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Exposure of *Salmonella* Typhimurium to guava extracts increases their sensitivity to acidic environments

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ABSTRACT

This study aimed to investigate the effect of exposure of *Salmonella* Typhimurium to the guava extracts on their resistance to acidic environments. To obtain guava extracts, the freeze-dried guava branch, fruit and leaf were treated with acetone, ethanol and methanol overnight, and each extract was dissolved in dimethyl sulfoxide (DMSO) to achieve a final concentration of 50 mg/ml. The acid resistance of *S.* Typhimurium exposed or unexposed to various guava extracts was measured by subjecting them to acidified media (pH 3.8) containing acetic, citric, lactic or hydrochloric acids. The bacterial injury by the guava extracts was also determined. Exposure of cells to the guava extracts decreased their *D*-values in acidified TSB by up to 70% compared with those of unexposed cells. Among the different types of solvents and parts of the guava plant, the ethanol extract of guava fruit had greater effect in decreasing the acid resistance of *S.* Typhimurium, making the cells more sensitive to various acidulants. Cells exposed to the guava extracts in acetic acid exhibited relatively low *D*-values compared to those of cells in other acidulants. Cells exposed to the ethanol extract of the guava fruit had the lowest *D*-value at 8.6 min when incubated in acidified TSB containing acetic acid. Exposure of cells to the guava extracts for 3 h caused sublethal injury (99%), indicating that a decrease in the resistance of the exposed *S.* Typhimurium to an acidic environment might be due to the damage on the bacterial membrane. The results obtained in this study demonstrate the potential of the ethanol extract of guava fruit as another hurdle, enhancing the antimicrobial effect of acid treatment.

1. Introduction

Salmonellosis is a worldwide infectious disease usually characterized by the acute onset of fever, abdominal pain, nausea, diarrhea, and (occasionally) vomiting (Hohmann, 2001). According to the scientific report of European Food Safety Authority (EFSA), *Salmonella* was identified as the leading causative agent and a total of 108,614 confirmed cases of human salmonellosis including 90 deaths were reported in 2009 (EFSA & ECDC, 2011). In Asia, a total of 1065 and 1480 confirmed cases of salmonellosis in 2011 and 2010 were reported in Korea (KFDA, 2012) and Singapore (MOH, 2011), respectively. Among approximately 2400 *Salmonella* serovars, *S.* Typhimurium is the most frequently isolated serotype that accounted for about 35% of reported human isolates (Wilmes-Riesenberg, Bearson, Foster, & Curtiss, 1996).

To control this foodborne pathogen during processing, transportation and storage of foods, the food industry has manipulated intrinsic or extrinsic factors such as temperature, pH, moisture content, antimicrobial agents, and water activity (Jay, 2000). Among them, the acidification of foods by adding an acidulant is one of the most effective control measures to reduce the risk of salmonellosis. However, several studies have shown that *Salmonella* spp. can enhance their resistance to acidic environment via acid tolerance response (Alvarez-Ordóñez, Fernández, Bernardo, & López, 2010; Baik, Bearson, Dunbar, & Foster, 1996; Mani-López, García, & López-Malo, 2011; Yuk & Schneider, 2006). To effectively control such an acid tolerant pathogen in foods, a combination of two or more antimicrobials such as essential oils and acidulants has been suggested. For instance, thymol or carvacrol combined with acetic acid successfully reduced populations of *S.* Typhimurium in broth, exhibiting their synergistic effect against the pathogen (Zhou et al., 2007).

Guava (*Psidium guajava* L.), a fruit plant belonging to the family Myrtaceae, is cultivated throughout the tropics and subtropics (Lutterodt, 1989) and previous studies have reported that solvent and aqueous extracts of different parts of guava plant effectively inhibited growth of foodborne pathogens including *Escherichia coli* O157:H7, *Listeria monocytogenes*, *S.* Enteritidis, *S.* Typhimurium and

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Staphylococcus aureus (Ibrahim, Yang, Song, & Tse, 2011; Jo, Ok, & Lee, 2009; Mahfuzul, Bari, Inatsu, Juneja, & Kawamot, 2007). The antimicrobial effect of guava may be due to some flavonoids such as morin and quercetin (Arima & Danno, 2002) since these phenolic compounds can disrupt the bacterial cell membrane, leaking the intracellular molecules (Rasooli, Brzaei, & Allameh, 2006). Although these studies have shown the antimicrobial effect of guava extracts on several foodborne pathogens, nothing has been reported regarding the effect of guava extracts on bacterial survival in an acidic environment. Thus, this study aimed to investigate the effect of the guava branch, fruit or leaf extracts on the resistance of *S.* Typhimurium in acidic environments containing various acidulants.

2. Materials and methods

2.1. Bacterial strain and culture condition

Salmonella Typhimurium ATCC 14028 strain was obtained from the Health & Environment of Gyeongsangnam-do Government Institute in Korea. Frozen stock culture was activated on 10-ml trypticase soy broth (TSB, Difco, Detroit, MI, USA.) at 37 °C with biweekly transfers to retain viability during the experiments. Prior to use, *S.* Typhimurium was incubated in 10 ml of TSB at 37 °C for 18 h with two consecutive transfers. The cultures were centrifuged at 2500 × g for 5 min at 4 °C and washed twice with phosphate buffered saline (PBS; pH 7.3) solution before exposure to the guava extracts.

2.2. Preparation of guava extracts

Guava extracts were prepared using the solvent extraction method described elsewhere (You, Park, Yuk, & Lee, 2011). Briefly, guava (*P. guajava* L.) plants (Guava Korea Co., Uiryeon, Korea) were separated into the branch, fruit and leaf using knives. Each part was freeze-dried and finely ground using a blender (MC 811C, Novita Co., Seoul, Korea), followed by passage through a sieve (25 mesh) to obtain a unified powder. A 10-g powder of each part was mixed with 300 ml of acetone, ethanol or methanol overnight at room temperature and then filtered with a Whatman No. 1 filter paper (Whatman PLC, Maidstone, Kent, UK). The filtrate was concentrated by removing the solvents under reduced pressure on a rotary evaporator (Eyela N-1000, Tokyo, Japan) at 37 °C. Dried extracts were dissolved in dimethyl sulfoxide (DMSO) (Junsei, Japan) to achieve a final concentration of 50 mg/ml and stored at 4 °C prior to use.

2.3. Determination of acid resistance

A 100-μl aliquot (ca. 10^7 CFU) of the aforementioned washed culture was inoculated into 1-ml of the guava extract in DMSO and left for 3 h at room temperature. Bacterial culture in DMSO without the guava extracts was served as controls in this study. After the exposure of cells to the guava extracts, the mixture was transferred into 9.9 ml of acidified TSB (pH 3.8) with sterile 5 N acetic acid (TSB-A; Samchun, Pyeongtaek, Korea), 5 N citric acid (TSB-C; Daejung, Siheung, Korea), 5 N lactic acid (TSB-L; Daejung) or 5 N hydrochloric acid (TSB-H; Daejung). The cell suspension was incubated in a water bath (MCB-3011D, Mono-tech, Korea) at 37 °C, and the survival was monitored periodically. Viable cell densities after each time interval were enumerated by pour-plating on trypticase soy agar (TSA, Difco) after proper serial dilutions with 0.1% peptone water. The plates were then incubated at 37 °C for 24 h and colonies were counted using an automatic counter

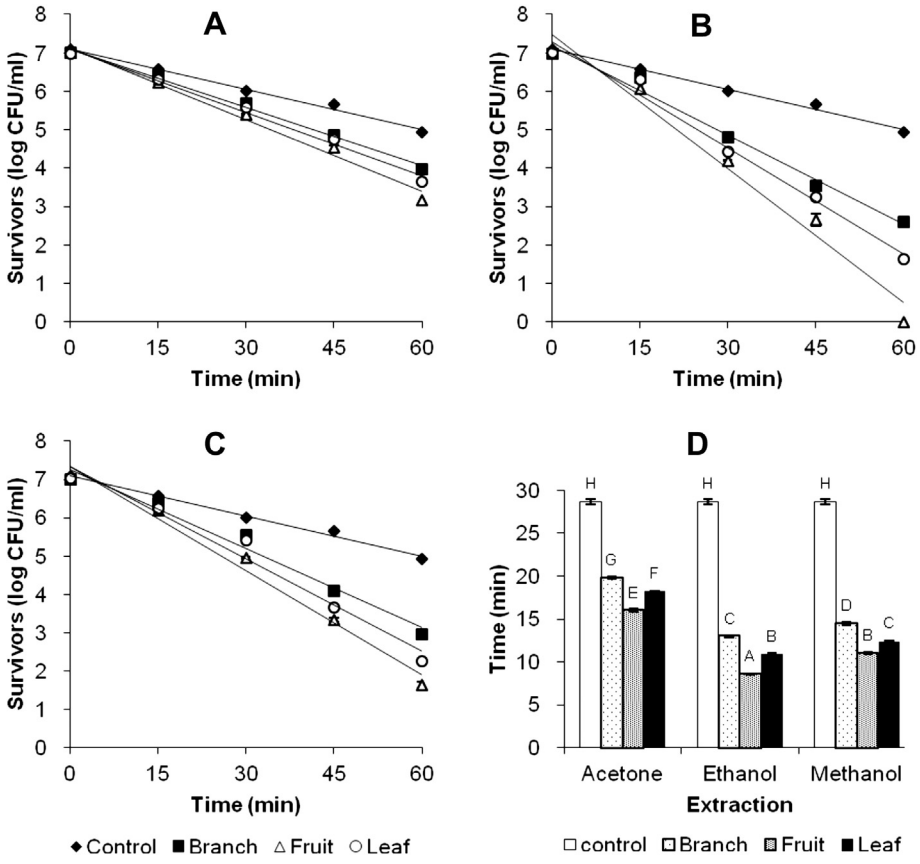

Fig. 1. Survival of *Salmonella* Typhimurium in acidified TSB with acetic acid at pH 3.8 after pre-exposure to acetone (A), ethanol (B) and methanol (C) extracts of different parts of guava plant. *D*-values were calculated based on inactivation curves (D). Mean values are mean ± SD of triplicates and different letters indicate a significant difference ($P < 0.05$).