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Penetration of Bacteria into Meat

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Bacteria are confined to the surface of meat during the logarithmic phase of growth. When proteolytic bacteria approach their maximum cell density, extracellular proteases secreted by the bacteria apparently break down the connective tissue between muscle fibers, allowing the bacteria to penetrate the meat. Nonproteolytic bacteria do not penetrate meat, even when grown in association with proteolytic species.

Although bacterial spoilage of meat at chill temperatures is generally regarded as a surface phenomenon, it has been reported that mesophilic bacteria can penetrate 10 to 15 cm into meat within 2 days, even at subzero temperatures (3). However, Thomas (7) observed that bacteria could not be detected at depths greater than 2 cm in normal meat held for 2 days at room temperature, whereas they were present throughout meat from animals that had been injected with papain before slaughter. As the extent to which bacteria invade the deeper levels of meat may be important for reasons of hygiene and understanding of the spoilage process, the factors affecting microbial penetration of meat were examined.

MATERIALS AND METHODS

Cultures. The bacteria used were *Escherichia coli* strain B, NCIB 9484; *Pseudomonas fluorescens*, NCIC 9053 and NCIC 8865; *Salmonella typhimurium* isolated from veal; and *Enterobacter* sp., *Microbacterium thermosphactum*, *Staphylococcus aureus*, and *Acinetobacter* sp., isolated from chilled mutton. All cultures were maintained on nutrient agar and grown in shake culture on Oxoid nutrient broth. Motility was determined by microscopic observation, and proteolytic activity was determined by hydrolysis of casein incorporated in nutrient agar plates.

Penetration of meat. *Sternomandibularis* muscles were removed from commercial bull carcasses immediately after slaughter. To prevent cold shortening, muscles were wrapped in plastic film, clamped at rest length, and stored at 15°C for 24 h and then at 2°C for a further 24 h to ensure completion of rigor. For shortened muscle, unwrapped meat was held for 48 h at 2°C (5). The muscles were then placed in a glove box, and formaldehyde vapor was passed through for 2 h to sterilize surfaces inside the box. The outer layer of muscle was removed, and 2-cm^3 blocks were cut from meat unaffected by formaldehyde. For experiments with uncut muscle surfaces, blocks were cut without prior treatment of the muscle with formaldehyde. Each muscle block was placed in the base of a sterile plastic petri dish over a hole 15 mm in diameter cut with a hot glass rod. The dish was placed inside a sterile lid, filled with 2% agar, and covered with a second lid. Bacteria were inoculated on the upper surface of the meat to give an initial cell density in excess of $10^7/cm^2$. After incubation, the bottom surface was swabbed and the swab was streaked on nutrient agar plates to determine whether penetration had occurred. Colonies from positive plates were further examined to confirm the identity of the bacteria on the uninoculated meat surface.

To determine the rate of penetration, strips of meat, 2 cm^2 in cross section and 25 cm in length, were embedded by immersing each strip in a trough of agar that was just beginning to set. This ensured that meat was not left exposed, because of contact with the trough base or walls, as the agar on the trough surfaces had already solidified. The solid agar was removed from one end of the meat with sterile instruments, and the trough was covered with a sterile lid before it was removed from the glove box. The exposed end was heavily inoculated with *S. typhimurium* scraped from a nutrient agar plate. For each determination, five strips of meat were inoculated at the same time. At hourly intervals, a strip was removed from the agar with sterile instruments, beginning at the uninoculated end after flaming the exposed agar surface. The strip was cut into 1-cm slices from the uninoculated end. Each slice was homogenized with 10 ml of peptone water, and 0.1-ml portions of the homogenates were spread on nutrient agar plates. Penetration was deemed to have commenced when a strip was found to contain bacteria at a depth of at least 2 cm. The rate of advance was calculated from the depths to which bacteria had penetrated in the remaining strips after further incubation.

Micrographs. Samples of meat were fixed in glutaraldehyde, stained with osmium tetroxide, and embedded in epoxy resin for sectioning (4). Sections were stained with toluidine blue and photographed with bright-field illumination, using an Orthoplan microscope (Leitz, Weltzar, West Germany).

RESULTS

Both motile and nonmotile proteolytic bacteria inoculated at $10^7/cm^2$ on the upper surface of

TABLE 1. *Motility, production of proteolytic enzymes, and ability to penetrate meat in eight strains of bacteria*

Species	Motility	Proteolysis	Penetration of meat
S. typhimurium	+	+	+
P. fluorescens NCIC 9053	+	+	+
Enterobacter sp.	+	+	+
Acinetobacter sp.	−	+	+
S. aureus	−	+	+
E. coli strain B	+	−	−
P. fluorescens NCIC 8865	+	−	−
M. thermosphactum	−	−	−

TABLE 2. *Time required for penetration of a 2-cm-thick block of meat after inoculation of one surface with bacteria at an initial cell density of $10^7/cm^2$*

Temp (°C)	Time required for penetration (h)	
	S. typhimurium	*P. fluorescens* NCIC 9053
37	16	NP (5)[a]
30	24	20
20	40	36
10	NP (14)	72
5	NP (14)	120

[a] NP, No penetration. Numbers in parentheses indicate days.

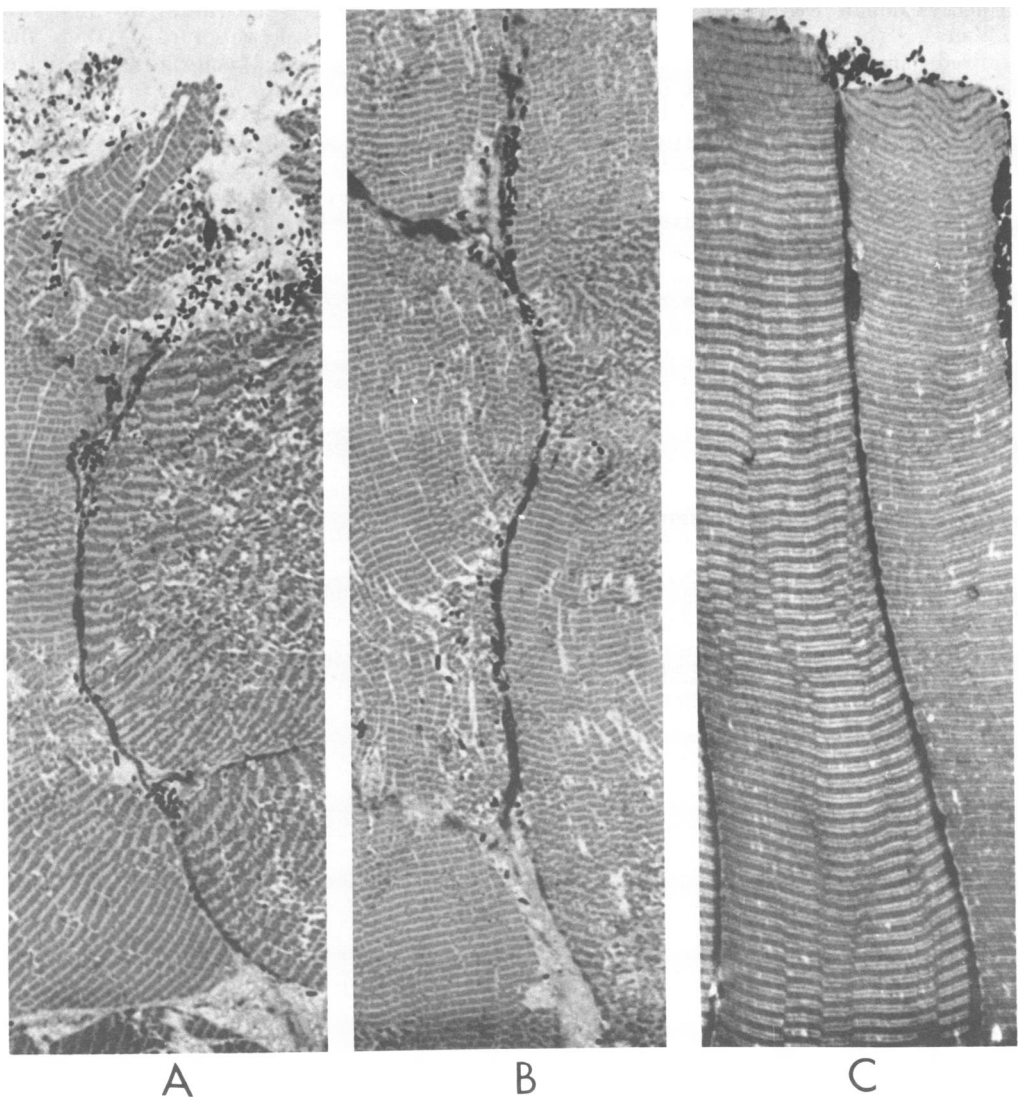

FIG. 1. *Sections vertical to the inoculated surface from 2-cm^3 blocks of meat inoculated on one surface with P. fluorescens, NCIC 9053 (proteolytic), or P. fluorescens, NCIC 8865 (non-proteolytic), at an initial cell density of $10^7/cm^2$ and incubated at 20°C for 4 days. (A) NCIC 9053, inoculated surface; (B) NCIC 9053, center of block; (C) NCIC 8865, inoculated surface. Direction of penetration was from top to bottom. ×1,600.*

2-cm^3 blocks of meat penetrated to the lower surface within 36 h at 30°C, but when inoculated at 10^3/cm^2, penetration did not occur until the bacteria had reached their maximum cell density at 3 days. Non-proteolytic bacteria had not penetrated after 7 days at 30°C (Table 1). The same results were obtained whether the inoculated surface was cut or uncut, the muscle was shortened or unshortened, and the muscle fibers were horizontal or vertical to the inoculated surface. When meat samples were inoculated with mixed cultures of a proteolytic and a non-proteolytic species, only the former bacterium could be isolated from the uninoculated surface, although both were present in similar numbers on the inoculated surface. The time required before penetration occurred was dependent upon the temperature of incubation, and penetration did not occur in the absence of bacterial growth (Table 2). The rate of advance of invading *S. typhimurium* was 10, 7, and 3 cm per h at 37, 30, and 20°C, respectively. The path of penetrating bacteria was determined by examination of sections from inoculated meat blocks that had been incubated at 20°C for 4 days. The proteolytic species were present between the muscle fibers throughout the meat, and some degradation of muscle fibers occurred. Non-proteolytic species did not penetrate beyond the uncut ends of the superficial muscle fibers (Fig. 1).

DISCUSSION

Penetration of meat by bacteria apparently results from the breakdown of the connective tissue between muscle fibers by proteolytic enzymes secreted by the bacteria. The endomysium is a delicate structure, whereas the protein of the muscle fibers is densely packed (1); therefore, the endomysium would be more rapidly disrupted by proteases, allowing invasion of bacteria by this route. Penetration of meat by nonmotile bacteria and the rapid rate of advance of invading microorganisms indicate that physical forces are involved in the movement of bacteria through meat. Non-proteolytic species do not invade in company with proteolytic species probably because, with mixed cultures, penetration originates in the area of growth of a microcolony of the proteolytic species so that the non-proteolytic bacteria are excluded. Protease production by bacteria does not occur until the end of logarithmic growth, when the meat is in an advanced stage of spoilage (2, 6). Therefore, unless the meat has been treated with a protease preparation to cause breakdown of the muscle structure (7), there should be no penetration of bacteria into organoleptically sound meat.

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