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Spoilage Association of Chicken Skin

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The bacterial succession on the skin of broiler chicken carcasses stored at 2°C was traced, and the ability of representative isolates to produce off-odors was determined by using sterile leg and breast muscle sections. Off-odors were identified by olfactory and chemical means. The inability of peptone-iron agar to detect many sulfide-producing strains was noted.

McMeekin (6, 7) has previously examined the spoilage associations of chicken leg and breast muscle. In this study similar methods were used to examine the spoilage association of chicken skin. Because of the inability to obtain sterile chicken skin, the spoilage potential of isolates from skin was assessed by inoculation onto sterile leg and breast muscle.

MATERIALS AND METHODS

Origin and isolation of bacterial strains. Five chicken carcasses taken from the end of an immersion chiller were placed on trays inside inflated polyethylene bags and stored at 2°C for 16 days. (The conditions of storage were arranged to provide an aerobic environment of high a_w similar to that found during commercial storage.) Skin samples were taken at 0, 4, 8, 12, and 16 days of storage (16 cm^2 of breast and leg skin) and immediately homogenized in 0.9% (wt/vol) saline with a Colworth Stomacher (A. J. Seward and Co. Ltd., London). Samples of a dilution series prepared from the macerate were plated on nutrient agar and peptone-iron agar and incubated at 22°C for 2 days and at 2°C for 14 days. For each sampling time, colony counts per 16 cm^2 of skin were recorded, and 50 colonies were selected at random and grown on nutrient agar. A total of 250 pure cultures were isolated and maintained on nutrient agar slopes at 2°C.

Characterization of isolates. Isolates were identified by using the determinative scheme of Shewan et al. (14), which required the determination of Gram reaction, morphology, motility, flagellation, oxidase reaction, mode of attack on glucose, and pigment production. Strains identified as *Acinetobacter/Moraxella*-like were previously termed *Achromobacter*.

Psychrophilic growth. Isolates were streaked onto nutrient agar and incubated at 2°C for 14 days. All isolates forming colonies within this period were deemed to be psychrophilic (12).

Detection of off-odor-producing isolates. Isolates were assessed for spoilage potential by their ability to produce off-odors from leg and breast muscle sections. Sterile sections of muscle were excised (7), inoculated with washed cell suspensions of each isolate ($\simeq 10^4$ cells per g), and incubated at 2°C for 14 days. Olfactory and visual examinations were made after 7 and 14 days.

Production of volatile sulfides by off-odor producers. The ability of spoilage bacteria to produce sulfide volatiles when grown in media supplemented with cysteine and/or methionine was examined. All cultures capable of producing off-odors on sterile muscle sections were grown on nutrient agar at 22°C for 48 h and inoculated into these media. Methanethiol production was tested by using a modification of the method of Sharpe et al. (13). A loopful of each nutrient agar culture was suspended in 1 ml of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.0) and incubated with 4 ml of filter-sterilized (12.5 mM) L-methionine and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) for 4 h at 22°C. Controls were prepared by replacement of methionine and cell suspensions by an equivalent amount of Ringer solution. The production of a yellow coloration indicated the presence of methanethiol. Hydrogen sulfide was detected by reaction with lead acetate above nutrient broth supplemented with 0.1% (wt/vol) L-cysteine hydrochloride. Several isolates which failed to produce off-odors on muscle were also tested for H_2S and CH_3SH production.

RESULTS

Bacterial flora on the skin of poultry carcasses stored at 2°C. The incidence of bacteria on the skin of poultry carcasses stored at 2°C is shown in Table 1. During the test period, estimated numbers of bacteria increased from 10^4 to 10^{10} cells per 16 cm^2 of skin. Similar numbers of organisms were recovered on nutrient agar and peptone-iron agar, but the latter failed to detect the presence of any H_2S-producing bacteria as described by Levin (5). The difference in numbers recovered at 22°C and at 2°C at each sampling time is reflected in the selection for psychrophilic bacteria, which rose during storage from 18 to 100% of the flora after 16 days at 2°C (Table 1).

The types of bacteria recovered are recorded in Table 2. A variety of genera were represented during the first 4 days of storage, with micrococci (autochthonous skin flora) and flavobacteria predominant. However, representatives of these genera were not recovered after 8 days at 2°C. After 10 days, off-odors and the appearance of

sliminess were evident, indicating spoilage of the carcass, and flora analyses at 12 and 16 days indicated a flora dominated by *Pseudomonas* group I and group II types. *Acinetobacter/Moraxella*-like organisms, *Pseudomonas* group III/IV, and enteric types were consistently recovered.

Production of off-odors on muscle sections. Numbers of bacteria capable of producing off-odors when inoculated onto sterile muscle sections increased from 2 to 16% of the total flora at 16 days of storage (or 11 to 16% of the psychorophilic flora) (Table 2). Off-odors produced by various genera are recorded in Table 3.

Production of volatile sulfides. A number of isolates were shown to produce volatile sulfides when grown in cysteine- and/or methionine-supplemented media. Hydrogen sulfide was produced from cysteine, and methanethiol was produced from methionine. All isolates which produced sulfide-like off-odors on muscle sections also produced H_2S and methanethiol, whereas other strains gave variable results (Table 3).

DISCUSSION

Before processing, chicken skin may carry loads of up to 10^6 organisms per 16 cm^2, most of which are gram-positive cocci (10). During proc-

TABLE 1. *Bacterial numbers and percentage of psychrophiles on chicken skin during storage at 2°C*

No. of days	No./16 cm^2 of skin after incubation for:				Psychrophiles (%)
	2 days at 22°C		14 days at 2°C		
	Nutrient agar	Peptone-iron agar	Nutrient agar	Peptone-iron agar	
0	3.5×10^{4a}	3.8×10^4	6.4×10^3	6.3×10^3	18
4	7.0×10^5	8.3×10^5	3.6×10^5	4.1×10^5	54
8	1.8×10^7	1.4×10^7	1.5×10^7	1.4×10^7	88
12	2.3×10^9	1.5×10^9	2.0×10^9	1.4×10^9	94
16	1.7×10^{10}	2.0×10^{10}	1.7×10^{10}	1.8×10^{10}	100

[a] Data represent mean count of duplicate samples.

TABLE 2. *Distribution of flora during storage at 2°C*

No. of days	% of population								
	Micrococci	Enteric types	*Flavobacterium/Cytophaga*	*Acinetobacter/Moraxella*-like	*Pseudomonas* group I	*Pseudomonas* group II	*Pseudomonas* group III/IV	Others	Off-odor producers
0	30	8	18	4	2	4	20	14	2 (11)[a]
4	10	8	16	4	10	18	16	18	6 (12)
8		2		2	28	60	8		8 (9)
12		2		2	30	62	4		12 (13)
16		2		2	28	60	8		16 (16)

[a] Numbers within parentheses indicate percentages of psychrophilic population.

TABLE 3. *Types of off-odors produced on muscle sections and volatile sulfide production by isolates*

Bacterial type	No. of strains	Type of off-odor produced on muscle substrate		H_2S production[a]	Methanethiol production[b]
		Breast	Leg		
Enteric type	1	Sulfide-like	Sulfide-like	+	+
Enteric types	3	Nil	Nil	Variable	Variable
Acinetobacter/Moraxella-like	1	Nil	Sulfide-like	+	+
Acinetobacter/Moraxella-like	3	Nil	Nil	Variable	Variable
Pseudomonas group I	5	Sulfide-like	Sulfide-like	+	+
Pseudomonas group I	5	Nil	Nil	+	Variable
Pseudomonas group II	1	Sulfide-like	Sulfide-like	+	+
Pseudomonas group II	5	Evaporated milk	Evaporated milk	Variable	Variable
Pseudomonas group II	7	Fruity	Fruity	Variable	Variable
Pseudomonas group II	5	Nil	Nil	Variable	Variable
Pseudomonas group III/IV	2	Sulfide-like	Sulfide-like	+	+
Pseudomonas group III/IV	5	Nil	Nil	Variable	Variable

[a] Nutrient broth, cysteine and lead acetate.
[b] Methionine and DTNB.

essing, many of these are removed by the scalding process, and subsequent contaminants are largely gram-negative bacteria which will be present at levels of 10^4 cells per 16 cm^2, even under optimum processing conditions (Table 1). Scanning electron microscopy has shown that these organisms are contained in a layer of material on the skin surface and may be located in spaces of capillary size (11). For these reasons, sterile skin cannot be consistently obtained without treatment which damages the substrate. Therefore, it was necessary to assess the spoilage ability of skin isolates by inoculation onto leg and breast muscle sections.

This study has shown that only a fraction of the flora present on the skin of chicken carcasses stored at 2°C was able to produce spoilage odors on muscle sections. When expressed as a percentage of the psychrophilic flora, off-odor producers remained a consistently small proportion of the flora during the storage period.

Psychrophilic off-odor-producing bacteria were represented by *Pseudomonas* groups I, II, and III/IV, *Acinetobacter/Moraxella*-like species, and enteric types. Similarly, Herbert et al. (4) reported that off-odor-producing bacteria on spoiled cod and haddock never accounted for more than 20% of the total flora. Studies by McMeekin (6, 7) have also shown that a restricted group of psychrophiles were responsible for the production of off-odors on breast and leg muscle stored at 2°C.

Members of *Pseudomonas* group II produced sulfide-like, fruity-ester, and evaporated-milk odors, whereas other strains produced only the sulfide-like odor. Freeman et al. (3) reported volatile sulfides to be major components of the head space above spoiling breast muscle at 2 and 10°C. A single *Acinetobacter/Moraxella*-like strain grew only on leg muscle, producing a sulfide-like odor. Its inability to develop on breast tissue may be attributed to the pH effect described by Barnes and Impey (1) and McMeekin (6, 7). Representatives of each group of bacteria making up the spoilage association did not produce detectable off-odors on muscle sections, and the contribution of their metabolism and growth in spoilage remains to be elucidated.

It is of interest that *Alteromonas putrefaciens* was not recovered in this study although it has been reported to be an important component of the spoilage flora of poultry carcasses (2) and spoiling leg muscle (7). Even peptone–iron agar, which has been widely used (4, 5, 9), failed to detect *A. putrefaciens* or indeed any other H_2S-producing bacterium. McMeekin et al. (8) have suggested that peptone–iron agar is limited in usefulness in that it detects only H_2S-producing strains and is less sensitive than tests based on reaction with lead acetate. They proposed a test with DTNB which allowed detection of volatile organic sulfides in addition to hydrogen sulfide. The detection of strains producing sulfide-like off-odors on muscle sections and producing volatiles from cysteine and methionine which react with lead acetate and DTNB confirms the inability of peptone–iron agar to recover many sulfide-producing bacteria.

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