BACTERIAL ATTACHMENT ONTO MEAT AND MEAT RELATED SURFACES

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INTRODUCTION

Initial interaction between bacterial cell and surface, termed as adhesion or attachment, is a direct consequence of their physicochemical properties and eventually involves special structures on the cell and the binding surface sites. An understanding of how common spoilage bacteria attach to meat and related surfaces should provide a clue on preventing or minimizing deleterious bacterial actions. Meat offers a rich medium for sustaining microbial growth and since contamination is primarily a surface phenomenon, attachment to meat, processing equipment and other surfaces is fundamental to the meat industry (Selgas, et.al., 1993). In studying bacterial attachment, however, is important to consider the actual conditions in which the bacteria and the surface occur naturally and the method to study attachment as well. Moreover, controversial reports on scanning electron microscopy (SEM) specimens preparation are found. The aims of this study were to set up an optimal procedure to carry out specimen preparation for viewing by SEM and to visualize actual attachment structures on beef muscle, stainless steel, polyethylene and polyurethane sponge.

MATERIALS AND METHODS

Pieces of approximately 2 by 2 by 2 mm of sterile beef *longissimus dorsi* , stainless steel, polyethylene and polyurethane sponge were immersed into beakers containing the attachment medium composed by 10^7 CFU/ml of *Escherichia coli*, *Pseudomonas fluorescens* and *Lactobacillus casei* respectively. Bacteria were put in contact with the target surface - and consequently allowed to attach, at room temperature (22°C) up to 24 hs. after being immersed. Attachment was tested at 0, 60, 120 minutes and 24 hs. Each experiment (sample/microorganism) was tested separately and the attachment medium was prepared by suspending the target bacterium in a 0.85% aqueous NaCl saline solution. Bacterial inoculum and attachment medium: *E. coli* and *P. fluorescens* were grown in tryptic soy broth and incubated at 25°C for 24 hs., while *L. casei* was grown in Man Rogosa Sharpe broth and incubated at 25°C for 48 hs. Cultures were refrigerated centrifuged at 3,000 g at 4°C for 10 min and supernatants were decanted and suspended in 0.85% aqueous NaCl saline solution and centrifugated again under the same conditions. Immediately after centrifugation, supernatants were again decanted and the microorganisms were finally suspended in the 0.85% Nacl saline solution. Once the samples (specimens) had completed the defined attachment times, they were removed and placed in a rinsing saline solution. In this step samples were standarized manually rinsed for two minutes; it was assumed that microorganisms loosely attached were transferred to the saline solution and those not released into the saline solution were considered attached to the targeted surface. Immediately after, samples were transferred to sterile stomacher bags and stomached for 2 min. The stomaching procedure was done for all samples except for the stainless steel, this particular specimen was rinsed twice according to the aforementioned procedure. Sample preparation for SEM: Three different methods were evaluated. Procedure 1- Freeze drying technique: Samples were placed into a glass petri dish and filled with liquid nitrogen -this last operation was repeated three times to compensate for the loss of nitrogen. Then, the petri dish was transferred to freeze dryer operated at -40°C. Vacuum was turned on immediately after all nitrogen had been evaporated. Samples were kept about 18 hs. under these conditions, and then immediately viewed by SEM. Procedure 2- Critical point drying (CPD) A (Lu, T-W, 1987): This procedure involved 5 steps. Samples were first fixated in a 2.5% glutaraldehyde in a 0.5% Na Cl aqueous solution and incubated for 12 hs. at 4°C. Samples were rinsed ten times with a 0.5% Na Cl aqueous solution. Post-fixation, after rinsing specimens were immersed in either a 1% osmium tetroxide in a 0.5% Na Cl aqueous solution or in 2% osmium tetroxide in water and incubated for 12 hs. at 4°C. Then they were ten time rinsed as described before and fixed in 1% of thiocarbohydrazide in 0.5% Na Cl aqueous solution for 30 minutes. Finally, specimens were post-fixed in a 1% osmium tetroxide in a 0.5% Na Cl aqueous solution and incubated for 1 h. at 4°C and subsequently rinsed as described before. Dehydration, it was carried out by immersing the samples in a graded series of ethanol/distilled water for 30 min in each one of the series. Ethanol used was 10, 20, 30, 40, 50, 60, 70, 80, 95 -twice, and 100% -twice. CPD, specimens were treated with a grades series of amyl acetate/ethanol in a ratios of 1:3, 3:1 and 100% amyl acetate twice for 30 min each. A Denton Vacuum DCP-1 Point Dryer Unit and liquid carbon dioxide were used for critical point drying. Mounting and coating, each specimen was mounted on an aluminum SEM flat top mushroom shape stub with conducting silver paint and it was coated with an approximately 80A thickness of gold-palladium in a Denton Vacuum Desk II Cold Sputter Etch Unit. Specimens were kept in a dessicator under vacuum until being seen on SEM. Procedure 3- Critical point drying B (Modification of procedure A): Fixation, samples were fixed into glass vials containing 2.5% glutaraldehyde either in a 0.85% Na Cl aqueous solution or in a buffer pH 7.2 and incubated 12 hs at 4°C. After fixation samples were rinsed, each time for a period of 15 min, as described in Procedure 2. Dehydration, it was done as described in Procedure 2. During dehydration, samples were kept at 4 °C. CPD: It was carried out in a Denton Vacuum DCP-1 Point Dryer Unit using liquid carbon dioxide as a transient medium. Mounting and coating, each specimen was coated on an aluminum SEM stub with double adhesive tape. Specimens were coated and subsequently kept under vacuum as described in Procedure 2. SEM Unit Conditions: Specimens were viewed on either a Jeol 820 or Jeol 35CF Scanning Microscopes at 20 KV and 25KV current beam respectively.

RESULTS AND DISCUSSION

Bacterial attachment to meat and meat related surfaces has been reported on model systems basically using a bath with some kind of attachment medium in which the microorganisms are suspended. Target tissues are allowed to stay in contact with the organisms over a certain period of time. Those loosely adhered bacteria are removed by rinsing or shaking the sample, while those firmly attached are counted after being stomached or blended. Moreover, bacterial attachment has been studied by SEM and other sophisticated procedures such as surface energy values (Mafu et al., 1990, Zottola, 1994). They stated that attachment capabilities may involve the presence of extracellular material. Attachment structures on *E. coli* on different surfaces are shown on Figures 1, 2 and 3. In the current study, attachment fibrils were demonstrated in all samples particularly after 120 min contact between the bacterium and the target surface. *E. coli* and *P. fluorescens* showed more fibrils structures than *L. casei*, at each one of the contact times assayed. There were no apparent differences among attachment structures on different surfaces assayed. Fibrils were particularly evident on polyethylene and polyurethane sponge. Since polyurethane sponge has been

extensively used in sampling surfaces for microbiological analysis (Lasta, et al. 1992, Anonymous 1996), it is important to standarize sampling protocols in order to avoid erroneous interpretations (lower counts due to poor bacterial recovering from sponge) due attachment of bacteria to the sponge. The goal of setting up a SEM procedure which could result in less distortion and less artifacts on the specimen and therefore it could be the most lifelike obtainable, was achieved with Procedure 3 (See Materials and Methods). The freeze drying procedure, despite its relatively simple feature, produced specimens with many distortions (particularly in polyurethane sponge and polyethylene film). The modification performed in the Procedure 3, resulted in comparable SEM views as the original CPD procedure. The modified procedure showed two additional advantages, first it was less time consuming and second no hazard materials (ie. osmium tetroxide) are needed. Bacterial attachment and biofilm formation, enhance microorganisms resitence to removal and inactivation (Zottola, 1994). Therefore, it should be carefully considered when implementing cleaning and sanitizing programs in the food industry. SEM proved to show excellent data on bacterial attachment in materials extensively used in the food industry, such as stainless steel, which may appear "visually cleaned" after the usual cleaning procedures.

CONCLUSIONS

The modified CPD procedure allowed to demonstrate attachment structures in all bacteria studied and in all target surfaces when viewing by SEM. This procedure is less time consuming and no hazard materials need to be handled.

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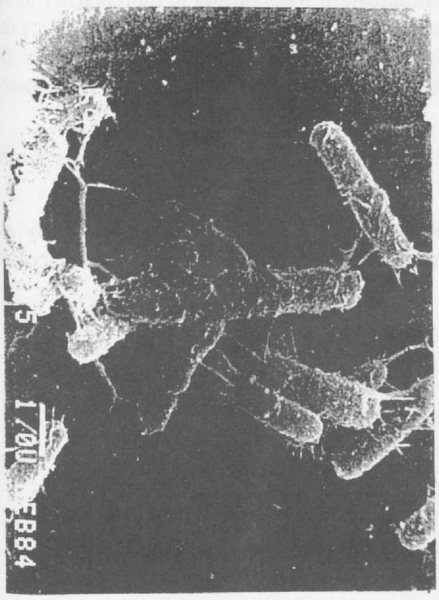

FIGURE 1
E. coli on polyethylene
60 min of contact time

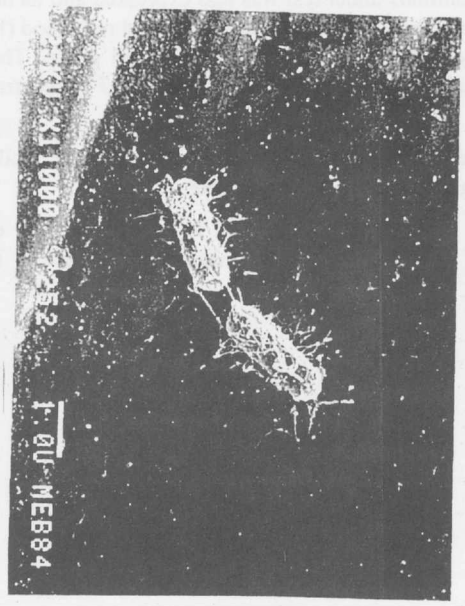

FIGURE 2
E. coli on stainless steel
60 min of contact time

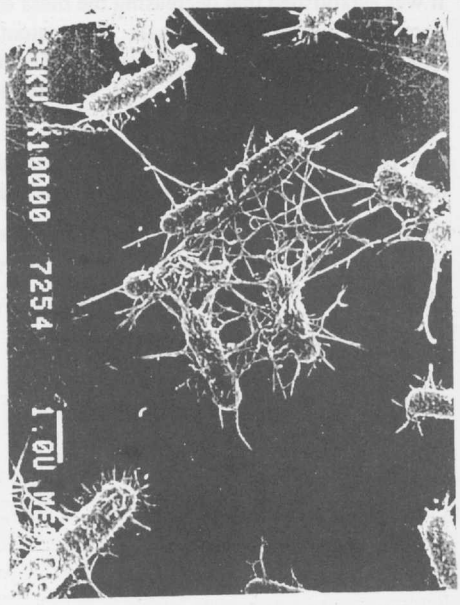

FIGURE 3
E. coli on polyurethane sponge
60 min of contact time