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Comparison of microbial transfer rates from *Salmonella* spp. biofilm growth on stainless steel to selected processed and raw meat

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ABSTRACT

Salmonella biofilm cells can serve as a serious source of cross-contamination, both in the home and at food production sites. The objectives of this study were to determine the transfer rates (RTs) of *Salmonella* biofilm cells and to model the transfer process of biofilm cells from stainless steel surfaces to raw meat. The results showed that the RTs were significantly influenced by the types of meat products, with bacon and emulsified sausage showing higher RTs and roast pork showing lower RTs. Higher RTs of biofilm grown in a meat-based medium, Meat Thawing-Loss Broth (MTLB), were observed as compared to that in a standard growth medium (TSB). The logistic, exponential and multi-roots models could be used to satisfactorily describe the transfer of biofilm cells as demonstrated by use of MSE, F-test and R^2. There was no difference in attachment strength (reflected by the coefficients of transfer models) of biofilm grown in TSB or MTLB, as shown by the coefficients of *r*, *D* and *B* in three models. Compared with the exponential and the multi-roots models, the logistic model was able to more accurately fit the whole transfer process of biofilm cells. Our findings highlight the occurrence of cross-contamination with biofilm cells, and the models may provide useful tools in quantitative microbiological risk assessment of meat products.

1. Introduction

Salmonella sp. is one of the most globally widespread foodborne pathogens and is mainly responsible for food poisoning in EU and in US (EFSA-ECDC, 2014; US-CDC, 2012), resulting in numerous food recalls every year. Although there are many sources for cross-contamination by food pathogens, the bacteria attached on food contact surfaces have been demonstrated to be the principal source (Shi & Zhu, 2009). In suitable environments, the bacteria can form micro-colonies on surfaces with 3-D structures and are commonly referred to as biofilm. Studies have demonstrated that *Salmonella* can form biofilm on a wide variety of contact surfaces (Chia, Goulter, McMeekin, Dykes, & Fegan, 2009; Nguyen, Yang, & Yuk, 2014) and it has been well documented that cells comprising the biofilm can survive long term and result in further, more serious cross-contamination of food. This is a significant issue for food processing industries involving meat, dairy and fish, etc as well as for the ready-to-eat foods industries (Srey, Jahid, & Ha, 2013), since biofilm cells are resistant to stresses such as desiccation and antibiotics (Hoiby, Bjarnsholt, Givskov, Molin, & Ciofu, 2010).

Cross-contamination with *Salmonella* biofilm cells during food processing has become a major concern to food manufacturers and it is frequently associated with the transfer, direct or indirect, of bacteria from contaminated products to non-contaminated products (Pérez-Rodrígue, Valero, Carrasco, García, & Zurera, 2008). Many outbreaks have been traced back to the transfer of *Salmonella* biofilm cells from contact-surfaces to food (Srey et al., 2013). Therefore, a clear understanding of the bacterial transfer of biofilm formed by food-borne *Salmonella* is essential for developing effective strategies and quantitative microbiological risk assessment frameworks. Current studies have mainly focused on the factors that affect the numbers of food pathogens being transferring from an inert surface (or food surface) to a food following contact (or food surface), such as nutrient limitation, surface types (Midelet, Kobilinsky, & Carpentier, 2006), bacterial stress (Keskinen, Todd, & Ryser, 2008), contact time, pressure (Rodriguez & McLandsborough, 2007), moistness and other factors (Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2002; Luber, Brynestad, Topsch, Scherer, & Bartelt, 2006; Papadopoulou et al., 2012; Vorst, Todd, & Ryser, 2006). Rodriguez, Autio and McLandsborough (2007) assessed the influence of inoculation levels, material hydration and roughness of stainless steel surfaces on

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the transfer of *Listeria monocytogenes* from inoculated bologna to stainless steel and polyethylene. They showed that the use of transfer rates rather than the absolute transfer numbers was essential to allow comparisons of transfer conditions or to make comparisons between works of different research groups. However, in most studies, the cells being transferred were grown under commercial growth media, which are favorable laboratory cultures for cells but not usually encountered in actual food processing plants. Only limited studies have focused on the transfer of pathogen cells grown in simulated food model systems (Midelet & Carpentier, 2002).

Recently, mathematical modeling of the transfer of pathogens on various food and processing surfaces during direct contact has received increased attention and it has been suggested that, for assessment of cross-contamination, transfer models are essential for correctly quantifying the risk. Some studies have developed models to predict the transfer numbers of food pathogens between food and contact surfaces; for example, the modeling of *L. monocytogenes* from a slicer blade to ready-to-eat meat surfaces during slicing (Aarnisalo, Sheen, Raaska, & Tamplin, 2007; Sheen, 2008). However, in most studies, the donor surfaces (food and processing surfaces) of transfer were contaminated by planktonic cells, and few studies have involved the positive-biofilm isolates of pathogens (Keskinen et al., 2008). It has clearly been documented that pathogens, such as *Salmonella* and *L. monocytogenes*, grow predominantly as biofilm in most of their natural habitats, rather than in planktonic states (Brooks & Flint, 2008; Steenackers, Hermans, Vanderleyden, & De Keersmaecker, 2012), thus the results of cell transfer obtained under such planktonic modes may not be applicable to the transfer of these cells under biofilm states. Considering the above, the present study was therefore carried out to (i) compare the transfer rates of *Salmonella* biofilm cells from stainless steel to several types of meat products; (ii) mathematically model the cross-contamination of *Salmonella* biofilm cells transferring from stainless steel surfaces to raw chicken meat; (iii) compare the attachment ability and transfer process of *Salmonella* biofilm grown in a standard medium and in a meat-based medium.

2. Materials and methods

2.1. Strains and growth conditions

Six strains of *Salmonella* obtained from National Center of Meat Quality and Safety Control in China (NCM) and previously isolated from meat and meat processing equipment in a chicken slaughter plant (*S.* Agona NCM1120, *S.* Typhimurium NCM1121, *S.* Derby NCM1122, *S.* Indiana NCM 1207, *S.* Heidelberg NCM 1204, and *S.* Infantis NCM 1212), were used in this study. The six-strain cocktail of *Salmonella* was prepared by each of the six strains of *Salmonella* grown in TSB for 24 h at 37 °C. The ratios of cell numbers of each strain in the cocktail were 1:1:1:1:1:1 and the final concentration of cells was approximately 10^5 CFU/mL. A standard growth medium (TSB) and a meat-based growth medium (chicken meat thawing-loss broth, MTLB) were used. MTLB was prepared as described previously (Midelet & Carpentier, 2002). The final concentration of protein in MTLB was 5 mg/mL, determined by the Biuret protein assay.

2.2. Meat and meat products

Five meat products (sliced ham, roast pork, bacon, salted sausage and Cantonese sausage) and refrigerated chicken breast meat were purchased in a local supermarket. Sliced ham, roast pork and salted sausage were ready-to-eat meat products, with sliced ham and salted sausage showing high fat contents (the information provided by the ingredients list on the packaging) and smooth surface characteristics (touch profile), roast pork showing low fat content and high roughness surface. Bacon and Cantonese sausage contained visible fat, bacon showed smooth surface and soft texture, but Cantonese sausage showed a dry and hard texture. Each sample was cut into pieces of 40 × 30 × 4 mm in a sterile environment. The outer-surfaces of the refrigerated meat were removed with sterile scalpel to obtain an "internal part", which was then cut into four pieces of 40 × 25 × 4 mm. Each type of meat product was randomly sampled to detect background levels of *Salmonella* spp. using XLD agar (Land bridge, China). Detection of *Salmonella* in all the prepared-samples was negative, and no typical black colonies appeared on XLD agar plates, indicating that the procedures of Pre-preparing samples were logical, and had no effects on the following enumeration.

2.3. Biofilm formation and viable cells numeration

Stainless steel plates (50 × 20 × 1 mm, food grade 304, 2B finish), a material commonly used in the manufacture of meat-processing equipment, were used for biofilm formation. Prior to use, the plates were cleaned as previously described by Belessi, Gounadaki, Psomas, and Skandamis (2011). For biofilm formation, 100 μL of cocktail suspension, prepared as described above, was transferred into a centrifuge tube containing 10 mL of MTLB (or TSB) containing a stainless steel plate, and then the tubes were incubated at 20 °C for 7 days, and viable cells in biofilm were enumerated at 3, 5 and 7 days. A single plate was removed for sampling and was rinsed three times with 0.85% NaCl solution to remove non-attached cells. The attached biofilm cells were removed with sterile cotton swabs and the swabs were then transferred to tubes containing 0.85% NaCl solution, vortexed with beads for about 5 min, and then serial dilutions were prepared (Midelet & Carpentier, 2002; Winkelströter, Gomes, Thomaz, Souza, & De Martinis, 2011). Numbers of viable cells were determined in four replicates using XLD agar plate. Results were expressed as Log CFU/cm^2.

2.4. Transfer of biofilm cells from stainless steel to meat products

The 5-day biofilm formed as described above was air-dried for 40 min at room temperature. The method of double meat surface sandwich (the meat surfaces were in the upper and the lower layer, and the stainless steel plate was in the middle layer) was used in transfer experiments described as follows. A stainless steel plate, having a 5-day formed biofilm, was placed on a prepared meat surface. The plate was then completely covered with another surface of the same meat product, and a sterilized stainless steel mass weighing 500 g (the contact area 10 cm^2) was placed on the upper meat surface for 30 s (Midelet & Carpentier, 2002; Midelet et al., 2006). After applying the transfer conditions, cells that had been transferred to meat product surfaces were determined by the plate count method using XLD agar plates. The transfer rate (RT) was calculated as previously described by Rodriguez and McLandsborough (2007); Rodriguez, et al. (2007); RT (%) = cells number (CFU/cm^2) transferred to meat products/cells number (CFU/cm^2) in biofilm before transferring × 100%.

2.5. Modeling biofilm cells transfer to chicken meat

The 3-, 5- and 7-day of biofilms grown in TSB and MTLB were used in the transfer test and the succession-blot method was applied to determine the attachment strength of each biofilm. The details of the first transfer blot were the same as described above (method of double surface sandwich), after the first transfer, the same stainless steel plate (in the middle layer) was placed on a second chicken meat surface while the cells transferred to the first two chicken meat