:H7/ and *Salmonella* Typhimurium were tested by adding the organic acid solutions at different concentrations (1.0, 2.0, 3.0, 4.0%). As described above, the CFS of treated spinach/cantaloupe homogenates were added to 96-well plates containing the reporter strain *V. harveyi* BB170. Combinations of LA and MA (LA1% + MA1%, LA4% + MA4%) were also

investigated. The plates were incubated at 30 °C for 3h with shaking. Luminescence readings were measured at 490nm using a plate reader.

Assay to measure the effect of organic acids on *V. harveyi* strain BB170 bacteria

Overnight AB medium cultures of *V. harveyi* BB170 were diluted (1:5000) with fresh AB medium. The subculture of *V. harveyi* BB170 and organic acids that showed the highest AI-2 inhibition activity were added to individual sterilized tubes at the ratio of 9:1 v/v (*V. harveyi* BB170: organic acids solution sample), and incubated at 30 °C for a similar time period as the AI-2 bioassay. Serial dilutions were made for enumeration of viable cells of *V. harveyi* BB170 on Marine agar (Becton Dickinson) and log counts were recorded.

Statistical analysis

The JMP 10.0 software (SAS Institute, Cary, NC) was used for one way analysis of variance to determine the effect of organic acids on AI-2 like activities, activity inhibitions, and the growth of reporter strain *V. harveyi* BB170. A factorial design was used in the study for the organic acid treatments. Analysis of variance (ANOVA) was performed and significant difference was determined at $P < 0.05$. Tukey HSD was used to compare means and all experiments were conducted in triplicates and repeated for reproducibility.

Results and discussion

Evaluation of the relative AI-2 activity by *E.coli* and *Salmonella* on spinach and cantaloupe

The pathogenic bacteria, *E. coli* and *Salmonella* were selected for study on spinach and cantaloupe respectively since they have been found to contaminate these produce causing

numerous cases of illness. Quorum sensing and biofilm formation in pathogenic bacteria have been shown to enhance their survival rate while affecting the severity of the contamination problem (Gandhi and Chikindas, 2007; Annous *et al.*, 2009; Silgayi *et al.*, 2009). The autoinducer molecules AI-1 and AI-2 were previously detected to induce quorum sensing (Surette and Bassler 1998; Surette *et al.*, 1999) and were used by researchers in studying quorum sensing and biofilm formation (Lu *et al.*, 2004; Uckoo *et al.*, 2015). To investigate the quorum sensing ability of a pathogen the relative AI-2 activity was determined as the ratio of the average value of the sample against that of the negative control.

The results of relative AI-2-like activity in spinach homogenate inoculated with six stains of *E. coli* followed by incubation for 12h are given in Table.1. The ED 14 strain produced the highest relative AI-2-like activity of 55 Relative Light Units (RLU, the units of measurement for the chemiluminescence or bioluminescence) which was significantly high in comparison to other tested strains ($p < 0.05$). Hence, ED 14 strain of *E. coli* O157:H7 was selected to investigate the effect of various organic acid concentrations, alone and/or in combination. Table.2 shows the relative AI-2 activity produced by two *Salmonella* Typhimurium strains (SD 10 and SD 11) in cantaloupe homogenate after 12 h incubation, where the SD 10 strain showed higher (53%) relative AI-2 activity in comparison to SD 11 (21%). Hence, SD 10 strain was chosen to further investigate the effect of organic acids on biofilm formation mechanism.

Determination of the effect of organic acids on the AI-2 activity

Lactic and malic organic acids at concentrations of 1.0, 2.0, 3.0, and 4.0% (alone and in combination) were tested for their ability to inhibit AI-2 activity in spinach and cantaloupe homogenates inoculated with *E.coli* O157:H7 (ED 14) and *Salmonella* Typhimurium (SD 10)

respectively. These concentrations were selected based on previous studies on effective pathogen inhibition by the organic acids on produce (Ganesh *et al.*, 2010; Ganesh *et al.*, 2012; Massey *et al.*, 2013).

Inhibition of *E. coli* O157:H7, ED 14 AI-2 activity in spinach homogenate is shown in Table 3. Overall results show that lactic acid was more effective than malic acid in inhibiting AI-2 activities. At its highest concentration of 4.0%, malic acid showed 37% inhibition of AI-2 and at the same concentration, lactic acid inhibited the AI-2 activity by 80% which was significant ($p<0.05$). The combined treatment of lactic and malic acid at 1.0% and 4.0% were not significant in inhibiting AI-2 activity. The treatment LA+MA (1.0% each) resulted in 17% inhibition while LA+MA (4.0% each) inhibited the relative AI-2 activity by 25%. Hence, a combination of organic acids did not have any synergistic or additive effect on *E.coli*'s ability to produce AI-2.

The Table 4 shows the inhibition of *Salmonella* Typhimurium, SD 10 strain by organic acid treatments. The combined treatment of LA+MA (4.0% each) had the highest inhibition by 80%. However, it is not significantly different from that shown by lactic acid alone at 4.0% concentration which resulted in an inhibition of 76%. These results on reduction of quorum sensing ability by organic acids can supplement previous studies which demonstrated their antimicrobial effect on produce (Ganesh *et al.*, 2010).

These outcomes can be explained by the fact that the inhibitory properties of the organic acids is due to their concentrations, ability to penetrate the bacterial cell wall and the capacity to alter the microbial cytoplasmic pH (Midolo *et al.*, 1995; Eswaranandam *et al.*, 2004). The differences in efficacy among the organic acids can be attributed to their molecular size (Ganesh *et al.*, 2010).

Inhibitory effect of organic acids on the growth of *V. harveyi* BB170

The influence of organic acids on the multiplication of the reporter strain is shown in Table 5. The treatments selected for this tests were: Lactic acid 4.0%, Malic acid 4.0%, and LA+ MA (4.0% each) since they caused significant inhibition of AI-2. This experiment was conducted to verify if the decline in AI-2 activity was due to the growth inhibition of the reporter strain *V. harveyi* BB170. For this purpose, a mixture of (1:9) of organic acids and the diluted reporter strain *V. harveyi* BB170 in AB medium was incubated as mentioned previously. There were no significant differences between the control (growth in AB medium) and the various treatments, suggesting that inhibition of reporter strain growth was not the basis for inhibition of AI-2 activity by the organic acids. Enumeration of the *V. harveyi* BB170 cells was conducted to determine the effect of organic acids on the report bacteria as an alternative to bioluminescence assay. This is due to the fact that organic acids may impact the redox reactions for NAD(P)H formation (energy source for bioluminescence) in the reporter bacteria there by affecting the bacterial metabolism and the response on AI-2 activity.

The AI-2 inhibition activity of the organic acids is considered to be lower in comparison to certain chemical food additives reported in the literature. As an example, turkey patties treated with sodium propionate showed inhibition of AI-2 by 99.8% (Lu *et al.*, 2004). However, naturally occurring organic acids have greater potential for application in foods since most consumers are avoiding chemical additives and preservatives in food products. Among other organic acids such as fatty acids including palmitic acid, steric acid, oleic acid, and linoleic acid isolated from poultry meat have also shown inhibition of the AI-2 activity up to 65% (Soni *et al.*, 2008). A mixture of steric, palmitic, oleic, and linoleic acids inhibited the AI-2 activity by 59.5% (Widmer *et al.*, 2007). Although these results show significant AI-2 inhibition, they are not

comparable to the higher inhibition levels of up to 80% shown by the naturally produced organic acids tested in this study. The overall significance of this study was the effect of organic acids in reducing quorum sensing ability of pathogenic bacteria on minimally processed produce.

Conclusion

Foodborne illness due to *E.coli* O157:H7 and *Salmonella* outbreaks on minimally processed produce are a consistent problem in the U.S. leading to economical and health consequences. These bacterial pathogens show virulence and sustained survival due to quorum sensing ability that provides a barrier against the effects of antimicrobials. For the first time, this study demonstrated the ability of natural antimicrobials like organic acids to inhibit the expression of autoinducer-2 molecule which thereby hinders the quorum sensing ability of pathogenic bacteria including *E.coli* O157:H7 and *Salmonella* Typhimurium. Lactic acid alone at 4.0 %, and a combination treatment of malic acid 4.0% + lactic 4.0% were able to inhibit AI-2 activity by *E.coli* O157:H7 and *Salmonella* by 80% on spinach and cantaloupe respectively. Therefore, food protection and safety in the minimally processed produce industry, dealing with frozen or refrigerated vegetables and fruits, and salad mixes, can be enhanced using these natural antimicrobials. The significance of the findings observed in this study lies in the potential capacity of natural antimicrobial agents which impact cell to cell signaling and potential biofilm formation by food-borne pathogens.

Acknowledgements

The project was funded by a grant from the National Research Initiative by the United States Department of Agriculture (CSREES/NRI - 2008-35201-18855).

References

1. Annous, B. A., Fratamico, P. M. and Smith, J. L. 2009. Quorum sensing in biofilms: why bacteria behave the way they do. *J. Food Sci*, 74(1): R1-R14.
2. Bainton, N. J., Bycroft, B. W., Chhabra, S. R., Stead, P., Gledhill, L., Hill, P. J., Rees, C. E., Winson, M. K., Salmond, G. P., Stewart, G. S., and Williams, P. 1992. A general role for the lux autoinducer in bacterial cell signaling: control of antibiotic biosynthesis in *Erwinia*. *Gene* 116: 87-91.
3. Bassler, B. L. and Greenberg, E. P. 1997. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J. Bacteriol.* 179: 4043-4045.
4. Bassler, B. L. 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.* 2: 582-587.
5. Bodini, S. F., Manfredini, S., Epp, M., Valentini, S. and Santori, F. 2009. Quorum sensing inhibition activity of garlic extract and *p*-coumaric acid. *Lett. Appl. Microbiol.* 49: 551-555.
6. Choo, J. H., Rukayadi, Y. and Hwang, J. 2006. Inhibition of bacterial quorum sensing by vanilla extract. *Lett. Appl. Microbiol.* 42: 637-641.
7. Costerton, J. W., Stewart, P. S. and Greenberg, E. P. 1999. Bacterial biofilms: a common cause of persistent infections. *Science*, 284: 1318-1322.
8. Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. and Greenberg, E. P. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*, 280: 295-298.
9. Eswaranandam, S., Hettiarachchy, N. S. and Johnson, M. G. 2004. Antimicrobial activity of citric, lactic, malic, or tartaric acids and nisin-incorporated soy protein film against *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella gaminara*. *J. Food Sci*, 69: 79–84.
10. Gandhi, M. and Chikindas, M. L. 2007. *Listeria*: a foodborne pathogen that knows how to survive. *Intl J Food Microbiol*, 113(1): 1-15.
11. Ganesh, V., Hettiarachchy, N. S. Griffis, C. L. Martin, E. M. and Ricke S. C. 2012. Electrostatic Spraying of Food-grade Organic and Inorganic Acids and Plant Extracts to Decontaminate *Escherichia coli* O157:H7 on Spinach and iceberg Lettuce. *J Food Science*, 77(7): M391–M396.
12. Ganesh, V., Hettiarachchy, N. S., Ravichandran, M., Johnson, M. G., Griffis, C. L., Martin, E. M., Meullenet, J. F. and Ricke. S. C., 2010 Electrostatic Sprays of Food-grade Acids and Plant Extracts are More Effective than Conventional Sprays to Decontaminate *Salmonella Typhimurium* on Spinach. *J Food Sci*, 75(9): M574-M579.
13. Kendall M. M. and Sperandio V. 2014. Cell-to-cell signaling in *E. coli* and *Salmonella*. *EcoSal Plus*. Doi:10.1128/ecosalplus.ESP-0002-2013.
14. Koh, K. H. and Tham, F. 2011. Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. *J Microbiol. Immunol. Infect*, 44: 144-148.
15. Lu, L., Hume, M. E. and Pillai, S. D. 2004. Autoinducer-2-like activity associated with foods and its interaction with food additives. *J. Food Prot.* 67: 1457-1462.
16. Lu, L., Hume, M. E., and Pillai, S. D. 2005. Autoinducer-2-like activity on vegetable produce and its potential involvement in bacterial biofilm formation on tomatoes. *Foodborne Pathol. Dis*, 2: 242-249.

17. Luo, Z. Q. and Farrand, S. K. 2001. The *Agrobacterium tumefaciens* *rnd* homolog is required for TraR-mediated quorum-dependent activation of Ti plasmid *tra* gene expression. *J. Bacteriol.* 183: 3919-3930.
18. Massey, L.M., Hettiarachchy, N.S., Martin, E.M. and Ricke, S.C. 2013. Electrostatic spray of food-grade organic acids and plant extract to reduce *Escherichia coli* O157:H7 on fresh-cut cantaloupe cubes. *J Food Safety*, 33(1): 71-78.
19. Midolo, P. D., Lambert, J. R., Hull, R., Luo, F. and Grayson, M. L. 1995. In vitro inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *J App Bacteriol*, 79(4): 475-479.
20. Moreira, C. G., Palmer, K., Whiteley, M., Sircili, M. P., Trabulsi, L. R., Castro, A. F. P. and Sperandio, V. 2006. Bundle-forming pili and EspA are involved in biofilm formation by enteropathogenic *Escherichia coli*. *J Bacteriol.* 188: 3952–3961.
21. Oger, P., Farrand S. K. 2002. Two opines control conjugal transfer of an *Agrobacterium* plasmid by regulating expression of separate copies of the quorum-sensing activator gene *traR*. *J Bacteriol.* 184: 1121-1131.
22. Reading, N. C., Torres, A. G., Kendall, M. M., Hughes, D. T., Yamamoto, K. and Sperandio, V. 2007. A novel two-component signaling system that activates transcription of an enterohemorrhagic *E. coli* (EHEC) effector involved in remodeling of host actin. *J Bacteriol.* 189: 2468–2476.
23. Silagyi, K., Kim, S., Lo, Y. M. and Wei, C. 2009. Production of biofilm and quorum sensing by *Escherichia coli* O157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. *Food Microbiol.* 26: 514-519.
24. Smith, J. L., Fratamico, P. M. and Novak, J. S. 2004. Quorum sensing: a primer for food microbiologists. *J. Food Prot.* 67: 1053-1070.
25. Soni, K. A., Jesudhasan, P., Cepeda, M., Widmer, K., Jayaprakasha, G. K., Patil, B. S., Hume, M. E. and Pillai, S. D. 2008. Identification of ground beef-derived fatty acid inhibitors of autoinducer-2-based cell signaling. *J Food Prot.* 71: 134-138.
26. Sperandio, V., Torres, A. G., Girón, J. A., and Kaper, J. B. 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol.* 183: 5187-5197.
27. Surette, M. G., Miller, M. B., and Bassler, B. L. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proceed Nat Acad Sci*, 96(4): 1639-1644.
28. Surette, M. G., and Bassler, B. L. 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proceed Nat Acad Sci*, 95(12): 7046-7050.
29. Taga, M. E. and Xavier, K. B. 2005. Methods for Analysis of Bacterial Autoinducer-2 Production. *Curr protocols microbiol*, 1C-1.
30. Uckoo, R. M., Jayaprakasha, G. K., Vikram, A., and Patil, B. S. 2015. Polymethoxyflavones isolated from the peel of Miray Mandarin (*Citrus miaray*) have biofilm inhibitory activity in *Vibrio harveyi*. *J Agri and Food Chem*, 63(32): 7180-7189.
31. Waters, C. M., and Bassler, B. L. 2005. Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* 21: 319-346.

- 353 32. Widmer, K. W., Soni, K. A., Hume, M. E., Beier, R. C., Jesudhasan, P. and Pillai, S. D.
354 2007. Identification of poultry meat-derived fatty acids functioning as quorum sensing
355 signal inhibitors to autoinducer-2 (AI-2). *J. Food Sci.* 72: M363-M368.

Table 1. Relative AI-2 like activity in spinach homogenate inoculated with various stains of *E.coli* for 12 h.

Strain*	Relative AI-2 like activity (%)**
ED 14	55.0 ± 2.1 ^A
ED 15	28.0 ± 2.6 ^B
ED 16	36.1 ± 2.6 ^C
MD 46	15.2 ± 2.3 ^D
MD 47	15.3 ± 0.9 ^D
MD 58	14.3 ± 0.9 ^D

* Strains of *E.coli* O157:H7 studied. Values provided are means ± standard deviation of triplicates and those conducted by the same letter are not significantly different (P<0.05). **Relative AI-2 like activity was calculated as the ratio of luminescence of the test sample to the negative control (AB medium).

Table 2. Relative AI-2 like in cantaloupe homogenate inoculated with stains of *Salmonella* for 12 h.

Strain*	Relative AI-2 like activity (%)**
SD 10	53.2 ± 0.9 ^A
SD 11	21.3 ± 0.7 ^B

*Strains of *Salmonella* Typhimurium studied. Values provided are means ± standard deviation of triplicates and those connected by the same letter are not significantly different (P<0.05).

**Relative AI-2 like activity was calculated as the ratio of luminescence by the test sample to the negative control (AB medium).

21 **Table.3. Reduction in quorum sensing activity of *E. coli* ED 14 strain based on AI-2 activity**
 22 **inhibition in spinach by organic acids**

Treatments (%)*	Inhibition %**
LA (1.0)	26.2 ± 1.0 ^E
LA 2.0	33.1 ± 0.3 ^{CD}
LA 3.0	49.5 ± 1.5 ^B
LA 4.0	80.2 ± 2.3 ^A
MA 1.0	23.4 ± 0.4 ^F
MA 2.0	26.9 ± 0.2 ^F
MA 3.0	28.0 ± 0.4 ^{DE}
MA 4.0	37.5 ± 1.4 ^C
LA MA 1	17.2 ± 0.3 ^E
LA MA 4	25.4 ± 0.8 ^E

23 Values are means ± standard deviations of triplicates and those connected by the same letter are
 24 not significantly different (P<0.05). *LA: lactic acid, MA: Malic acid. **Calculated in
 25 comparison to positive control (relative activity of AI-2 by *V. harveyi* BB152) values.

Table 4. Reduction in quorum sensing activity of *Salmonella* Typhimurium SD 10 strain based on AI-2 activity inhibition in cantaloupe by organic acids

Treatments (%)*	Inhibition % **
LA (1.0)	27.5 ± 0.8 ^F
LA 2.0	36.2 ± 0.6 ^{DE}
LA 3.0	58.0 ± 2.1 ^B
LA 4.0	76.6 ± 0.2 ^A
MA 1.0	16.1 ± 0.4 ^G
MA 2.0	26.3 ± 1.2 ^F
MA 3.0	28.8 ± 1.8 ^{DE}
MA 4.0	46.7 ± 1.4 ^C
LA MA 1	39.9 ± 1.7 ^D
LA MA 4	80.4 ± 2.9 ^A

Values provided are means ± standard deviation of triplicates and those connected by the same letter are not significantly different (P<0.05). *LA: lactic acid, MA: Malic acid. **Calculated in comparison to positive control (relative activity of AI-2 by *V. harveyi* BB152) value.

Table 5. Effect of organic acids on the growth of the reporter strain *V. harveyi* BB170

Treatment*	Growth (log CFU/mL)
AB medium	5.4 ± 0.1^A
LA 4 %	5.3 ± 0.2^A
MA 4%	5.3 ± 0.0^A
LA MA 4%	5.2 ± 0.2^A

Values provided are means $\pm$ standard deviation of triplicates and those connected by the same letter are not significantly different ($P < 0.05$). *AB medium: Auto-inducer bioassay medium; LA: Lactic acid; MA: Malic acid.

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