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Microbial Ecology of Decontaminated and Not Decontaminated Beef Carcasses

Xianqin Yang[1], Frances Tran[1] & Taylor Wolters[1]

[1]Agriculture & Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, Canada

Correspondence: Xianqin Yang, 6000 C & E Trail, Lacombe, Alberta T4L 1W1, Canada. Tel.: 1-403-782-8119. E-mail: xianqin.yang@agr.gc.ca

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Abstract

The objective of this study was to investigate spoilage microflora of decontaminated and not decontaminated beef carcasses. Carcasses after skinning and after chilling at two beef plants (A, a small plant where no interventions but dry chilling are used; B, a large plant where multiple antimicrobial interventions and spray chilling are used) were swab-sampled for determination of carcass microflora. The numbers of aerobes, *Pseudomonas*, *Brochothrix thermosphacta*, lactic acid bacteria (LAB), and *Enterobacteriaceae* (EB) on carcasses at plant A after skinning were 5.2, 3.7, 2.8, 3.9, and 1.6 log cfu 15,000 cm^{-2} and they were not significantly different ($p>0.05$) from those on carcasses at plant B at equivalent times. The numbers of EB were significantly lower ($p<0.05$) on carcasses after chilling at both plants. However, no difference was observed for the other four groups of microorganisms ($p>0.05$). At plant A, the microflora on carcasses after skinning and after chilling included 18 and 19 bacterial species, with *Psychrobacter* (30.9%) and *Psychrobacter* (42.9%) being the respective predominant genus, respectively. At plant B, the microflora after skinning and after chilling included 21 and 17 bacterial species, with *Chryseobacterium* (18.6%), *Kocuria* (18.6%) and *Brevibacterium* (18.6%), and *Pseudomonas* (33.3%) being the respective predominant genera. The spoilage microflora of decontaminated beef carcasses is similar in numbers to that of conventionally produced carcasses, suggesting the decontamination treatments for beef carcasses may not cause major difference in storage life of chilled vacuum packaged beef, the major form of beef for international and domestic trading in North America.

Keywords: microflora, beef carcasses, dry chilling, spray chilling, decontamination

1. Introduction

The muscle tissue of a healthy animal is largely free of bacteria (Grau, 1986), but the meat is inevitably contaminated with bacteria when the carcass surface is exposed to the environment during hide removal and downstream operations in the carcass dressing process (Bell, 1997). To control the contamination of beef carcasses, particularly with the high profile enteric pathogen *E. coli* O157:H7 for which cattle are the primary reservoir (CDC, 1993; Hancock, Besser, Lejeune, Davis, & Rice, 2001), the US Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) promulgated that all federally inspected meat packing plants in the US and foreign establishments that export meat to the US establish hazard analysis critical control point (HACCP) systems (USDA/FSIS, 1996). The great majority of cattle slaughtered in North America are processed in large beef packing plants where the line speed is high (MacDonald, 2003). In addition to implementation of HACCP, the management of those large beef packing plants have also incorporated multiple antimicrobial interventions, particularly during the carcass dressing process (C. O. Gill, 2009). Consequently, the microbiological efficacy of antimicrobial interventions has been the subject of investigation of numerous studies (C. O. Gill, 2009; Loretz, Stephan, & Zweifel, 2011) which primarily focus on the effect of such treatments on the numbers of aerobes, a commonly accepted hygiene indicator, and the numbers/prevalence of enteric pathogens such as *E. coli* O157:H7. The microflora initially present on meat surfaces are of great importance to not only the safety, but also the storage stability of meat because meat spoilage is primarily caused by the growth of bacteria and their metabolic activities (C.O. Gill, 1996). However, studies on whether or not antimicrobial interventions impact the carcass spoilage microflora are lacking. Important meat spoilage bacteria include *Pseudomonas*, *Enterobacteriaceae* (EB), lactic acid bacteria (LAB) and *Brochothrix thermosphacta* (A. Gill & Gill, 2009). The objective of this study was to examine the spoilage microflora of carcasses produced at large

beef packing plants where multiple antimicrobial interventions and spray chilling are a common practice in relation to that of carcasses produced conventionally, i.e. without antimicrobial intervention but with dry chilling.

2. Method

2.1 Sample Collection and Processing

On each of four days, samples were collected from groups of 5 beef carcasses selected at random before and after chilling at two federally inspected Canadian beef packing plants (A, a small plant where no antimicrobial interventions are used and the carcasses are dry chilled for 72 h; B, a large plant where multiple antimicrobial interventions including spraying carcasses with 5% lactic acid and pasteurization of carcasses with hot water are implemented and the carcasses are spray chilled for approximately 48 h). For plant A, samples were collected right before carcasses entered the chiller and when they were about to enter the fabrication floor after chilling. For plant B, samples were collected after hide removal, prior to any antimicrobial treatment, and when carcasses were about to enter the fabrication floor after chilling. The two sampling points at both plants are referred as skinned carcasses and chilled carcasses, respectively. Each sample was collected by swabbing an area of 1000 cm^2 on the brisket, the flank and the rump of a carcass side. The samples were kept on ice until they were processed within 4 h of collection.

2.2 Enumeration of Bacteria

Five ml of 0.1% peptone was added to each sponge which was pummelled for 2 min. Fluid from all five sponges of each sample type was combined and treated as one composite sample. The sponge fluid was centrifuged at 10,000 x *g* for 10 min. Each resulting pellet was resuspended in 5 ml of 0.1% peptone water. Portions of 100 µl of appropriate serial ten-fold dilutions in 0.1% peptone water were spread-plated in duplicate on plate count agar (PCA), de Man Rogosa-Sharpe agar, streptomycin sulfate/thallium acetate/actidione agar, and cephaloridine, fucidin, and cetrimide agar, for enumeration of aerobes, LAB, *B. thermosphacta* and *Pseudomonas*. The plates were incubated as described previously (Badoni, Rajagopal, Aalhus, Klassen, & Gill, 2012). One ml portions of each dilution and the undiluted suspension were mixed with 15 ml of violet red bile glucose agar and the plates were incubated at 25 ℃ for 48 h, for enumeration of EB. All microbial counts were transformed to log values. Counts of the same type from each sampling point at each plant were considered as a set to which a Ryan-Joiner test for normal distribution was applied using Minitab version 16 (Minitab Inc., State College, PA). Mean counts were separated using the Tukey function in Minitab.

2.3 Microflora Identification by 16S rRNA Gene Sequencing

A total of 20 colonies on a PCA plate from each of three composite samples for each sampling point at each plant were selected at random and each was resuspended in 200 µl of trypticase soy broth (TSB) supplemented with 15% glycerol solution. The isolates were purified and subsequently subjected to 16S rRNA gene sequencing, as described previously (Youssef, Gill, & Yang, 2014). Identification was assumed when the similarity of the query and Genbank sequence was ≥ 99%.

3. Results and Discussion

The numbers of aerobes on skinned and chilled carcasses at plant A were about 5.2 and 4.6 log cfu 15,000 cm^{-2}, 0.7 log unit more than those on skinned carcasses at plant B (Table 1). However, those differences were not significant ($p > 0.05$). EB on chilled carcasses at both plants were ≤ 0.1 log cfu 15,000 cm^{-2}, >1.6 log units less than EB on the respective skinned carcasses. The mean numbers of *Pseudomonas*, *B. thermosphacta*, and LAB on skinned carcasses at plant A were somewhat higher than those at plant B. However, the numbers of these three groups of microorganisms were not significantly different between skinned and chilled carcasses at the same plant or on the same type of carcasses at the two plants ($p > 0.05$). Total aerobic counts between 10^4 and 10^{10} cfu/cm^2 on hides have been reported in studies from various countries (Antic et al., 2010; Bacon et al., 2000; Blagojevic, Antic, Ducic, & Buncic, 2011; Yang, Badoni, Tran, & Gill, 2015; Zweifel, Capek, & Stephan, 2014). Some of those bacteria will inevitably be transferred to the meat during hide removal, from direct contact of hides with carcasses, aerosols generated during pulling of the hides and/or equipment that is used for opening cuts (C. O. Gill, 2005). In fact, a recent study has investigated the microbial ecology of feces, hides and eviscerated carcasses at an Australian abattoir and found that bacteria on eviscerated carcasses are mainly from hides (Chandry, 2013). A recent Irish study has reported levels of aerobes, EB, *Pseudomonas*, LAB and *B. thermosphacta* on skinned carcasses at 4.0, 2.0, 3.1, 3.4 and 2.7 log cfu 100 cm^{-2} and on dry chilled carcasses at 4.7, 2.6, 2.9, 3.3 and 4.2 log cfu 100 cm^{-2}, respectively (Reid et al., 2017). An earlier Swedish study noted levels of aerobes, EB, *Pseudomonas*, and *B. thermosphacta* on the adipose tissue of brisket before chilling at 7.4, 4.4,

5.2 and 5.4 log cfu 100 cm^{-2} (Lasta, Pensel, Masana, Rodr ǵuez, & Garc á, 1995). The numbers of all five groups of organisms on both skinned and dry chilled carcasses observed in this study are much lower than those previously reported. Published accounts on the levels of *Pseudomonas*, *B. thermosphacta* and LAB on chilled decontaminated carcasses are largely unavailable. The levels of aerobes on skinned and decontaminated carcasses can range from 4.0 to 9.1 log cfu 100 cm^{-2} and 1.4 to 5.3 log cfu 100 cm^{-2}, respectively (Arthur et al., 2004; Bacon et al., 2000; Yang, Badoni, Youssef, & Gill, 2012). The numbers of aerobes and EB on both skinned and chilled carcasses at the large plant involved in this study were comparable with those lower levels reported in the literature.

Table 1. Numbers of five groups of bacteria recovered from skinned and chilled carcasses from two beef packing plants[a]

Plant	Stage	Bacteria (log cfu 15,000 cm^{-2})*				
		Aerobes	*Pseudomonas*	*B. thermosphacta*	LAB	EB
A	Skinned	5.2 ± 0.4^{A}	3.7 ± 0.7^{A}	2.8 ± 2.5^{A}	3.9 ± 0.8^{A}	1.6 ± 0.7^{AB}
	Chilled	4.6 ± 0.4^{AB}	2.8 ± 0.5^{A}	1.5 ± 1.4^{A}	3.3 ± 1.1^{A}	-0.1 ± 0.8^{C}
B	Skinned	4.4 ± 0.9^{AB}	3.0 ± 1.2^{A}	1.7 ± 1.5^{A}	2.5 ± 0.5^{A}	2.8 ± 0.3^{A}
	Chilled	3.9 ± 0.3^{B}	3.2 ± 0.7^{A}	1.8 ± 1.6^{A}	3.2 ± 0.7^{A}	0.1 ± 1.1^{BC}

[a]Samples were collected from plant A where no decontaminating treatment was applied and plant B where multiple decontaminating treatments were applied.

*Mean values denoted by the same superscript letters are not significantly different ($p > 0.05$).

From skinned carcasses, 68 and 59 isolates from plant A and B were identified to species level and they belonged to 18 and 21 different groups, respectively. From chilled carcasses, 63 isolates from each plant were identified to species level. The isolates from plant A and B belonged to 19 and 17 species, respectively. The bacterial species isolated from skinned and chilled carcasses at plant A, and skinned and chilled carcasses at plant B, were of 16, 13, 16 and 11 genera, respectively (Fig. 1). Most genera (63%) found on skinned carcasses at both plants were unique. However, most genera on chilled carcasses were shared with their respective skinned carcasses, 61.5 and 54.5% for plant A and B, respectively.

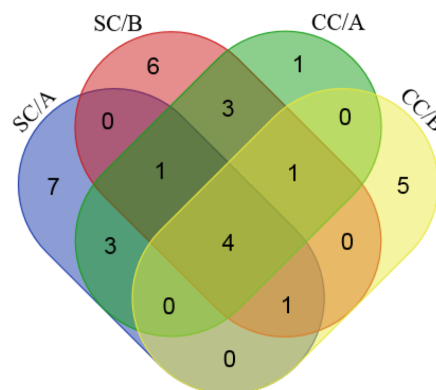

Figure 1. Venn diagram of the genera isolated from skinned carcasses (SC) and chilled carcasses (CC) at two federally inspected beef packing plants (A and B)

Table 2. Groups and genera of bacteria recovered from skinned carcasses at two beef packing plants

Bacteria		Number of isolates		Fraction of isolates	
Group	Genus	Plant A	Plant B	Plant A	Plant B
GNAE	*Acinetobacter*	9	3	13.2	5.1
	Brevundimonas	-[a]	1	1.5	1.7
	Chryseobacterium	1	11	1.5	18.6
	Diaphorobacter	-	2	-	3.4
	Ochrobactrum	6	-	8.8	
	Kaistella	-	1	-	1.7
	Pseudomonas	6	1	8.8	1.7
	Psychrobacter	21	-	30.9	-
	Rhizobium	-	1	-	1.7
	Sphingobium	1	-	1.5	-
	Stenotrophomonas	2	-	2.9	-
Group total		46	20	67.6	33.9
GNFAN	*Brucella*	1	-	1.5	-
	Enterobacter	6	-	8.8	-
	Serratia	-	1	-	1.7
Group total		8	1	10.3	1.7
GPAE	*Arthrobacter*	1	9	1.5	15.3
	Bacillus	1	1	1.5	1.7
	Brachybacterium	-	1	-	1.7
	Brevibacterium	2	11	2.9	18.6
	Frigoribacterium	1	-	1.5	-
	Kocuria	-	11	-	18.6
	Microbacterium	-	2	-	3.4
	Rothia	1	-	1.5	-
Group total		7	35	10.3	59.3
GPFAN	*Aerococcus*	7	-	10.3	-
	Paenibacillus	-	1	-	1.7
	Staphylococcus	-	2	-	3.4
	Streptococcus	1	-	1.5	-
Group total		8	3	11.7	5.1

[a]- not recovered.

GNAE, Gram-negative aerobes; GNFAN, Gram negative-facultative anaerobes; GPAE, Gram-positive aerobes; GPFAN, Gram-positive facultative anaerobes.

The isolates from both plants consisted primarily of strict aerobes, accounting for 77.9% and 90.4% of the total isolates from Plant A and B, respectively (Table 2). Even so, most of the aerobes from skinned carcasses at plant A were Gram-negative bacteria, 67.6%, while those from plant B were largely Gram-positive bacteria, 59.3%. The predominant genera were *Psychrobacter* (30.9%) on carcasses from plant A and *Chryseobacterium* (18.6%), *Kocuria* (18.6%) and *Brevibacterium* (18.6%) on carcasses from plant B. Isolates from both plants had a very small fraction of EB. The bacterial species found on skinned carcasses at both plants were mostly soil or animal associated organisms and were among those which have been previously reported on meat immediately after slaughter (Corry, 2007).

Table 3. Groups and genera of bacteria recovered from chilled carcasses at two beef packing plants

Bacteria		No of isolates		Fraction of isolates	
Group	Genus	Plant A	Plant B	Plant A	Plant B
GNAE	*Acinetobacter*	4	7	6.3	11.1
	Brevundimonas	4	-	6.3	-
	Caulobacter	-[a]	1	-	1.6
	Chryseobacterium	-	5	-	7.9
	Janthinobacterium	-	1	-	1.6
	Pedobacter	-	2	-	3.2
	Pseudomonas	1	21	1.6	33.3
	Psychrobacter	27	-	42.9	-
Group total		36	37	57.1	58.7
GNFAN	*Enterobacter*	3	-	4.8	-
Group total		8	-	4.8	-
GPAE	*Arthrobacter*	10	8	15.9	12.7
	Bacillus	3	2	4.8	3.2
	Brevibacterium	1	-	1.6	-
	Kocuria	1	-	1.6	-
	Microbacterium	5	-	7.9	-
	Rhodococcus	-	1	-	1.6
	Rothia	1	-	1.6	-
Group total		21	11	33.3	17.5
GPFAN	*Carnobacterium*	-	1	-	1.6
	Enterococcus	1	-	1.6	-
	Staphylococcus	2	14	3.2	22.2
Group total		3	15	4.7	23.8

[a]- not recovered.

GNAE, Gram-negative aerobes; GNFAN, Gram negative-facultative anaerobes; GPAE, Gram-positive aerobes; GPFAN, Gram-positive facultative anaerobes.

At plant A, similarly to skinned carcasses, the microflora of chilled carcasses was made up of 88.8% aerobes (Table 3), with *Psychrobacter* (42.9%) being the predominant genus. The fractions of Gram-negative and Gram-positive aerobes on chilled carcasses were about 10% less and 25% more, respectively, than those on skinned carcasses. The increase of the latter was mainly attributed by species of *Arthrobacter*. This shift in the microflora was likely a result of the dry chilling used at the plant as it has been widely accepted that Gram-negative bacteria are, in general, more susceptible to drying than Gram-positive bacteria (Yang, 2016). Species of *Arthrobacter* are soil organisms and some are able to grow at low temperatures and can be resistant to desiccation (Busse, 2016). The increase in the fraction of this genus is then not surprising. At plant B, 58.7% of the microflora on chilled carcasses was Gram-negative aerobes, compared to 33.9% on skinned carcasses. This change could be, in part, at the expense of the decrease of Gram-positive aerobes (59.3% to 17.5%). In addition, the fraction of Gram-positive facultative anaerobes increased, from 5.1 to 23.8%, mainly contributed by species of *Staphylococcus*. The most predominant genera on chilled decontaminated carcasses were *Pseudomonas* (33.3%) and *Staphylococcus* (23.8%). The higher fraction of *Pseudomonas* on chilled decontaminated carcasses than on skinned carcasses could be caused by the growth of *Pseudomonas* on carcasses during spray chilling (C. O. Gill & Landers, 2003). The relatively large fraction of *Staphylococcus* may have originated from the hands of workers (Chandry, 2013).

In brief, decontaminated and non-decontaminated carcasses had similar levels of all five groups of spoilage bacteria. The absence of species of *Enterobacteriaceae* that are of high spoilage potential and a very small fraction of lactic acid bacteria on both decontaminated and non-decontaminated carcasses indicate the storage life of chilled, vacuum packaged beef derived from such carcasses could be similar and long.

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