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Assessment of antimicrobial effects of food contact materials containing silver on growth of *Salmonella* Typhimurium

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ABSTRACT

Food contact materials containing antibacterial properties are progressively appearing in the market. Items intended to provide antimicrobial effects such as increased shelf life and food safety are incorporating silver materials during the manufacture of such products. This study examined the total silver content, release capacity, and antibacterial activity of three different silver-containing food contact materials: plastic food storage containers, a plastic cutting board, and food wrapping paper. Silver content and release were determined by Inductively Coupled Plasma Mass Spectrometry, and the results showed that, although the amount of silver in each product was similar, migration varied considerably with kind of material and simulant choice. Antimicrobial effect was tested by measuring the growth of *Salmonella* Typhimurium during or after exposure to the different food contact materials. The results showed that the food storage containers and wrapping paper delayed the growth of *S.* Typhimurium under certain conditions, but that these effects were short-lived. The strain of *S.* Typhimurium used in this study was found to be negative for the presence of tested silver resistance genes. The results of this study suggest that a thorough investigation should be required to show/claim the efficacy of silver-containing food contact materials for food safety purposes.

1. Introduction

Commercialization of the antibacterial properties of silver is beginning to move from the wound care area to the consumer world, with an emphasis on food safety and personal hygiene. This shift can be visualized in the increasing numbers of nanomaterial-containing products available for purchase. In addition, the form of silver used in many of these products increasingly falls into the nanomaterial range. Currently, there are no silver nanoparticles (AgNP)-impregnated food contact materials (FCM) that are permitted to be marketed in the US. However, some AgNP-impregnated FCM, such as food storage containers, plastic bags, cutting boards, and food wrapping paper have been marketed globally (Addo Ntim et al., 2015; Duncan, 2011). Silver nanoparticles were detected in some of these consumer use products upon systemic analysis of migration of silver into food simulant (Echegoyen and Nerin, 2013). However, it can be difficult to determine whether a consumer product incorporates silver nanomaterials (Contado, 2015). Use of nanotechnology is often not declared in such products—an omission which could be intentional or unintentional. Many of these products advertise claims about their purported ability to improve food safety or shelf-life, but there is little information about the nanoparticle content or true efficacy of such products. Silver and silver nanoparticles (AgNPs) exert strong antimicrobial properties and have demonstrated antimicrobial effect against multiple foodborne pathogens, including *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Shigella flexneri*, *Vibrio cholera*, and *Vibrio parahaemolyticus* (Duncan, 2011; Paredes et al., 2014; Rai et al., 2012; Xu et al., 2012; Zarei et al., 2014). There is evidence that

Abbreviations: Ag-containers, Silver infused containers; AgNP, Silver Nanoparticles; CFU, Colony-Forming Units; EPA, Environmental Protection Agency; FDA, Food and Drug Administration; FCM, Food Contact Materials; ICP-MS, Inductively Coupled Plasma-Mass Spectrometry; MDL, Minimum Detection Limit; LB, Luria-Bertani; ppb, parts per billion.

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silver in the form of AgNPs may also inactivate common foodborne viral pathogens (Bekele et al., 2016; Duncan, 2011). In addition, the broad-spectrum antimicrobial activity of silver and AgNP suggests these materials may also reduce levels of non-pathogenic bacteria and fungi that are common agents of food spoilage (Duncan, 2011; Jo et al., 2009; Lara et al., 2011; Panacek et al., 2009; Rai et al., 2009).

In such products, nanoparticles and/or silver ions could be released by diffusion, dissolution, and desorption processes as described by Duncan and Pillai (2015). Furthermore, a number of bacterial organisms, including *E. coli* and *Salmonella* strains, have been shown to be capable of developing resistance to the antimicrobial activity of silver, either through spontaneous mutations or the acquisition of specific genes (McHugh et al., 1975; Randall et al., 2015; Silver, 2003).

This study evaluated the total silver content, silver release potential, and antimicrobial activity for silver-impregnated plastic food storage containers, food wrapping paper, and cutting boards. The objective of this study was to assess the antimicrobial potential of FCM containing silver (Table 1) against common foodborne pathogen *Salmonella enterica* serovar Typhimurium. For the food storage containers, this analysis was extended to include evaluations of antimicrobial effect at different temperatures and pH levels. The form of the silver present in the wrapping paper and cutting boards is unknown, but the food storage containers used in this study were shown to contain AgNP in a previous experiment (Echegoyen and Nerin, 2013). Additionally, screening of *S.* Typhimurium for genes associated with silver resistance was performed.

2. Materials & methods

2.1. Food contact material

FCM used in this study were obtained via the internet from an online vendor. Two different food storage containers were used for this experiment - unmodified plastic food storage containers purchased at a grocery store (Control containers) and plastic food storage containers infused with silver (Ag-containers). Both containers (control and Ag-containers) matrix material is polypropylene. The packaging associated with the Ag-containers claims that *"micro-sized silver particles infused into containers protect against bacterial growth"* by the manufacturer. Antimicrobial properties of silver-coated cutting board and food wrapping paper with silver were also evaluated.

2.2. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis of silver infused food containers

An Agilent 7700 series ICP-MS (Agilent Corp, Santa Clara, CA) was used for the analysis of total silver in the FCM. The instrument parameters were: 1550 W, Rf power, gas carrier: 099 L/min, auxiliary gas argon: 0.90 L/min, plasma gas, argon: 14.99 L/min, isotope measurement: 107Ag, nebulizer pump speed: 0.1 rps, nebulizer: micromist, ion lenses model: x-lenses, sample introduction: peri pump, sample depth: 10 mm, 0.3 s integration time per mass and 3 points per mass. For each FCM, 10 samples, approximately 50–80 mg (7 mm of diameter with 2 mm of thickness) were taken using random criteria, from different parts of container, cutting board, or food contact paper. The samples were acid digested followed by microwave digestion method with a Mars 6 (one touch technology) (CEM, CEM Corporation, Matthews, NC) as described (Artiaga et al., 2015; Echegoyen and Nerin, 2013; Huang et al., 2011). After the complete digestion, the transparent solution (resulted from the digestion process) was transferred into a 15 mL plastic conical tube and diluted to 10 mL with 2% nitric acid. The instrument was calibrated using silver ICP-MS standard solutions prepared at 0, 3, 5, 10, 50, 100, and 500 ppb (silver standard in 2% HNO3). Indium was used as an internal standard. The minimum detection limit (MDL) was 0.126 ppb, calculated by multiplying the standard deviation by the Student's value of 3.143 for seven replicates as described in Environmental Protection Agency (EPA) Compendium Method IO-3.5 (EPA, 1999).

2.3. Ag migration study

Migration studies were conducted following the guidance of premarket submissions for food contact substances (chemistry recommendations) from the U.S. Food and Drug Administration (FDA/CFSAN, 2014). The test was conducted in four food simulants: water (Ultrapure Water System, 0.2 μm filtered 18 MΩ distilled water, Barnstead, Thermo-Scientific, Waltham, MA, USA), 3% acetic acid (>99.99% Sigma-Aldrich), 50% methanol/water (200 proof HPLC/spectrophotometric grade, Sigma-Aldrich), and commercial household olive oil. A silver release study was conducted for ten days at 40 °C with three replicates for each of the FCM The test samples (FCM) were weighed with a surface contact area of 18 cm^2 for cutting board (plastic/simulant ratio ~0.6 cm^2/mL), 22 cm^2 for containers (plastic/simulant ratio ~0.7 cm^2/mL), and 196 cm^2 for paper (paper/simulant ratio ~6.5 cm^2/mL).

2.4. Bacterial strain and growth media

The strain of *Salmonella enterica* serovar Typhimurium used in this study was a food isolate that has been used in previous work by our group (Williams et al., 2015). *S.* Typhimurium was grown overnight in Luria-Bertani (LB) broth at 37 °C. *E. coli* strain J53 and *E. coli* strain J53 with plasmid (pMG101) containing the *sil* resistance operon were obtained from Anne O. Summers at the University of Georgia in Athens, Georgia. J53 and J53 (pMG101) were cultured on LB agar plates.

2.5. Evaluation of antimicrobial activity of food containers

Minimal Essential Media (MEM) comprised of a mixture of M9 salts, glucose, magnesium sulfate, calcium chloride, thiamine, and amino acids was utilized in the containers as a food simulant. For experiments evaluating effects of pH on antimicrobial effect, a 1 M hydrochloric acid (HCl) solution was used to adjust the MEM as needed. Each container was sanitized by a combination of 70% ethanol treatment and 1 h UV exposure and allowed to dry completely before use. For the spiking study, 30 mL of MEM was dispensed into each container using aseptic technique. This amount was chosen because it was the volume necessary to cover the

Table 1
Description of food contact materials (FCM) evaluated in this study.

Material	Country of origin	Exterior labelling on product packaging	Type of silver mentioned on packaging
Plastic Food Storage Containers	Korea	"Make food last longer and stay fresher"	micro-sized
Plastic Food Cutting Board	USA	"High density polyethylene with agent to inhibit bacterial growth. Infused with silver for antibacterial protection"	silver
Food Contact Paper	USA	"Antimicrobial protected product"	silver