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Spoilage Association of Chicken Breast Muscle

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The ability of pure cultures of bacteria isolated from spoiling chicken breast muscle to produce strong off-odors was tested by using sterile breast muscle sections. The incidence of organisms capable of producing strong off-odors and changes in flora during storage of naturally spoiling muscle at 2 C was traced, and the relationship between bacterial type and off-odor production was noted.

Studies with sterile muscle sections allow assessment of the ability of pure bacterial cultures to produce off-odors associated with spoilage. Studies have been described recently in relation to the spoilage of porcine muscle (4) and fish muscle (5). The work described traces the flora of chicken breast muscle during low-temperature storage and makes use of sterile sections to characterize organisms capable of producing strong off-odors on this substrate.

MATERIALS AND METHODS

Origin and isolation of strains. The breast skin of chilled, eviscerated chicken was removed, and the underlying muscles (pectoral proper and supra coracoid) were excised in approximately 5-g portions. These were stored in sterile petri dishes at 2 C. The muscle was not homogenized in order to preserve the cellular and physical integrity of the substrate.

Three portions (15 g total) were selected at random at each sampling time (0, 4, 8, 12, and 16 days). Samples were homogenized in 135 ml of saline peptone diluent by using a Colworth Stomacher (A. J. Seward and Co. Ltd., London). Serial dilutions were prepared and plated in nutrient agar (Lab-Lemco [Oxoid L20], 1.0% [wt/vol]; peptone [Oxoid L37], 1.0% [wt/vol]; NaCl, 0.5% [wt/vol]). Counts per gram of muscle were recorded at each sampling time, and all colonies on an appropriate dilution plate (i.e., one showing about 50 colonies) were removed and purified on nutrient agar. The 250 strains isolated were maintained on agar slopes (as for nutrient agar with Lab-Lemco and peptone reduced to 0.2% [wt/vol]).

Production of off-odors on chicken breast muscle. A modification of the technique described by Gardner and Carson (4) was used to excise sterile sections (about 2 g) from chicken breast muscle. The muscle was not roasted with a bunsen burner since this would have cooked into the depth of the tissue. Sections were stored in sterile screw-capped bottles.

A number of checks for sterility were employed. (i) Sections were stored at refrigeration temperatures for at least 14 days before use and examined visually and olfactorily for growth before use. (ii) To provide an index of the efficiency of excision of each batch of sections, 10% were selected at random as sterility controls; each of these was incubated at 22 C after addition of 5-ml amounts of nutrient broth. After incubation of the pure culture inoculum on the muscle section, a streak plate was prepared and colonial purity was checked.

The 250 cultures isolated from naturally contaminated chicken breast muscle stored at 2 C were grown in nutrient broth for 48 h at 22 C, harvested by centrifugation (4,000 × *g* for 20 min), and washed twice in sterile phosphate buffer (0.1 M, pH 6.8) to give a final concentration of approximately 5×10^4 cells/ml. Portions of washed-cell suspensions (0.1 ml) were inoculated onto muscle sections to give approximately 2.5×10^3 cells/g and incubated at 2 C for 14 days. Sensory examinations were carried out at 7 and 14 days.

Analysis of head space vapors. An objective description of the odors produced by inoculation of the sterile muscle sections was provided by gas chromatographic analysis of the head space vapors. The instrument was a Phillips PV 4000 series gas chromatograph fitted with a Tenax GC column (Field Instruments Co. Ltd., Tetrapak House, Richmond, Surrey) 3 m in length with an internal diameter of 2 mm. The gas (oxygen-free nitrogen) flow rate was 20 ml/min, the injection port temperature was 240 C, and the oven temperature was 200 C.

Characterization of isolates. The scheme of Shewan et al. (11) was used to identify the isolates. Eighteen-hour nutrient agar slope cultures were Gram stained (12), and motility was examined by the hanging-drop technique. Motile cultures were stained for flagella by the Liefson technique as cited by Norris and Swain (10). Oxidase reaction (8), mode of attack on carbohydrates (6), and fluorescent-pigment production (7) were also examined.

RESULTS

Numbers and incidence of different bacteria during spoilage of naturally contaminated muscle. Counts on nutrient agar at each sampling time are as follows. On days 0, 4, 8, 12 and 16 of storage, 1.97×10^3, 6.80×10^5, $3.20 \times$

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10^8, 1.25×10^9, and 1.44×10^9 organisms were recovered, respectively. All of the 250 strains isolated from naturally contaminated muscle stored at 2 C were gram-negative, motile rods which included representatives of *Pseudomonas* groups I, II, and IV (11) and an enteric type. The salient features of each group are shown in Table 1. Table 2 illustrates the distribution of these types (as a percentage of the flora) during the period of storage. The initial flora consisted almost exclusively of *Pseudomonas* group I organisms. The proportion of these decreased steadily with time, being replaced by *Pseudomonas* type II. Although the proportion of *Pseudomonas* group I strains fell consistently during storage, the actual numbers increased until the 12-day stage.

Pseudomonas group IV organisms were also recovered as a small fraction of the flora up to 8 days, but not after 12 or 16 days of storage. These types, however, should not be discounted as insignificant; in terms of numbers, group IV types represented 2% of 3.2×10^8 organisms per g at the 8-day stage, i.e., a count of 6.4×10^6/g. A number of enteric organisms were also recovered after 8 days (10%) and 12 days (2%).

Incidence of off-odor producers. The incidence of organisms producing strong off-odors when inoculated onto sterile breast muscle sections is shown in Table 3. Initially, 15% of the isolates produced strong odors, and this increased steadily to the 16-day stage when 45 of the 56 isolates (80%) were in this category.

Within both major groups there appears to be a progressive selection for organisms able to produce strong off-odors (Table 3).

Types of off-odor produced. Three distinctive types of odor were recognized. These were described as "sulfide-like, fruity, and evaporated milk." Because of the difficulties in interpretation of subjective descriptions of odors, an objective analysis of the smells was provided by gas chromatography (Table 4). It is interesting to note that all of the peaks produced by the sulfide-like samples were also present in the fruity samples—the former showed a number of additional peaks.

Sulfide-like odors rose to a peak (22% of samples) with the strains isolated after 8 days of storage, fruity types remained uniformly low throughout, and strains giving the evaporated-milk odor increased steadily during storage, but particularly rapidly between the 12- and 16-day stages.

Relationship between taxonomic position and off-odor produced. Within each of the *Pseudomonas* groups were found strains that caused no detectable off-odor and each of the three types of odor described above. Expressed as an overall percentage, a greater proportion of *Pseudomonas* type II (73%) caused strong off-odors than *Pseudomonas* type I (22%). This, however, may simply be a reflection of the selection for *Pseudomonas* type II during the period of storage. At the point where a general off-odor was first detectable on the naturally spoiling sample (8 days at 2 C), *Pseudomonas*

TABLE 2. *Distribution of Pseudomonas type I and Pseudomonas type II during storage at 2 C*

Days	% of Population	
	Pseudomonas I	*Pseudomonas* II
0	95	2
4	89	6
8	56	32
12	43	55
16	4	96

TABLE 3. *Incidence and distribution of off-odor producers during storage at 2 C*

Days	% Off-odor producers		
	All strains	*Pseudomonas* type I	*Pseudomonas* type II
0	15	14	[a]
4	26	17	[a]
8	46	33	47
12	60	43	75
16	80	[a]	83

[a] Percentage not expressed for small numbers of strains.

TABLE 1. *Characteristics of gram-negative motile rods isolated*

Group	Oxidase reaction	Flagellation	Fluorescent pigment	Reaction in HL medium[a]
Pseudomonas group I	+	Polar	+	Oxidative
Pseudomonas group II	+	Polar	–	Oxidative
Pseudomonas group IV	+	Polar	–	No change
Enteric	–	Peritrichous	–	Fermentative

[a] Hugh and Liefson medium (glucose).

TABLE 4. *Gas chromatographic results for representative strains*

Strain and odor	Retention time (s)	Peak
Pseudomonas group II	75	969
Strain 91	98	1,778
"Sulfide-like"	124	244
	140	544
	160	114
	206	677
	251	36
Pseudomonas group II	48	3,002
Strain 136	65	2,247
"Evaporated milk"	76	389
	83	1,051
	94	1,124
	112	509
	138	1,967
Pseudomonas group II	75	824
Strain 160	96	1,006
"Fruity"	124	563
	139	143
	206	695

type I represented 56% of the flora (1.79×10^8/g), and *Pseudomonas* type II represented 32% of the flora (1.02×10^8/g). Thirty-three percent of the *Pseudomonas* I strains isolated at 8 days produced strong off-odors when inoculated onto sterile muscle sections. The corresponding figure for *Pseudomonas* II 8-day isolates was 47%.

The predominant odor produced by the *Pseudomonas* II organisms was the evaporated-milk type. This was particularly evident with those strains isolated during the latter part of the storage period. Forty-one of the 54 *Pseudomonas* II strains isolated after 16 days of storage at 2 C produced this odor when inoculated onto sterile muscle sections.

All of the enteric types produced strong off-odors, whereas only sulfide-like odors were recorded for *Pseudomonas* type IV. (The number of representative strains of the latter two groups was low.)

DISCUSSION

It is generally accepted that off-odors and other changes associated with the spoilage of poultry meat stored at chill temperatures are caused by the growth of a few groups of psychrophilic bacteria (2, 3). In this study, inoculation of certain strains onto sterile breast muscle sections produced strong off-odors which may be indicative of the ability of these strains to contribute to the spoilage of naturally contaminated muscle.

Adams et al. (1) traced the incidence of spoilers during the course of spoilage of fish press juice. These normally remained less than 20% of the population. Similarly, Herbert et al. (5) showed that, although organisms capable of causing strong off-odors on cod muscle increased considerably in numbers during chill storage, they never accounted for more than 10 to 20% of the total flora. This is not the situation with chicken breast muscle, where there is a consistent increase in the fraction of the population able to cause strong off-odors. Indeed, it appears that within both numerically dominant groups there is a selection for these types as storage progresses.

The preponderance of *Pseudomonas* group I and II strains on chicken breast muscle and the faster growth of the *Pseudomonas* type II agree with the results of Barnes and Impey (3). These workers inoculated breast and leg muscle with a mixture of *Pseudomonas* I and II, *P. putrefaciens*, and *Acinetobacter* strains in proportions similar to those expected on a carcass immediately after processing. *P. putrefaciens* and *Acinetobacter* types were not recovered from breast samples after 4 days at 1 C, although both increased considerably on leg samples. The nonpigmented pseudomonad, although initially present as only 2% of the flora, rapidly became predominant on breast samples. The differential effect on the growth of the various types was attributed to the influence of the relatively low pH of chicken breast muscle (5.7 to 5.9). No *P. putrefaciens* or *Acinetobacter* strains were recovered in this study.

Lerke et al. (9) characterized the spoilers of sole press juice and found an extremely close correlation between taxonomic position and spoilage ability. Herbert et al. (5) linked fruity odors to *P. fragi* (group II) and sulfide-like odors to *P. putida* (group I) and III/IV types including *P. putrefaciens*. The results above indicate that off-odor producers and strains that cause no detectable organoleptic change on sterile chicken breast muscle occur within the same taxonomic group.

It may be concluded that the flora of naturally spoiling chicken breast muscle at 2 C is dominated by *Pseudomonas* groups I and II, with *Pseudomonas* IV and an enteric type also present. The *Pseudomonas* II strains are most favored by the conditions and increase rapidly to become the major portion of the flora. During spoilage there is a selection for types able to produce strong off-odors when inoculated onto sterile muscle sections.

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LITERATURE CITED

1. Adams, R., L. Farber, and P. Lerke. 1964. Bacteriology of spoilage of fish muscle. II. Incidence of spoilers during spoilage. Appl. Microbiol. **12**:277-279.
2. Ayres, J. C. 1960. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. J. Appl. Bacteriol. **23**:471-486.
3. Barnes, E. M., and C. S. Impey. 1968. Psychrophilic spoilage bacteria of poultry. J. Appl. Bacteriol. **31**:97-107.
4. Gardner, G. A., and A. W. Carson. 1967. Relationship between carbon dioxide production and growth of pure strains of bacteria on porcine muscle. J. Appl. Bacteriol. **30**:500-510.
5. Herbert, R. A., M. S. Hendrie, D. M. Gibson, and J. M. Shewan. 1971. Bacteria active in the spoilage of certain seafoods. J. Appl. Bacteriol. **34**:41-50.
6. Hugh, R., and E. Liefson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. **66**:24-26.
7. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. **44**:301-307.
8. Kovacs, N. 1957. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) **178**:703.
9. Lerke, P., R. Adams, and L. Farber. 1965. Bacteriology of spoilage of fish muscle. III. Characterisation of spoilers. Appl. Microbiol. **13**:625-630.
10. Norris, J. R., and H. Swain. 1971. Staining bacteria, p. 106-134. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 5A. Academic Press Inc., London.
11. Shewan, J. M., G. Hobbs, and W. Hodgkiss. 1960. A determinative scheme for the identification of certain genera of bacteria with special reference to the *Pseudomonadaceae*. J. Appl. Bacteriol. **23**:379-390.
12. Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Book Co. Inc., London.