Bacterial adhesion to cooked sausages: development of a model experimental system

J.-P. G. PIETTE and J.-Y. POSTEC

Agriculture Canada, Food Research and Development Centre, St-Hyacinthe, Québec, Canada

SUMMARY

An experimental system was developed to study bacterial adhesion to cooked sausages, using natural sheep casing and processed collagen casings as model surfaces. Square pieces (ca. 1 cm^2) of the casings were inserted into specially designed flow chambers. Bacteria were introduced (ca. 10^8 $CFU.mL^{-1}$) and allowed to sediment on the casings for 20 min. The chambers were then rinsed under controlled conditions (shear stress of 0.05 $N.m^{-2}$) and the remaining (adherent) bacteria were stained and enumerated under light microscopy. Typically, *Brochothrix thermosphacta* cells adhered to casings in large numbers (ca. 10^5 $CFU.cm^{-2}$), regardless of the casing composition (100% collagen or collagen-sorbitol-cellulose) and mode of rehydration (30 min in water or 5 min in a 10% NaCl solution; room temperature). Lower numbers of *Lactobacillus* sp. cells were found adhering to the casings (ca. 10^4 $CFU.cm^{-2}$) and this was due to a low level of sedimentation rather than to weak adhesive properties. Preliminary results indicate that the method developed will be applicable to pieces of casings peeled off cooked sausages.

INTRODUCTION

Shelf lives of several months can be achieved by cooking meat products in their final packages (Ingram and Simonsen, 1980). For practical reasons, however, use of this process is restricted to the case of large size unsmoked products. Most other products are vacuum-packed after cooking. These get typically spoiled within a few weeks due to the rapid growth of a strictly or facultatively anaerobic psychrotrophic flora (generally *B. thermosphacta*, *Lactobacillus*, *Leuconostoc*, and/or *Streptococcus*) which recontaminates the product surface prior to packaging (Tompkins, 1986).

At the present time, limitation of the recontamination relies entirely on strict adherence to proper cleaning and sanitation procedures in packaging rooms. Additional means to limit bacterial recontamination might be developed if the mechanisms involved in the transfer and adhesion of bacteria to product surfaces were known, and this could lead to substantial increases of current shelf lives.

As a first step in the study of the post-cooking recontamination of meat products, the present article describes the development of an experimental system to investigate bacterial adhesion to cooked sausages. The procedure proposed is adapted from a method previously developed to quantify bacterial adhesion to model meat surfaces (Piette and Idziak, 1989, 1991).

MATERIALS AND METHODS

B. thermosphacta and *Lactobacillus* sp. were isolated from refrigerated beef and refrigerated vacuum-packed cooked ham, respectively. Standardized cultures were prepared by growing the organisms in brain heart infusion broth (*B. thermosphacta*) or lactobacillus MRS broth (*Lactobacillus* sp.) for three consecutive 24 h periods, with transfer in fresh medium each day. All incubations were done at 25°C witout agitation. For the adhesion experiments, the standardized cultures were diluted 1:50000 in fresh medium, incubated for 24 h, the cells were harvested by centrifugation, washed twice in deionized water, and resuspended in deionized water to the desired concentration. Cell counts were determined by the standard dilution and plating techniques.

The pure collagen (100% of the dry mass) and collagen-glycerol/sorbitol-cellulose (73%-21%-5% of the dry mass) casings were obtained from Naturin Canada. They were rehydrated at room temperature, either in water for 30 min or in a 10% NaCl solution for 5 min, and cut in 1 cm^2 square pieces. The inside surface was then blotted dry with absorbent paper and glued to a 24 x 60 mm glass cover slip using a transparent double-sided adhesive tape. The natural sheep casing was obtained from Independent Products Canada. It was thoroughly rinsed in running water to remove excess salt and soaked in deionized water for 30 min at room temperature. The casing was then cut in square pieces and the wet pieces were mounted directly on a glass

cover slip to which they adhered strongly upon drying. The cover slips bearing the casing samples were subsequently inserted into specially designed flow chambers described elsewhere (Piette and Idziak, 1989).

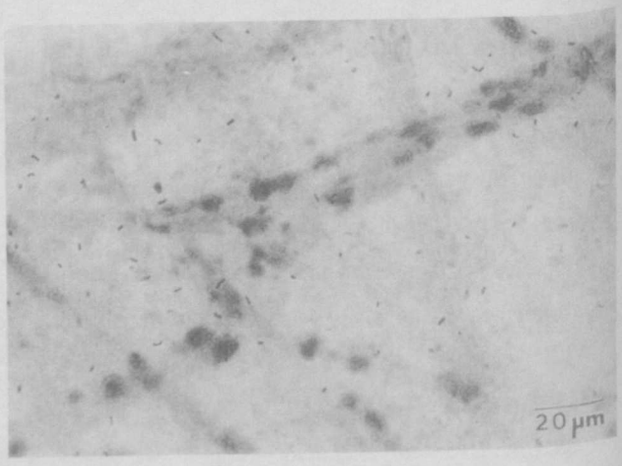
In adhesion experiments, the chambers were positioned to place the casing samples at the bottom, face up, and filled with the prepared bacterial suspensions. The suspensions were left in the chambers for 20 min during which time the bacterial cells sedimented onto the casings. The chambers were then rinsed with deionized water for 5 min at a flow rate of 110 $\mu L.s^{-1}$, corresponding to a shear stress of 0.05 $N.m^{-2}$ along the casings surface. The bacteria remaining on the casings (adherent) were stained with basic fuchsin and enumerated with a photon microscope. In separate experiments, the rate of sedimentation of the bacteria onto the chamber bottom (and therefore the casings) was evaluated from a series of photomicrographs.

Fine emulsion sausages were made in the meat processing plant of the Food Research and Development Centre (Saint Hyacinthe, Canada), using pilot-size industrial equipment and a standard commercial recipe. The sausages were stuffed in collagen or natural casings and cooked in a smokehouse to an internal temperature of 71°C. During cooking, the sausages were smoked with natural smoke produced by the wet smouldering of wood sawdust. After cooling, the sausages were peeled, the inside face of the casings was carefully cleaned of adhering meat particles, and the casings were mounted onto glass cover slips using transparent double-sided adhesive tape. The cooked casing samples were subsequently inserted into flow chambers and adhesion experiments were performed.

RESULTS AND DISCUSSION

The method described in this work has been successfully used to study bacterial adhesion to meat, with fat and tendon slices as model surfaces (Piette and Idziak, 1989, 1991). In fact, the method can be applied to other surfaces provided three conditions are met. Firstly, owing to the flow chamber geometry, the sample used must be flat and thin. Secondly, one must be able to mount the sample onto a glass cover slip for insertion into the flow chamber and the sample must adhere to glass strongly enough to stay in place while the chamber is filled with the bacterial suspension, rinsed, and emptied. Thirdly, the sample must be transparent so that its surface can be observed with a light microscope to detect adherent bacteria.

According to the criteria listed above, all casings selected for the present study were found to be suitable for use in the flow chambers. The casings were all thinner than 100 μm and could be fixed strongly to glass cover slips. Natural casing adhered to glass on its own, as was previously observed for fat and tendon slices (Piette and Idziak, 1989). Collagen casings were different in this respect since they did not adhere to glass. In this case, however, adhesion was achieved by use of double-sided adhesive tape. Once in place, all casings remained adherent to the supporting cover slips during the whole adhesion experiment. In addition, all three casings were transparent which resulted in surface adhering bacteria being clearly visible (Picture 1).



Picture 1: *B. thermosphacta* cells adhering to raw natural sheep casing.

Adherent bacteria were found on the surfaces of collagen casings prior to their introduction into the flow chambers (Table 1, controls). These bacteria amounted to less than 5% of the surface population after exposure of the casings to bacterial suspensions (Table 1), indicating that extensive adhesion of *B. thermosphacta* and *Lactobacillus* sp. occurred during the experiments. Both organisms also adhered to natural casing in large numbers (Table 1) but in that case the bacteria originally on the casing constituted a substantial portion (5.2-52%) of the final adherent population.

The results of several studies have shown that the presence of sodium chloride in the adhesion medium could increase or decrease the extent of bacterial adhesion to meat, although the mechanisms involved are not fully understood. In particular,

Table 1: Adhesion of *Brochothrix thermosphacta* and *Lactobacillus* sp. cells to the surfaces of collagen or natural casings

Organism	Number of bacteria (log_{10} CFU.cm^{-2})			
	Introduced in the chamber [1]	Adherent to pure collagen casing	Adherent to mixed collagen casing	Adherent to natural sheep casing
	A. Casings rehydrated in saline; bacteria suspended in water			
B. thermosphacta	6.32 ± 0.37 [2]	5.48 ± 0.12 A [3]	5.46 ± 0.09 A	nt [4]
Lactobacillus sp.	6.01 ± 0.10	4.49 ± 0.28 B	4.68 ± 0.25 B	nt
	B. Casings rehydrated in water; bacteria suspended in water			
None (controls)	none	2.74 ± 0.41	2.12 ± 0.17	3.85 ± 0.10
B. thermosphacta	6.48 ± 0.29	5.07 ± 0.34 A	5.06 ± 0.38 A	5.13 ± 0.08 A
Lactobacillus sp.	5.98 ± 0.09	4.08 ± 0.08 B	4.29 ± 0.07 B	4.55 ± 0.09 B
	6.58 ± 0.01	nt	5.01 ± 0.13 A	nt

[1]. Expressed as the number of cells poised over one unit area.
[2]. Means ± standard deviation (N ≥6).
[3]. Within the same treatment group (A, B, or C), different letters in the same column indicate significantly different adhesion ($P < 0.05$).
[4]. Not tested.

Pseudomonas fluorescens was found to adhere more to tendon slices in a 5 g.L^{-1} saline solution than in water (Piette and Idziak, 1991). In contrast, the adhesion of *Salmonella typhimurium* and *Salmonella singapore* to chicken muscle fascia (Thomas and McMeekin, 1981) and the adhesion of *Moraxella osloensis* to slices of tendon (Piette and Idziak, 1991) were sharply reduced in the presence of NaCl. In view of these results, the method selected to rehydrate the collagen casings (30 min in water or 5 min in a 10% saline solution; both methods recommended by the casing manufacturer) could possibly influence bacterial adhesion to the casing surface. Indeed, higher numbers of adherent bacteria were found on collagen casings rehydrated in saline than on casings rehydrated in water (Table 1). However, the difference was small (ca. 0.4 log_{10} CFU.cm^{-2}) indicating that the mode of rehydration of the casings is expected to have little practical consequence on subsequent bacterial contamination.

Several reports have suggested that *Lactobacillus* cells had a limited ability to adhere to meat surfaces. For exemple, *Lactobacillus* was found to adhere to pork skin in much lower numbers than *Pseudomonas putrefaciens* (Butler *et al.*, 1980). Also, the adhesion of *Lactobacillus* to model meat surfaces was much lower than the adhesion of *Acinetobacter* and *P. fluorescens*, when all three bacteria were suspended in their spent growth media (Piette and Idziak, 1989). It was later shown that the relative ability of various organisms to adhere to meat surfaces was greatly influenced by the composition of the suspending medium and the nature of the meat surface (Piette and Idziak, 1991). In the present study, the number of *Lactobacillus* cells adhering to casings was 0.58-0.99 log_{10} CFU.cm^{-2} lower than the number of adherent *B. thermosphacta* cells (Table 1). However, the number of *Lactobacillus* cells introduced in the flow chamber was also slightly lower than the number of *B. thermosphacta* cells introduced (0.31-0.50 log_{10} CFU.cm^{-2} difference). This difference resulted in less *Lactobacillus* (4.90 log_{10} CFU.cm^{-2}) than *B. thermosphacta* (5.67 log_{10} CFU.cm^{-2}) cells reaching the casing surface during the regular 20 min exposure time (Figure 1). When the population of *Lactobacillus* was increased to the same level as that of *B. thermosphacta*, similar numbers of cells of both organisms reached the casing surface and became adherent (Figure 1, Table 1). These results point to no substantial difference between the extents of adhesion of *B. thermosphacta* and *Lactobacillus* sp. to casings, under the experimental conditions selected.

The ultimate purpose of this study is to develop an efficient methodology to study bacterial contamination of cooked meat products prior to packaging. For practical reasons, raw casings were chosen to demonstrate the feasibility of using flow chambers and direct microscopic enumeration to study bacterial adhesion to the surface of sausages. Attempts were subsequently

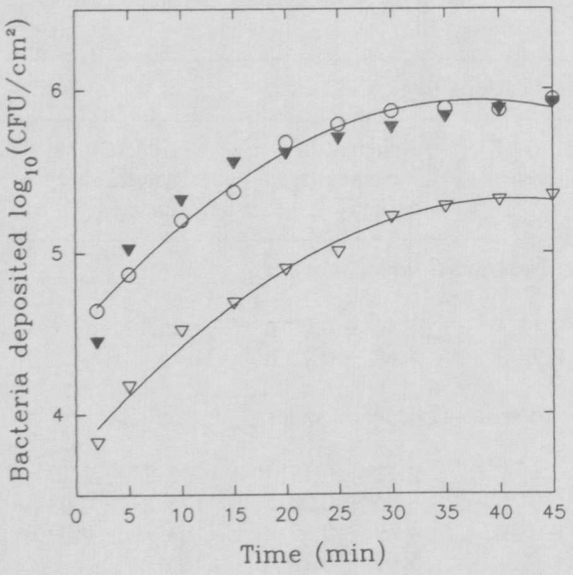
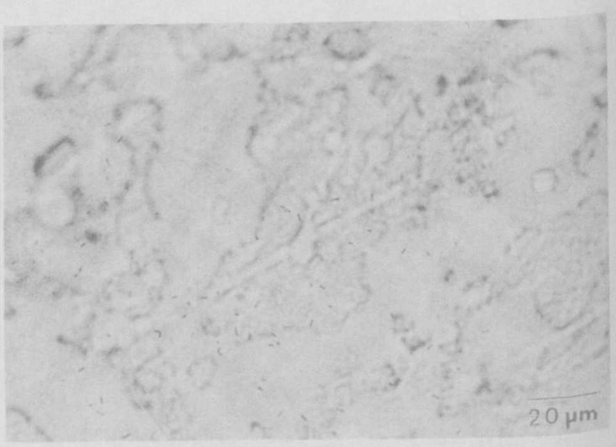


Figure 1: Deposition rates of *B. thermosphacta* (circles) and *Lactobacillus* sp. (triangles) cells on the bottom surface of the flow chamber. Points are the averages from two replicate experiments. Average populations introduced in the chamber (CFU poised over 1 cm^2 of surface) were: 1.4×10^6 (○); 7.0×10^5 (▽); 3.9×10^6 (▼).



Picture 2: *B. thermosphacta* cells adhering to natural sheep casing peeled off a cooked sausage. Note the alterations of the casing surface compared to Picture 1.

made to apply the method developed to pieces of casings peeled off cooked sausages. Preliminary results indicate that casings recovered from cooked sausages could be mounted onto glass cover slips, introduced into flow chambers, exposed to bacterial suspensions, and that the casings were still transparent enough to obtain sharp images of the adhering bacteria (Picture 2). However, the casing pieces frequently detached from the supporting cover slips during the experiments and better methods to mount the cooked casings onto glass must now be found before going further.

CONCLUSION

The combined use of specially designed flow chambers and light microscopy was found to be convenient and useful to study bacterial adhesion to pieces of raw casings. Preliminary results also indicated that, after minor improvements, the method will be applicable to pieces of casings peeled off cooked sausages. This will provide the means to study efficiently the pre-packaging contamination of cooked sausages by bacteria.

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