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Accumulation of Spores in Hot Waters Recirculated in Beef Carcass Pasteurizers

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Abstract

Concerns have been raised about the possible accumulation of bacterial spores in hot water recirculated in beef carcass pasteurizers. Therefore, waters from pasteurizers at two large beef packing plants were examined for the presence of spores. At both plants the numbers of spores in pasteurizer waters tended to increase non-uniformly as they were reused on carcasses, but the maximum numbers recovered from any samples were 101 cfu/100 ml. All the organisms recovered from pasteurizer waters grew aerobically, and most were identified as species of Bacillus or Paenibacillus, which are commonly found in milk at numbers ≥ 10 cfu/ml. The small numbers of bacterial spores in recirculated waters in carcass pasteurizers seemingly pose no health risks to consumers.

Key words: *Beef carcasses; Pasteurizer water; Bacillus; Paenibacillus; Spores*

Introduction

At many large beef packing plants in North America, carcasses are pasteurized with recirculated hot water at the end of the carcass dressing process (Gill, 2009). Meat from carcasses decontaminated in that way is not acceptable in European markets because, at present, the EU prohibits pasteurizing of carcasses with other than potable hot water or steam (EFSA, 2010). However, an expert scientific committee of the European Food Safety Authority (EFSA) has recently published an opinion on the use of recycled hot water for carcass pasteurizing, in which it is stated that "the decontaminating efficacy of recycled hot water does not differ significantly from that of hot potable water" (EFSA, 2010). Even so, the committee considered that the accumulation of heat resistant bacterial spores in recirculated pasteurizer water might occur, and could be a cause for concern.

In the only previous study in which recirculated water used to carcass pasteurizing was examined for the presence of spores, there was no indication of accumulation of clostridial spores in water used for treating several thousand pig carcasses (Gill et al., 1997). The data obtained in that study were limited, and it could not be assumed that the condition of water recirculated in beef carcass pasteurizers would be the same. Therefore, a study was undertaken to obtain initial information on the presence of spores in waters recirculated in commercial beef carcass pasteurizers.

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Material and Methods

Sample collection. Water samples were collected from the carcass pasteurizer at each of two beef packing plants, A and B, which each processes between 800 and 1200 carcasses during a daytime work shift. The pasteurizers, which were constructed by the same manufacturer (Chad Co., Olathe, KS), each consists of a spray cabinet fitted with oscillating sprays, a basin for collection of sprayed water, a hot water tank and a water heating system. Water from sprayed carcasses collected in the basin is pumped back to the tank, and is pumped from the tank through the heating system to the spray heads. The water passing through the heating system is heated to 95 ℃. The water tank is fed continuously with make-up water, with excess water flowing to waste. At plant A, water is drained from the pasteurizer and the insides of the tank and basin are washed down during the mid-day break and at the end of

the shift. At plant B the pasteurizer is drained and washed down only at the end of the shift. At each plant, on one day, samples were obtained from the spray water collection basin, before any carcasses were treated, and at approximately 2 h intervals after carcasses treatment commenced until the end of the day shift. At each sampling time, two containers were each filled with approximately 600 ml of the recirculated water. On another day, at each plant, 12 samples were collected during a 1 h period in mid-afternoon. The sealable 1 L plastic containers (VWR Canada, Mississauga, Ontario, Canada) used for sample collection each contained 100 g of NaCl. Dissolution of the salt in the sampled water gave a 17 % NaCl solution in which spoilage bacteria would not grow, but in which bacterial spores would be preserved in a viable condition. After their receipt at the laboratory, within 48 h of their collection, the samples were stored at 2 ºC until they were processed for spore enumeration.

Enumeration of spore-forming bacteria. A 400 ml portion of each water sample was filtered through four layers of cheese cloth to remove large particles and flakes of fat. The filtrate was collected and centrifuged at 4 ºC and 10,000 x g for 30 min to pellet spores. Each pellet was washed once with 10 ml 0.1% (w/v) peptone water (Difco; Becton Dickinson, Sparks, MD), then the washed pellet was resuspended in 10 ml of 0.1% peptone water. The suspension was heated at 75 ºC for 20 min (Austin, 1998), then cooled in ice water. Eight 1 ml portions of the suspension were each used to prepare dilutions of 10-1 and 10-2. The entire content of each dilution was filtered through a hydrophobic grid membrane filter (Oxoid, Mississauga, Ontario, Canada). Four filters used with each dilution were each placed on plates of trypticase soy agar (TSA). The other eight filters were each placed on plates of Columbia agar (Oxoid) supplemented with 5% of defibrinated sheep blood (CBA; Oxoid). Duplicate TSA plates with filters used with each dilution were incubated aerobically, and the other four TSA plates were incubated anaerobically, at 35 ºC for 48 h. Duplicate CBA plates with filters used with each dilution were incubated aerobically, and the other four CBA plates were incubated anaerobically, at 4 ºC for 4 weeks. Squares on filters on TSA plates that contained colonies were counted for enumeration of spores of aerobic and anaerobic mesophilic spore formers. Squares on filters on CBA plates that contained colonies were counted for enumeration of aerobic and anaerobic psychrophilic spore formers. Colonies recovered from filters on TSA plates that were incubated anaerobically were spread on duplicate plates of TSA, which were incubated aerobically and anaerobically, as before, to determine if the isolates were facultative or obligate anaerobes.

Identification of isolates. Five types of colony could be distinguished on filters that were incubated on TSA. Isolates were obtained from 5 or 10 colonies of each type that were picked at random from filters used with samples from plant A or B, respectively. Each isolate was streaked on duplicate plates of TSA that were incubated at 35 ºC for 20 h or 72 h. Colonies from plates incubated for 20 h were used for Gram staining. Colonies from plates incubated for 72 h were used for the preparation of wet mounts, which were examined microscopically, under phase contrast illumination (BX-50 microscope; Olympus, Markham, ON, Canada) for the presence of spores. All spore-forming Gram positive isolates were identified from patterns of carbohydrate utilization, using API 50 CH strips (bioMérieux, Inc, Durham, NC) according to the manufacturer's instructions.

Table 1. Numbers of mesophilic spore-forming bacteria recovered on plates of trypticase soy agar incubated aerobically or anaerobically, from samples of water obtained from the carcass pasteurizer at each of two beef packing plants at various times of carcass processing, during a day time work shift

Time of processing (h)	Numbers of bacteria (cfu/100 ml)			
	Plant A		Plant B	
	Aerobic incubation	Anaerobic incubation	Aerobic incubation	Anaerobic incubation
0	0	0	5	0
1	19	7	-[a]	-
2	57	16	18	6
5	11	0	18	14
6	15	4	-	-
7	16	4	27	11
8	-	-	101	39

[a] No samples obtained at that time.

Results

No colonies were recovered on filters incubated on CBA plates. Colonies developed on filters used with most samples of pasteurizing water when filters were incubated aerobically or anaerobically on plates of TSA. The numbers of colonies on corresponding filters from duplicate samples were mostly similar. For samples obtained at plant A after various times of carcass processing, the numbers of bacteria increased with time during the first 2 h of processing, but after ≥ 5 h of processing the numbers were similar and less than the numbers recovered from samples obtained after 2 h of processing (Table 1). For samples obtained similarly at plant B, the numbers of bacteria increased erratically with the time of processing.

For samples collected on one day after between 5 and 6 h of processing, the numbers of colonies on filters on plates of TSA incubated aerobically were 5 ± 7 cfu/100 ml and 88 ± 33 cfu/100 ml for filters used with samples from plant A and B, respectively. The corresponding numbers of colonies on filters on plates of TSA that were incubated anaerobically were 3 ± 2 cfu/100 ml and 31 ± 8 cfu/100 ml.

All the colonies recovered from filters on plates of TSA that were incubated anaerobcaiily grew aerobically. Three isolates from plant A and one from plant B did not form spores. Five and six species of spore forming bacteria were identified among isolates from plant A and B, respectively, with Bacillus licheniformis being the predominant species isolated from both plants (Table 2).

Table 2. Species of spore-forming bacteria isolated from samples of water from carcass pasteurizers at two beef packing plants

	Number of isolates	
Species	Plant A	Plant B
Bacillus licheniformis	16	26
Bacillus pumilus	1	15
Bacillus subtilis	1	-[a]
Bacillus lentus	-	2
Bacillus firmus	1	-
Bacillus laterosporus	1	-
Bacillus stearothermophilus	-	1
Paenibacillus amylolyticus	-	1
Paenibacillus polymyxa	-	1
Not identified	2	3
Total	22	49

[a] Species not identified among the tested isolates.

Discussion

Bacterial spores can evidently be present in the hot waters recirculated in beef carcass pasteurizers. As large volumes of water are applied to the carcass sides in a pasteurizer, the water is circulated relatively rapidly. This would seem to ensure that the water is generally well mixed. Consequently, the numbers of spores in samples of water drawn from a pasteurizer at any one time should not vary greatly. The findings with samples of water drawn from each pasteurizer during a one hour period confirmed that expectation. The extent of the study was unfortunately limited by commercial factors. However, the findings with samples of water collected from each pasteurizer at various times during one day indicate that waters circulated in routinely cleaned pasteurizers before carcasses are processed are likely to contain few spores. The numbers of spores in pasteurizer water apparently tend to increase during processing, but the observed increases were relatively small and non-uniform. That any increases would likely be small could be expected, because the carcasses entering the pasteurizer had been washed twice, once as skinned carcasses before evisceration, then again as dressed carcass sides. If the numbers of bacteria on carcasses are relatively high washing will remove bacteria and, presumably, bacterial spores (Gill and Landers, 2003). However, repeated washing will have little or no effect on bacterial numbers. Thus, well washed carcass sides entering a pasteurizer would be expected to carry few spores that can be removed by the washing action of the pasteurizing water. Even so, the findings of this study indicate that occasionally relatively large numbers of spores are added to the pasteurizer water; but the circumstances in which that occur are not readily apparent.

Apart from spores from species that were not identified, the spores recovered from pasteurizer water were all those of species of Bacillus or Paenibacillus that are commonly found in milk (Durak et al., 2006; Scheldeman et al., 2004). The numbers of spores of these mesophilic organisms in raw milk are commonly ≥ 10 cfu/ml, and can exceed 105 cfu/ml (McGuiggan et al., 2002; Ranieri and Boor, 2009). As the spores of these organisms survive usual pasteurizing temperatures, the psychrotolerant members of the group are commonly the cause of spoilage of pasteurized milk (Ranieri et al., 2009). The presence of these organisms in milk is not regarded as a health risk.

The findings of this study are limited, and obviously the numbers of spores in recirculated pasteurizer waters at other plants may differ from the numbers found in this study. Further study of the matter would then be desirable. Nonetheless, the findings of this study indicate that accumulation of bacterial spores in recirculated pasteurizer waters is unlikely to pose a health risk to consumers.

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