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Nature, Characteristics, and Proteolytic Properties of Beef Spoilage Bacteria At Low and High Temperatures

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As a preliminary step towards elucidating the basic mechanism by which meats undergo bacterial spoilage at refrigerator temperatures, it seemed desirable to characterize the bacterial flora of both fresh and low-temperature spoiled meats. Accordingly, five samples of fresh, retail ground beef wrapped in aluminum foil were allowed to undergo spoilage at 5 to 6 C. At the time of purchase, the five samples had an average ERV (extract-release volume) of 43, which decreased to an average of 4 upon the attainment of frank spoilage. Upon the plating of both fresh and spoiled meats by use of tryptone-glucose-extract agar and 30 C incubation, a total of 69 cultures were selected from all samples. Of this number, 50 were recovered from fresh beef and only 19 from spoiled beef. Employing conventional methods of identification, the 50 fresh-beef isolates represented nine genera; 34 of the isolates were *Pseudomonas* spp. (Table 1). Of the 19 spoiled-beef isolates, 16 were of this genus. Of the nine genera recovered from the beef while fresh, only four could be recovered from the same beef after spoilage: *Pseudomonas*, *Achromobacter*, *Aeromonas*, and *Flavobacterium*. When the duplicates among the 69 isolates were eliminated, 34 strains resulted (22 from fresh beef and 12 from spoiled), with pseudomonads, representing 19, or 56%. Of these 19 strains, 10 were fluorescent under black-light, and all showed polar flagella.

Table 2 presents biochemical characterizations of the fresh- and spoiled-beef strains. All 12 strains from spoiled beef were gram-negative rods that fermented glucose and grew well at 5 C. It can be noted further that fewer of these strains produced acid reactions in litmus milk than those from fresh beef, whereas considerably more produced acetylmethylcarbinol (Voges-Proskauer positive).

The capacity of 20 of the 34 strains to degrade proteins at 5 and 30 C is shown in Table 3. More proteolysis occurred at the lower temperature than at the higher. With respect to gelatin reactions, some of these strains liquefied nutrient but not Frazier's gelatin; others were effective at 30 and not at 5 C and vice versa. When both incubation temperatures were employed along with both gelatin sources, all 20 strains were gelatinase-positive under at least one set of the four conditions. In regard to beef proteins, all except one of the strains that degraded at 30 C digested litmus milk at the same temperature. There was poor correlation between gelatin reactions and beef proteins, and between gelatin and litmus milk digestion. In addition, when the 34 strains were inoculated into purified and filter-sterilized beef actomyosin in *p*H 5.8 buffer and incubated at 5 C for 14 days, average percentage of breakdown by the 33 that grew was 58, with a range of 0 to 82. The 19 strains of *Pseudomonas* effected an average breakdown of 61%, with 8 of the 12 most effective strains belonging to this genus. Actomyosin breakdown was determined by optical-density measurements at 2,800 A. Determinations were made with a Beckman DB-G spectrophotometer after the bacteria had been removed by filtration.

These findings confirm the reports of A. D. Brown and J. F. Weidemann (J. Appl. Bact. **21**:11, 1958), J. C. Ayres (Food Res. **25**:1, 1960),

TABLE 1. *Generic distribution of isolates between fresh and spoiled beef, and the number of strains of each genus*

Genus	No. of beef isolates			No. of strains
	Total	Fresh	Spoiled	
Pseudomonas	50	34	16	19
Achromobacter	5	4	1	3
Aeromonas	3	2	1	3
Proteus	3	3	0	2
Flavobacterium	2	1	1	2
Alcaligenes	2	2	0	2
Sarcina	2	2	0	1
Streptococcus	1	1	0	1
Corynebacterium	1	1	0	1
Total	69	50	19	34

TABLE 2. *Summary of biochemical and other characteristics of the 22 strains from fresh and the 12 strains from spoiled beef given as percentage of totals*[a]

Source	Total no.	Gram-negative	Rods	Off-white pigmentation	Gelatinase	Litmus milk			Hydrolysis of beef protein	Glucose	Sucrose	MR-positive	VP-positive	Nitrate-positive	Motile	Amylase-positive	Growth at 5 C	Fluorescent
						NC	A and ACo	Digestion										
Fresh.....	22	86	91	86	59	55	18	23	61	64	18	36	5	36	73	5	91	23
Spoiled	12	100	100	92	67	58	8	33	58	100	50	25	33	33	75	0	100	33

[a] NC = no change; A = acid; Co = coagulation; MR = methyl red; VP = Voges-Proskauer.

TABLE 3. *Digestion of gelatin, beef proteins, and casein of litmus milk by 20 strains at 5 and 30 C for 7 days, given as percentage of total tested*

Temp (C)	Frazier's gelatin	Beef proteins[a]	Digestion of litmus milk
5	83	80	55
30	70	55	50

[a] Filter-sterilized, distilled water extracts of fresh beef in *p*H 5.8 buffer.

and others that *Pseudomonas* strains constitute the predominant group among fresh meat spoilage bacteria. The finding of more proteolysis at 5 than 30 C also tends to confirm the report of J. A. Alford (J. Bacteriol. **79**:591, 1960), who found that more strains of *Pseudomonas* and *Achromobacter* liquefied gelatin and digested litmus milk at 20 C than at higher temperatures.

Overall, it would appear from the above that bacteria which effected low-temperature beef spoilage were somewhat more proteolytic, and more produced alkalizing reactions in litmus milk at 5 than at 30 C. The extent to which the primary proteins of beef are attacked by these bacteria in the presence of the many low-molecular-weight compounds normally present in fresh beef is minimal at most (J. M. Jay, p. 387, E. J. Briskey et al. [ed.], *The Physiology and Biochemistry of Muscle as a Food*, University of Wisconsin Press, Madison, 1966), and the possibility exists that their ultimate breakdown is caused by beef cathepsins which are released by bacterial actions. The existence of nonproteolytic strains in spoiled beef may be taken to indicate that nutrients other than the primary proteins are utilized by the spoilage flora. Data confirming the latter have already been reported (J. M. Jay and K. S. Kontou, Appl. Microbiol. **15**:759, 1967).

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