Food Control 85 (2018) 42–47

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Dynamics of microflora on conveyor belts in a beef fabrication facility during sanitation

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ARTICLE INFO

Article history:
Received 6 June 2017
Received in revised form
2 September 2017
Accepted 13 September 2017
Available online 14 September 2017

Keywords:
Conveyor belt
Contamination
Sanitation
Microbial ecology
Active drying

ABSTRACT

The sanitation process commonly used for meat processing facilities includes three major steps: removal of soil (meat debris), cleaning and degreasing with detergent, and sanitization. The microbiological effect of such sanitation practice in commercial setting is largely unknown. Samples were collected from food-contact surface (CS) and non-food-contact surface (NCS) of two conveyor belts (Belt 1 and Belt 2, the latter of which was actively dried with air movers after sanitization) in a commercial beef fabrication facility at five time points: before cleaning (BC), after soil removal (ASR), after cleaning with detergent (ACD), 1.5 h after sanitization (AS), and before work the next day (BW), for enumeration of aerobes, lactic acid bacteria (LAB) and *Enterobacteriaceae* (EB). Selected isolates from each group of organisms from NCS of Belt 2 were also identified by 16 S rRNA gene sequencing. The mean numbers of aerobes, LAB and EB recovered BC were mostly about 6.0, 3.0 and 2.0 log CFU 1000 cm^{-2}, respectively. None of the individual steps alone resulted in significant changes in numbers of the three groups of microorganisms, with the exception of ACD of both surfaces of Belt 1 and AS of NCS of Belt 2 where $\geq$1.5 log reduction was observed for aerobes and LAB, respectively. However, the overall sanitation process resulted in significant reductions ($p < 0.05$) of all three groups by up to 3 log units. Air drying of Belt 2 did not result in further reduction in numbers of any of the three groups of bacteria. A total of 567 bacterial isolates were identified to the genus level. The aerobes from BC, ASR, ACD, AS and BW included 75, 89, 90, 69 and 81% of Gram-negative bacteria, with *Pseudomonas* (27%), *Pseudomonas* (34%), *Brevundimonas* (24%), *Stenotrophomonas* (19%), and *Stenotrophomonas* (24%) being the most prevalent genus, respectively. Five and 8 genera of LAB and EB were identified BC, with *Carnobacterium* (48%) and *Yersinia* (42%) being the most prevalent genus, respectively. *Carnobacterium* spp. were present at all five time points, mostly as a dominating component. Unlike EB whose diversity remained largely unchanged from BC to AS, the diversity of LAB decreased to only 1 genus.

1. Introduction

Like many foods, the microbiological safety and storage stability of beef is largely dictated by the type and numbers of bacteria present on the meat at the time of packaging, given other conditions such as storage temperature are properly managed. The production of beef at slaughtering plants involves three major processes: carcass dressing including the slaughtering of animals, skinning and evisceration, and carcass chilling and carcass breaking (Romans, Costello, Carlson, Greaser, & Jones, 2000). Hides of cattle may carry a variety of bacteria which may come from fecal materials, the environment, lairage and transportation trucks. Up to 10 log CFU per cm^2 of total aerobic counts on hides have been reported in studies from several European countries, the USA and Canada (Antic et al., 2010; Blagojevic, Antic, Ducic, & Buncic, 2011, 2012; Bacon et al., 2000; Serraino et al., 2012; Yang, Badoni, Tran, & Gill, 2015a; Zweifel, Capek, & Stephan, 2014). Some of those bacteria, including both pathogenic and spoilage bacteria may directly or indirectly transfer to the initially sterile carcasses during carcass dressing process (Bell, 1997; Grau, 1986). Historically, bacteria from carcasses are the primary source of contamination on beef (Cassin, Lammerding, Todd, Ross, & McColl, 1998). In recent years, the microbiological condition of dressed beef carcasses produced in Canada has improved drastically, resulting from the implementation of hazard analysis critical control point (HACCP) programs and effective multiple antimicrobial hurdles such as

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https://doi.org/10.1016/j.foodcont.2017.09.017
0956-7135/

pasteurization of carcasses with steam/hot water and spraying carcasses with solutions of short-chain organic acids (Gill, 2009; Yang, 2016). Some recent studies using genotyping and metagenomic analysis have demonstrated that conveyor belts can be a primary and recurring contamination source for beef from dressed carcasses carrying very few *E. coli* (Yang, He, Badoni, Tran, & Wang, 2017a; Yang, Tran, Youssef, & Gill, 2015b) and that meat processing environment is a primary contamination source of spoilage bacteria on meat (Stellato et al., 2016). Thus, the hygienic condition of equipment is of crucial importance to both safety and storage stability of meat, more so with the recent improvement in the microbiological condition of dressed carcasses.

The sanitation process currently employed by most meat processing plants includes three major stages: removal of soil, cleaning and degreasing with detergent, and sanitization, to achieve two main objectives: visibly clean equipment and reducing the numbers of bacteria to acceptable levels (Heinz & Hautzinger, 2007; Keener, 2005). To be approved by health authorities, food-contact sanitizers have to be able to reduce bacteria by $\geq$ 5 log within 30 s or $\geq$3 log within $\leq$5 min for a sanitizer with or without disinfectant claim (EPA, 1999; Gaulin, Lê, Shum, & Fong, 2011). Numerous laboratory studies on the efficacy of sanitizers are available and most of them use single species bacterial cultures. However, the efficacy of a sanitizer so determined may not reflect its efficacy for bacteria on processing equipment in meat plant because the microflora on equipment is likely to be a complex community of many species (Bagge-Ravn et al., 2003; Møretrø, Langsrud, & Heir, 2013). Furthermore, treatments before sanitization, for instance foaming with detergent, may also have impact on how bacteria respond to a sanitizer. However, published accounts on the overall microbiological effect of the sanitation practice or whether or not the individual steps of the sanitation process including sanitization have microbiological effects are largely lacking. Therefore, the objective of this study was to determine the microbiological effects of a commercial sanitation process for conveyor belts at the carcass breaking facility of a beef packing plant, by monitoring changes in numbers of total aerobes, lactic acid bacteria (LAB) and *Enterobacteriaceae* (EB) and changes in composition of each group. In addition, whether additional active drying of equipment after sanitization would be microbiologically beneficial was also investigated.

2. Materials and methods

2.1. Sanitation procedure for the fabrication facility of a beef packing plant

A beef packing plant that processes 120 cattle per day was involved in the study. Beef is fabricated at the plant on Monday and Tuesday of each week. Large portions of carcasses are conveyed by two belts (Belt 1 and Belt 2) perpendicular to each other at one end, from which they are taken to cutting tables alongside the belts for the removal of bones and fabrication into primal/subprimal cuts. The ambient temperature in the fabrication facility is maintained at 6–7 °C during production and it may go up to 15 °C during the sanitation process.

For the fabrication facility, the packing plant uses a generic sanitation protocol that is commonly employed by meat packing plants. During the study, the sanitation process was carried out by our research crew along with the cleaning crew. Briefly, the conveyor belts and the floor were first dry-cleaned to remove as much soil as possible and were subsequently washed with high pressure water (40–50 °C) to further remove soil. The cleaning started from the side of the fabrication room where the free end of Belt 1 is located and ended at the opposite side of the room where the two belts are connected. After dry-cleaning, the belts were rinsed with running water for 2 h, during which the surfaces of the belts were scrubbed manually using a motor scrubber (Caddy Clean Twin Brush Scrubber, Caddy Clean America, MA). Then, the belts were sprayed with a 2–5% solution of Powerfoam Plus T-624 (Epsilon, Edmonton, Alberta, Canada), a strong alkaline, self-foaming liquid cleaner and degreaser containing chlorine. The foam was rinsed off after a contact time of 30 min and an inspection was performed to make sure all surfaces were free of visible contamination. The belts were turned on briefly to run off water, and then were sprayed with a solution of 200 ppm E-San (Epsilon), a quaternary ammonium compounds (QACs) based sanitizer with benzalkonium chloride (BAC) as the main active component. After a contact time of 30 min, both belts were turned on briefly to evenly distribute, and run off excess sanitizer. Belt 2 was then actively dried for approximately one hour using two air movers (Viking Equipment, Solon, OH). During the sanitation process, equal attention was given to both food-contact surface (CS) and non-food-contact surface (NCS) at all stages. The entire process took approximately 8–9 h to complete and the time between the completion of sanitation and start of work the next day was approximately 4–5 h. The belts were generally dry before work started with or without active drying.

2.2. Collection of samples

On each of three days, the two conveyor belts were sampled at five time points: before cleaning (BC), after soil removal (ASR), after cleaning with detergent (ACD), 1.5 h after sanitization (AS), and before work the next day (BW). At each sampling point, three areas each approximately 1000 cm^2 of CS and NCS of each belt were swabbed using a synthetic sponge (Whirl-Pak™ Speci-Sponge™ Bags; Nasco, WI). The sponges were premoistened with 7 ml of 0.1% peptone water (w/v). Upon collection of each sample, an additional 7 ml of double strength neutralizing buffer (Difco, BD, Sparks, MD) was immediately added to each sponge that had been used for swabbing the surfaces. The samples were kept on ice until the completion of sample collection before work. In total, 180 swab samples were collected, with 9 from each sampling point for each surface type of each belt.

2.3. Processing of samples

All samples were processed for enumeration of aerobes, EB, and LAB. Each swab sample was pummeled for 2 min in a stomacher (Seward, West Sussex, UK) operated at low speed. The stomacher fluid expelled by squeezing the sponge from the three sponges collected from each surface type at each time was combined and treated as one composite sample. A 1 ml portion of each composite sample was used to prepare serial dilutions in 0.1% peptone water to 10^{-4}. A 1 ml portion of the undiluted stomacher fluid and the dilutions were each used to inoculate Petrifilm aerobic count plates (3 M Corp., St. Paul, MN, USA) which were incubated at 25 °C for 72 h. The colonies were counted, following manufacturer's instructions, for enumeration of total aerobes. A 10 ml portion of the stomacher fluid was centrifuged at 14,000 $\times$ *g* for 10 min. The pellet was resuspended in 1 ml of deMan Rosaga Sharp (MRS) broth (Difco). A portion of 0.1 ml of the suspension was used to prepare dilutions in MRS broth. The remaining 0.9 ml of the MRS suspension and dilutions were each used to inoculate Petrifilm aerobic plates. The plates were incubated at 25 °C for 72 h under anaerobic condition, and the colonies on plates were counted, following manufacturer's instructions, for enumeration of LAB. The final 10 ml portion of the undiluted stomacher fluid and the remaining of the 10^{-1} dilution in 0.1% peptone water were each filtered through