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Effects of meat juice on biofilm formation of *Campylobacter* and *Salmonella*

Jiaqi Li[a], Jinsong Feng[a], Lina Ma[a], César de la Fuente Núñez[b,c,d,e], Greta Gölz[f], Xiaonan Lu[a,*]

[a] Food, Nutrition, and Health Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada
[b] Synthetic Biology Group, MIT Synthetic Biology Center, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States
[c] Research Laboratory of Electronics, Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, 02139, United States
[d] The Center for Microbiome Informatics and Therapeutics, Broad Institute of MIT and Harvard, Cambridge, MA 02139, United States
[e] Harvard Biophysics Program, Harvard University, Boston, MA, United States
[f] Institute of Food Safety and Food Hygiene, Freie Universität Berlin, Berlin 14163, Germany

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ABSTRACT

Campylobacter and *Salmonella* are leading causes of foodborne illnesses worldwide, vastly harboured by raw meat as their common food reservoir. Both microbes are prevalent in meat processing environments in the form of biofilms that contribute to cross-contamination and foodborne infection. This study applied raw meat juice (chicken juice and pork juice) as a minimally processed food model to study its effects on bacterial biofilm formation. Meat juice was collected during the freeze-thaw process of raw meat and sterilized by filtration. In 96-well polystyrene plates and glass chambers, supplementation of over 25% meat juice (*v*/v) in laboratory media led to an increase in biofilm formation of *Campylobacter* and *Salmonella*. During the initial attachment stage of biofilm development, more bacterial cells were present on surfaces treated with meat juice residues compared to control surfaces. Meat juice particulates on abiotic surfaces facilitated biofilm formation of *Campylobacter* and *Salmonella* under both static and flow conditions, with the latter being assessed using a microfluidic platform. Further, the deficiency in biofilm formation of selected *Campylobacter* and *Salmonella* mutant strains was restored in the presence of meat juice particulates. These results suggested that meat juice residues on the abiotic surfaces might act as a surface conditioner to support initial attachment and biofilm formation of *Campylobacter* and *Salmonella*. This study sheds light on a possible survival mechanism of *Campylobacter* and *Salmonella* in meat processing environments, and indicates that thorough cleaning of meat residues during meat production and handling is critical to reduce the bacterial load of *Campylobacter* and *Salmonella*.

1. Introduction

Campylobacter and *Salmonella* are both common causes of foodborne diseases worldwide. They usually invade into the digestive system, causing diarrhea, abdominal pain, malaise, fever, nausea, and vomiting. In some rare cases, systemic infections can be induced and lead to death (Bolton, 2015; Majowicz et al., 2010). In 2014, *Salmonella* maintained the highest incidence rate of 15.45 per 100,000 surveillance populations in the United States among all common foodborne pathogens. The second most common pathogen was *Campylobacter*, with an incidence rate of 13.45 per 100,000 people (CDC, 2015). For both *Salmonella* and *Campylobacter*, livestock is the most common food animal reservoir, mainly poultry, cattle and swine. During meat processing, *Campylobacter* and *Salmonella* can be transmitted to the environment and final meat products through cross-contamination. Humans may get infected through the consumption of undercooked meat and inappropriate kitchen hygiene practices (CDC, 2013; Hansson et al., 2015).

The high incidence of *Campylobacter* and *Salmonella* infections is likely attributed to their high prevalence in meat processing environments (Pouillot et al., 2012). In their natural state, these microbes are believed to exist mainly as biofilms that can enhance their survival rates. Biofilms are consortia of bacteria in which cells are aggregated with each other in a self-produced polymeric matrix and adhere to an inert or living surface (Branda and Kolter, 2004). Sessile bacterial cells within biofilms are more resistant to various stresses compared to their planktonic counterparts (Dykes et al., 2003; Ferreira and Fernanda, 2016; Mah and O'Toole, 2001). The biofilm formation process is generally divided into four steps, including initial attachment, proliferation, maturation and dispersion, with the initial attachment phase regarded as the most important one (Römling et al., 2014). Whether

* Corresponding author.
E-mail address: xiaonan.lu@ubc.ca (X. Lu).

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Table 1
Bacterial strains used in this study.

Species	Strain	Source/mutation	Reference
C. jejuni	F38011	Human clinical isolate	(Klena et al., 1998)
	ATCC33560	Bovine isolate	(Volokhov et al., 2003)
	11168	Human clinical isolate	(Parkhill et al., 2000)
	81116	Human clinical isolate	(Pearson et al., 2007)
	Human10	Human clinical isolate	The current study
	F38011 ΔFlaAB	Aflagellated mutant	(Konkel et al., 2004)
	F38011 ΔFlgl	Aflagellated mutant	(Konkel et al., 2004)
	F38011-GFP	Green fluorescence mutant	(Mixter et al., 2003)
S. enterica	Enteritidis OEA2669	Human clinical isolate	The current study
	Enteritidis 3512H	Human clinical isolate	(Seo et al., 2004)
	Enteritidis ATCC43353	Bovine isolate	(Draz and Lu, 2016)
	Enteritidis ME14	Human clinical isolate	(Guard-Petter et al., 1996)
	Enteritidis PT30	Human clinical isolate	(Isaacs et al., 2005)
	Typhimurium SL1344	Human clinical isolate	(Mills et al., 1995)
	Typhimurium SL1344-GFP	Green fluorescence mutant	(Tampakakis et al., 2009)
	Typhimurium ATCC14028	Chicken isolate	(Teplitski et al., 2006)
	Typhimurium CA513	14028 Δ*barA*	(Teplitski et al., 2006)
	Typhimurium BA1557	14028 *fimI1557*::MudJ	(Teplitski et al., 2006)
	Typhimurium BA746	14028 *sirA*3::cam	(Teplitski et al., 2006)

bacterial cells attach to a surface or not depends upon the chemical and physical interaction between the surface of the cell and adhering surface (Palmer et al., 2007). Biofilms can form at various surfaces, such as wood, glass, stainless steel, and plastics. Biofilms formed on the direct or indirect food contact surfaces are considered to be one of the major risk factors of cross contamination in food processing, including those on a cutting board and a sewage vessels (Guyard-Nicodème et al., 2013; Kusumaningrum et al., 2003; Nguyen et al., 2012; Reuter et al., 2010; Yang et al., 2016).

Salmonella and *Campylobacter* could develop intense biofilms in well-defined laboratory conditions that has been mostly studied (Joshua et al., 2006; Srey et al., 2013; Steenackers et al., 2012; Verstraeten et al., 2008). However, this *in vitro* condition is very different from the condition encountered in food processing environment. Hence, biofilms in real food processing environment may exert different growth and/or survival behavior compared to those evaluated under laboratory condition (Cappitelli et al., 2014). Currently, there is limited access to conduct the investigation of biofilms in the actual meat-processing environment due to technical challenge. Therefore, meat fluid residues have been used to create a cultivation environment in laboratory that mimics the real situation (Hood and Zottola, 1997; Rantsiou et al., 2012; Somers and Wong, 2004).

The exudate of frozen raw meat, also referred to as meat juice, has been identified as an important source of bacterial contamination on food processing surfaces (Guyard-Nicodème et al., 2013). Meat juice (sterilized by filtration) has been used as a food-based model to mimic the nutrient in meat processing scenario (Birk et al., 2004; Ferreira and Fernanda, 2016; Milillo and Ricke, 2010; Wang et al., 2013). Several studies have indicated that chicken juice could influence the growth and induce transcriptional response of *C. jejuni* and *S. Typhimurium* (Birk et al., 2004; Brown et al., 2014, 2013; Cloak et al., 2002; Ligowska et al., 2011; Luber, 2009; Milillo and Ricke, 2010). Most of these studies were conducted on planktonic culture and the understanding of the interplay between meat juice and microbial biofilms is limited. Brown et al., (2014) identified that chicken juice could enhance cell survival and biofilm formation levels of selective *C. jejuni* strains. Wang et al., (2013) found that the growth of *Salmonella* within a biofilm was slower when it was formed in chicken juice compared to the laboratory culture medium of tryptic soy broth. Meat juice may therefore be a complex nutrient matrix that could play various roles in biofilm formation and bacterial cell survival. Therefore, more comprehensive studies are required to elucidate the effects of meat juice on *Campylobacter* and *Salmonella* biofilm development and their survival within the biofilms.

This study aimed to identify if meat juice is an effective promoting factor for biofilm formation of *Campylobacter* and *Salmonella*, and to investigate the possible mechanisms of the interplay between meat juice and biofilm formation. In order to mimic the situation of a food processing surface pre-coated with meat juice, biofilm formation levels of *Campylobacter* and *Salmonella* on meat juice pre-coated microfluidic device were also investigated.

2. Materials and methods

2.1. Preparation of meat juice

Chicken juice and pork juice were collected using a frozen-thaw method described in a previous study, with minor modifications (Birk et al., 2004). In brief, whole chicken and pork chops purchased from local grocery stores in Vancouver, B.C. were frozen at − 20 °C and the thaw liquid was collected at 4 °C. To eliminate large particles, the collected meat exudates were centrifuged (12,000 × *g*, 10 min) and the supernatant was subsequently sterilized using a 0.22-μm syringe filter (Millipore). Meat juice from several chickens and pork chops was mixed, respectively. The sterilized meat juice was stored at − 80 °C and thawed overnight at 4 °C before use. The concentration of meat juice was evaluated and standardized based on the content of proteins. The protein content in the chicken juice and the pork juice was determined by the Bradford assay (Sigma-Aldrich) and adjusted to 5 mg/ml with sterile water (Wang et al., 2013).

2.2. Bacterial strains and cultivation

Bacterial strains used in this study are listed in Table 1. *C. jejuni* strains were routinely cultivated either on MH agar plate (BD Difco) supplemented with 5% defibrinated sheep blood (Alere) or in MH broth (BD Difco) at 37 °C under microaerobic (10% CO_2) conditions. *S. enterica* strains were grown either on LB agar plate or in LB broth (BD Difco) at 37 °C under aerobic conditions. Appropriate antibiotics used for flagellar/fimbriael mutants were supplemented for the preparation of overnight bacterial cultures.

2.3. Growth of planktonic cells

Overnight *C. jejuni* or *S. enterica* cultures (stationary phase) were adjusted to an OD_{600nm} = 0.3, referring to 10^9 CFU/ml of *C. jejuni* and 10^8 CFU/ml of *S. enterica*, followed by centrifugation at 8000 × *g* for 2 min. The collected cell pellets were washed once with phosphate buffered saline (PBS, pH = 7.4) and re-suspended in PBS with the same