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Incidence of *Acinetobacter* spp. and Other Gram-Negative, Oxidase-Negative Bacteria in Fresh and Spoiled Ground Beef†

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A total of 1,409 gram-negative bacterial colonies were randomly selected from 19 samples of fresh and spoiled ground beef plated on six media. Only 137 (9.7%) were oxidase negative, and 20 (14.6%) of these were *Acinetobacter* spp., all of which were recovered from fresh meat samples. The importance of this group in both fresh and spoiled beef is less than is generally believed.

When fresh meats undergo open-air spoilage at refrigerator temperatures, the spoilage flora is almost exclusively gram negative, with *Pseudomonas* spp. being the single most dominant group. Among other gram-negative bacteria often found are psychrotrophic *Enterobacteriaceae* and *Acinetobacter-Moraxella* spp. The latter genera have been reported by numerous investigators to be a part of the normal flora of fresh meats (2). Because of the morphological, biochemical, and colonial similarities of these genera, many investigators do not separate them but designate them *Acinetobacter-Moraxella*, even though the former are oxidase negative and the latter are oxidase positive.

Before the publication of the 8th edition of *Bergey's Manual of Determinative Bacteriology* in 1974, the next most dominant bacteria in refrigerator-spoiled meats were regarded as being *Achromobacter* spp. Since this genus was not recognized in the 8th and 9th editions (8), the organisms which would have been so classified are now placed largely in the genera *Alcaligenes*, *Moraxella*, and *Acinetobacter*. While attempting to more fully characterize the flora of fresh and spoiled ground beef by use of currently used identification methods, we examined 19 samples of meats specifically for their content of *Acinetobacter* spp. and applied the API 20E system (Analytab Products, Plainview, N.Y.) to the species identification of all gram-negative, oxidase-negative isolates and used pseudo-palette plates (Micro-Palettes, Chicago, Ill.) for the further identification of gram-negative nonfermentative rods.

Beef samples were obtained from 10 different stores and consisted of ground round, ground chuck, and hamburger meat. Their overall microbial quality was assessed by use of the extract-release volume test (5). Each sample was homogenized by use of a Stomacher 400 and plated with the following media: plate count agar, violet red bile agar, eosin-methylene blue agar, MacConkey agar (all from Difco Laboratories, Detroit, Mich.), and the acinetobacter media described by Mandel et al. (9) and Holton (4). The fresh meats were allowed to spoil at 7°C until the extract-release volume was zero (10 to 14 days). The number of colonies randomly selected from all media plates was the square root of the mean count of duplicate plates.

The fresh samples had a mean extract-release volume of 30, which decreased to zero after spoilage at 7°C, whereas mean aerobic plate counts of fresh samples increased from 1.6×10^6 to 18×10^9 per g after spoilage (Table 1). From all samples, 1,409 colonies were selected and tested for oxidase reaction, and only 137 (9.7%) were oxidase negative. From fresh meat samples, 125 of 663 (18.9%) were oxidase negative, whereas from the same samples after spoilage, only 12 of 746 (1.6%) were oxidase negative, suggesting no significant role for this group under the conditions used. An organism that contributes significantly to spoilage would be expected to be present in high numbers well beyond incipient spoilage, and this is why the 10- to 14-day storage period was used.

The 137 oxidase-negative isolates were identified to 12 genera by use of the API 20E system and pseudo-palette plates, and the genera and species are noted in Table 2. Of the isolates, 84% were represented by the following four genera: *Serratia* (35.8%), *Enterobacter* (21%), *Acinetobacter* (14.6%), and *Providencia* (12%); 80% were *Enterobacteriaceae*, a finding in agreement with those of other investigators who used miniaturized identification systems (1, 10). Of the *Acinetobacter* isolates, 16 were *A. lwoffii* and 4 were *A. calcoaceticus*, and all were confirmed by the interspecies transformation method of Juni (6). All *Acinetobacter* spp. were recovered from fresh meats.

Regarding media for the recovery of *Acinetobacter*, 17 of the 20 isolates were recovered from plate count agar plates. Our inability to recover them on the media used by Mandel et al. (9) and Holton (4) was probably due to their low incidence relative to other gram-negative bacteria and to the presence of inhibitors in these media which in general prevent the recovery of low numbers of cells. Each of four control strains of *Acinetobacter* (ATCC 9036, 17904, 17909, and 23220) grew on all media from pure culture inocula.

Findings from this study suggest that the incidence of *Acinetobacter* spp. in fresh ground beef is considerably lower than is generally believed and that their role in spoilage is overstated. That *Acinetobacter-Moraxella* spp. contribute little to the spoilage process in a microbial flora dominated by pseudomonads has been noted previously (3). Although the gram-negative, nonpigmented, oxidase-positive, psychrotrophic coccobacilli found in meats are generally regarded as being *Moraxella*, findings by Juni and Heym (7) suggest that organisms of this type belong to yet another group, which these investigators referred to as achromobacters and which were shown to be unrelated to *Moraxella* and *Acinetobacter*. Although dominance of the spoilage flora of fresh refrigerated meats by pseudomonads is unquestioned, the next most important group is uncertain. Research is

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TABLE 1. Summary microbiological quality of the 19 samples, source of the 1,409 isolates tested, and number and percentage of oxidase-negative isolates

Parameters and media tested	Mean of 19 samples[a] of:		No. of isolates tested (no. oxidase negative) in:		Percent oxidase-negative isolates in	
	Fresh meat	Spoiled meat	Fresh meat	Spoiled meat	Fresh meat	Spoiled meat
Extract-release volume	30	0				
Plate count agar	1.60	18,000	213 (95)	257 (6)	45	2
Violet red bile agar	0.51	9.20	121 (18)	93 (1)	15	1
Eosin-methylene blue	2.60	22,000	46 (10)	60 (2)	22	3
MacConkey agar	0.04	13,000	32 (2)	93 (0)	6	0
Holton (4) medium	0.60	16,000	118 (0)	123 (0)	0	0
Mandel et al. (9) medium	0.85	14,000	133 (0)	120 (3)	0	3

[a] Except for extract-release volume values, bacterial counts $\times 10^6$/g.

TABLE 2. Identification of oxidase-negative meat isolates from fresh and spoiled beef on five media

Isolate	No. of oxidase-negative isolates on:									
	Plate count agar		Violet red bile agar		Eosin-methylene blue		MacConkey agar		Mandel et al. (9) medium	
	Fresh meat	Spoiled meat	Fresh meat	Spoiled meat	Fresh meat	Spoiled meat	Fresh meat	Spoiled meat	Fresh meat	Spoiled meat
A. lwoffii	14						2			
A. calcoaceticus	3		1							1
Serratia liquefaciens	20	3	9							
S. odorifera	11									
Serratia sp.	4		1							
Enterobacter agglomerans	15		2							
E. aerogenes					1					
E. cloacae	6		2							
Enterobacter sp.			1							2
Providencia stuartii	8	2		1	4	2				
Hafnia alvei	8		1							
Escherichia coli	2				3					
Pseudomonas cepacia	1	1								
Proteus vulgaris					1					
Citrobacter freundii	2									
Cedecea lapagei	11									
Yersinia enterocolitica			1							
Chromobacterium sp.					1					

under way to determine the incidence of oxidase-positive, coccobacillary types.

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