ATTACHMENT OF SPOILAGE BACTERIA TO BEEF MUSCLE TISSUE

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Summary

There were no differences ($p>0.05$) in the attachment of Pseudomonas fluorescens, Escherichia coli and Lactobacillus casei at 0 min. No differences were also found ($p>0.05$) in the percentage of attachment between P. fluorescens and L. casei for every attachment time tested. Results showed that P. fluorescens was the most readily attached and L. casei the least, while, E. coli was intermediate. The strain of B. thermosphacta used did not show attachment. SEM microphotographs showed well-defined attachment fibrils at 120 min.

Introduction

Microbial attachment is relevant to the meat industry since contamination and spoilage of meat are primarily a surface phenomena. Initial steps in microbial meat spoilage involve some sort of attraction between the microorganisms and the meat surface, which will eventually result in adhesion or attachment of the microbial cell to the meat surface. Bacterial attachment to meat surfaces has been considered from different angles. Rinsing procedures to liberate bacteria not firmly attached, has been used by Delaquis and McCurdy (1990) in the study of colonization of beef muscle by Pseudomonas. Most reports have used "bath immersion models" to demonstrate microbial attachment to animal tissues (Benedict, 1988).

Noterman and Kampelmacher (1974) have suggested that the attachment of several common spoilage organisms to broiler skin was dependent on the presence and functionality of the bacterial flagella. However, Thomas et al (1987) have reported that non-motile bacteria were retained by poultry skin as well as the motile types. Controversies on the attachment of bacteria to meat surfaces have also been stated by Benedict (1988). Early studies have reported, however, that the higher the initial population in the attachment medium the greater the adherence (Noterman and Kampelmacher, 1974). Nonetheless, it is difficult to compare the different reports because adherence to meat and meat related surfaces has been measured by different methods. The aim of this paper is to evaluate, under standardized conditions and at room temperature, the attachment of most common spoilage bacteria to sterile beef muscle tissue.

Materials and Methods

Beef muscle tissue: Beef animals were slaughtered at the OSU Meat Lab facility. Carcasses were aged for 48hs at 1°C and the rib area was removed by common deboning procedures. The coring technique described by Hone et al. (1977) was used to collect sterile longissimus dorsi (LD) muscle. A sterile LD sample was defined as having a bacterial count <1 CFU/g. Bacterial strains: Pseudomonas fluorescens (Pf), Lactobacillus casei (Lc) and Escherichia coli (Ec) were obtained from the OSU Department of Microbiology. Brochothrix thermosphacta (Bt) ATCC 11509 was obtained from the American Type Culture Collection. Pf and Ec were grown on Tryptic Soy Broth (Difco Lab.) and incubated at 25°C during 18-24hs. Bt was grown in Brain Heart Infusion (Difco Lab.) and incubated at 25°C under stirring (250 rpm) during 48hs. Lc was grown in MRS Broth (Beckton Dickinson Mic. Sys.) and incubated at 35°C for 48hs. Cultures were centrifuged (3,000 g at 4°C for 10min) and washed with saline solution (0.85% aqueous solution of NaCl) 2 times. Target bacterium was suspended in 20 ml of sterile saline solution given a concentration of approximately 10^7 CFU/ml (attachment medium). The surface spread plate method was used to enumerate the microorganisms. Tryptic Soy Agar (25°C-24hs) was used for the enumeration of Pf and Ec; MRS Agar (35°C-48hs) was used for Lc and Brain Heart Infusion Agar (25°C-48hs) was utilized for Bt. Attachment experiment: Pieces

of lean 1 by 1 by 1 cm sterile LD muscle were aseptically placed into 100 ml beakers containing the attachment medium and attachment was tested up to a period of 120 min at room temperature (ca. 22° C). Beginning at zero time and each 30 min one piece of meat was taken out and was aseptically transferred to a 250 ml beaker containing 20 ml of saline solution. The sample was thoroughly rinsed during 2 min and immediately after was transferred to a sterile stomacher bag containing 50 ml of 0.1% peptone water. The sample was homogenized in a Lab-Blender Stomacher 400 (Tekmar Co.) for 2 min. Aproppriated decimal dilutions (0.1% pepetone water) were made and bacteria enumerated. Each attachment experiment for each of the four target organisms was replicated 6 times with six individual animals. Percentage attachment: Transformations of CFU/ml in inoculum and CFU/cm² on muscle tissue to CFU/g were done to calculate percentage attachment (Rodríguez, 1990). Scanning electron microscope (SEM) studies: Specimens were viewed on a JEOL 820 Scanning Microscope (JSM-201, Japan Elec. Opt. Lab., Tokyo, Japan) at 20 kv beam current. Samples were prepared as described by Rodríguez (1990). Data Analysis: Bacterial counts were transformed to decimal log and analysis of variance was performed by using the SAS GLM procedure.

Results and discussion

Many factors affect bacterial attachment and some organisms are able to attach more readily than others (Firstenberg-Eden et al 1979). Furthermore, kinetics of attachment depend on individual specie and the meat surface as well. In this study, three of four species tested attached to the LD muscle surface instantly and one of them (Bt) did not show attachment. There were no differences ($p>0.05$) in the attachment of Pf, Ec and Lc at zero min. Pf readily attached to the LD muscle surface, and at 30 min and afterward its attachment was significantly higher than Lc (Fig. 1A). When considering the attachment rate over time, there were differences ($p<0.05$) in the rate of attachment of the three spoilage organisms between 0 and 30 min of attachment time. Ec and Lc also continued having a significant increase after the 30 min (Fig. 1B).

Despite that Farber and Idziak (1984) have reported that Brochothrix thermosphacta attach to meat surfaces, the strain collect used in our research did not. This can be explained considering that bacteria may loose their "adherent capabilities" when maintained for long periods on culture media. However, the non-attachment showed by Bt under the experimental conditions being tested should prove the significance of the attachment of the rest of the organisms and should validate the attachment experiment.

There were differences ($p<0.05$) in the percentage of attachment between Pf and Lc for every one of the considered attachment times. The data suggest that Pf was the most readily attached organism and Lc the least, while Ec was intermediate (Fig. 2A). There were no differences ($p>0.05$) after 0 min for the three organisms but all of them increased in percent attachment between 0 and 30 min (Fig. 2B). Butler et al (1979) have reported that most of the bacterial attachment to animal tissue surfaces occurred during the first minute of contact between sample and the attachment medium, and also reported that Pseudomonas accomplished a high rate of attachment. Adherence of this organism is particularly important because about 80% became attached at zero min and this bacterium is the main responsabile for the spoilage of meat stored under aerobic conditions.

Specimens showed well-defined fibrils particularly in cases of Pf and Lc at 120 min when viewed on SEM (Pictures 1 and 2). This result supports the theory of formation of extracellular materials on attached bacteria after short contact periods, and agrees also with the report of Mafu et al (1990) who found extracellular materials surrounding Listeria monocytogenes on glass surfaces after 1 h contact at 20° C.

Conclusions

Spoilage bacteria become attached to muscle surfaces almost instantly and this will have implications in meat hygiene and in meat shelf-life. Taking into account that under the

current slaughtering practices some sort of bacterial contamination should be expected, it seems to be appropriate to seek some way of preventing or blocking bacterial attachment.

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Figure 1

Attachment of spoilage microorganisms to beef muscle tissue

Graph A- Differences at each attachment time.

Points bearing the same letters at the same attachment time showed no significant differences (P>0.05).

Graph B- Attachment rate over time.

Points within individual bacterium attachment fate bearing the same letter showed no significant differences (P>0.05).

Pf= P. fluorescens, Ec= E. coli, Lc= L. casei

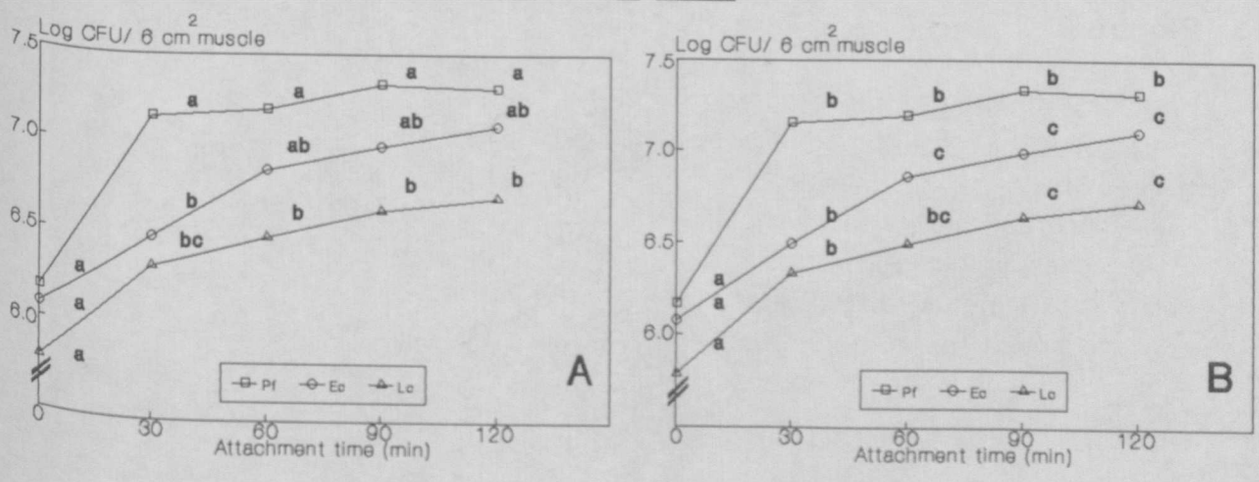


Figure 2

Percentage of microbial attachment to beef muscle tissue

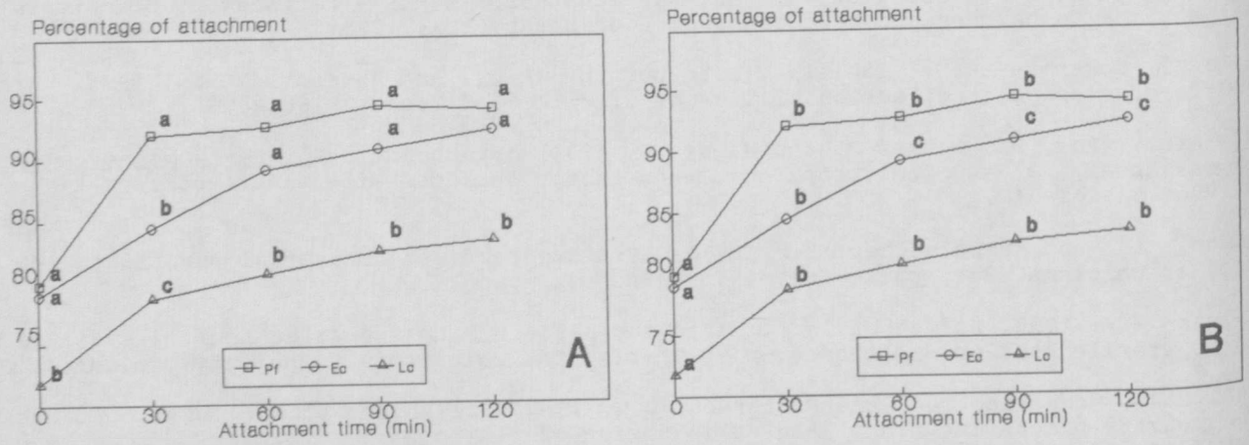
Graph A- Comparison among bacteria at each individual attachmet time

Points bearing the same letters at the same attachment time showed no signficant differences (P>0.05).

Graph B- Comparison among attachment times within each microorganism

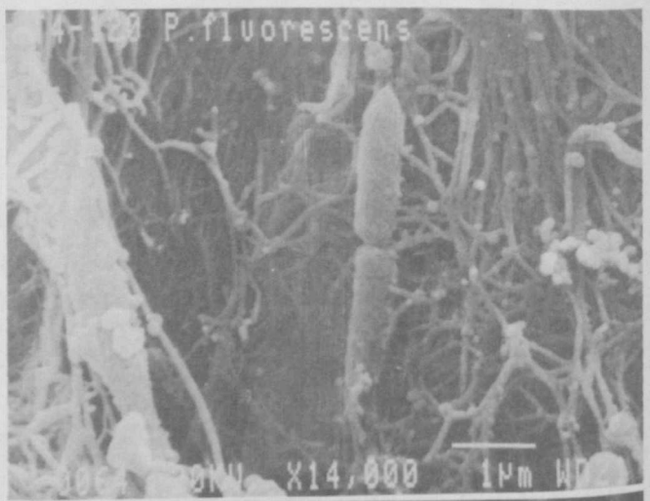
Points within individual microorganism attachment fate bearing the same letters showed no significant differences (P>0.05).

Pf: P. fluorescens, Ec: E. coli, Lc: L. casei



Picture 1

High magnification microphotograph of Pseudomonas flourescens on conective tissue fibers of LD muscle. One hundred and twenty min. of attachemnt time. Probable points of bacterial attachment and attachment fibrils are shown.



Picture 2

High magnification microphotograpg of Lactobacillus casei on conective tissue fibers of LD muscle. One hundred and twenty min. of attachemnt time. Probable points of bacterial attachment and attachment fibrils are shown.