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Identification of Volatile Organic Compounds Produced by Fluorescent Pseudomonads on Chicken Breast Muscle

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Four different fluorescent pseudomonads were isolated from spoiled, uncooked chicken breasts and were grown in pure culture on initially sterile chicken breast muscle at 2 to 6°C for 14 days. The volatile compounds produced by each culture were concentrated on a porous polymer precolumn and separated and identified by high-resolution gas chromatography–mass spectrometry.

McMeekin has examined the spoilage association of chicken leg and breast muscle (10, 11). Daud et al. (2) described the spoilage association of chicken skin. These studies confirm that the spoilage of poultry is due largely to the action of psychrotrophic gram-negative bacteria. There appears to be little information available which correlates the presence of volatile components with the presence of individual strains of bacteria growing on chicken muscle. Freemen et al. (4) identified volatile organic compounds produced by several microorganisms allowed to grow on radiation-sterilized chicken breasts. The identification of volatiles produced by pure cultures of spoilage organisms appears to be a useful approach in attempting to chemically define spoilage (15). In the present study, one *Pseudomonas putida* isolate and three *Pseudomonas fluorescens* isolates from the skin of uncooked, spoiled chicken breasts were allowed to grow separately on sterile chicken breast muscle at 2 to 6°C. Many of the compounds produced by these organisms during growth on the chicken muscle were separated and identified by combined gas chromatography-mass spectrometry (GC-MS).

Fresh chicken breasts were obtained from a local distributor and stored in polyethylene bags at 2 to 6°C for 14 days. After incubation, a 6.45-cm^2 surface area of the chicken breast was swabbed for 15 s with a moistened (sterile deionized water) sterilized cotton swab. The swab end was then broken into a test tube containing 9 ml of 3% (wt/vol) Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.5% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.). The same skin area was swabbed a second time in an identical manner, and the swab end was broken into the test tube containing the first swab end (13). The contents were then shaken, and serial dilutions were prepared and plated over the surface of prepoured agar plates containing 4% Trypticase soy agar (BBL Microbiology Systems) supplemented with 0.5% yeast extract (TSY agar). Isolates were randomly removed from appropriate dilution plates after 14 days of incubation at 2 to 6°C and purified by streaking on TSY agar.

The scheme of Shewan et al. (14) was used to classify the isolates. The tests performed were Gram stain, motility, oxidase reaction (8), mode of attack on glucose (6), fluorescent pigment production (7), and gelatin digestion (3).

A modification of the technique described by McMeekin (10) was used to aseptically remove sections of the pectoralis thoracicus and supracoracoideus muscles, which were subsequently cut into small muscle blocks of about 8 to 10 g.

Seven fluorescent pseudomonad organisms were isolated from spoiled chicken breasts held at 2 to 6°C for 14 days. Each of these cultures was grown on TSY agar slants for 24 h at 20°C, after which they were washed from the slants with 10 ml of sterile Ringer solution. The suspensions were diluted to a predetermined optical density to give a final concentration of approximately 8×10^8 cells per ml. A 2- to 3-ml amount of bacterial suspension was inoculated onto 40 g of muscle sections to give approximately 5×10^7 cells per g and incubated at 2 to 6°C for 14 days. The three *P. fluorescens* organisms (numbered 003, 041, and 060) and the one *P. putida* organism (numbered 014) were selected for further study based upon their off-odor production.

After 14 days of incubation, the 40 g of muscle containing the specific culture was transferred to a water-jacketed headspace sampling bottle, and the volatiles were collected and analyzed as described by Lee et al. (9). Purified dry helium was swept over the sample for 45 min at a rate of 100 ml/min while the temperature of the water-

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jacketed sample bottle was held at 55°C. The volatile compounds were trapped on 15.0 mg of Tenax-GC (60- to 80-mesh; Applied Science Laboratories, State College, Pa.), which was packed in a 62-mm precolumn (inside diameter, 1.5 mm).

After sample collection, the precolumn was inserted into the injection port (250°C) of a Perkin-Elmer model 3920B series gas chromatograph equipped with a glass capillary column (length, 80 m; inside diameter, 0.25 mm) coated with SF-96 methyl silicone and purged with helium carrier gas. During the 10-min purge of the compounds from the precolumn, a U trap at the beginning of the glass capillary column was submerged in liquid nitrogen (−196°C) to trap and concentrate the desorbed compounds. After purging and trapping, the liquid nitrogen was removed from the U trap. The oven temperature was maintained at 25°C for 4 min and then programmed from 25 to 190°C at 4°C/min. The final temperature of 190°C was maintained for 16 min to complete the recording of the chromatographic profiles.

Identification of compounds was accomplished by combined GC-MS with a Hewlett-Packard model 5982A-GC-MS controlled by a Hewlett-Packard 5934A data system. The glass capillary column used in this system had the same specifications as the one used in the Perkin-Elmer 3920B series gas chromatograph described above. The ion source energy was 70 eV. Spectra were obtained at a scan rate of 138 atomic mass units per s.

The compounds identified in this study are listed in Table 1. Compounds 7 (2-butenal), 15 (methyl thiol *n*-butyrate), and 23 (3-octanol) were produced by several fluorescent pseudomonads studied, but these organisms were not originally isolated from spoiled chicken breasts. Compound 28 (4-methyl-2,6-di-*tert*-butylphenol) results from decomposition of the Tenax-GC while in the injection port of the gas chromatograph. No compound detected in this study was found to be unique to culture 003 (*P. fluorescens*). Another *P. fluorescens* organism, culture 060, produced many of the same compounds as culture 003 except that 060 produced dimethyl sulfide (compound 3), methyl thiolacetate (compound 9), and 3-pentanone (compound 10), and 003 produced cyclo-heptene (compound 25). Compound 18 (2-octanol) was unique to the third *P. fluorescens* organism, culture 041. Compound 6 (2-butanol) and compound 17 (1-nonene) have been detected in several other fluorescent pseudomonad cultures grown on sterile muscle, but again, these cultures were not originally isolated from spoiled chicken breasts. The chromatogram produced by culture 041 was more unique than the chromatograms of cultures 003 and 060 due to the quantitative differences of several peaks, the absence of compounds 1, (methanethiol), 14 (ethyl methyl disulfide), and 21 (3-octanone), and the presence of compounds 6 (2-butanol), 17 (1-nonene), 18 (2-octanol), and 27 (*n*-nonanal). Compound 27 (*n*-nonanal) was also produced by culture 014 (*P. putida*).

Culture 014 produced many of the same compounds as the above three cultures but in differing amounts. The one unique compound produced by culture 014 was carbon disulfide (compound 4). Moreover, compound 26 (1-undecene) was not detected, but all three of the *P. fluorescens* cultures produced it.

Four individuals described the odors of cultures 003, 041, and 060 to be similar, the odor of culture 014 was very intense, but different from the odors of the other three organisms. Culture 003 gave the weakest odor of the four organisms, while culture 041 gave the strongest.

TABLE 1. Compounds identified[a]

No.	Compound	Production in culture no.:			
		003	060	041	014
1	Methanethiol	+	+		+
2	Acetone	+	+	+	+
3	Dimethyl sulfide		+	+	
4	Carbon disulfide				+
5	2-Butanone	+	+	+	+
6	2-Butanol			+	
7	2-Butenal[b]				
8	2-Pentanone	+	+	+	+
9	Methyl thiolacetate		+		+
10	3-Pentanone		+		+
11	Dimethyl disulfide	+	+	+	+
12	Toluene	+	+	+	+
13	Dimethyl benzenes	+	+	+	+
14	Ethyl methyl disulfide	+	+		+
15	Methyl thiol *n*-butyrate[b]				
16	2-Heptanone	+	+	+	+
17	1-Nonene			+	
18	2-Octanol			+	
19	Dimethyl trisulfide	+	+	+	+
20	Trimethyl benzenes	+	+	+	+
21	3-Octanone	+	+		+
22	4-Octanone	+	+	+	+
23	3-Octanol[b]				
24	2-Nonanone	+	+	+	+
25	Cycloheptene	+		+	
26	1-Undecene	+	+	+	
27	*n*-Nonanal			+	+
28	4-Methyl-2,6-di-*tert*-butylphenol	+	+	+	+

[a] The following compounds were detected in the uninoculated sterile breast muscle after incubation for 14 days at 2 to 6°C: acetone, toluene, *n*-hexanal, pentanol, 1-octen-5-ol, dimethyl and trimethyl benzenes, and 4-methyl-2,6-di-*tert*-butylphenol

[b] Produced by several fluorescent pseudomonads not originally isolated from spoiled chicken breasts.

Several compounds detected on sterile controls were not detected when pure cultures were allowed to grow on the muscle. This has been observed in previous studies (4), and the absence of these compounds may be due to their utilization by the microorganisms.

Using similar experimental conditions, Lee et al. (9) identified volatiles produced by the *P. putida* culture 014 and the *P. fluorescens* culture 060 when these organisms were allowed to grow in pure culture on TSY agar for 14 days at 2 to 4°C. Acetone, 2-butanone, methyl thiolacetate, dimethyl disulfide, toluene, dimethyl trisulfide, and 2-nonanone were detected when the *P. putida* culture 014 was grown on either chicken breast muscle or TSY agar. However, acetone and toluene were also found on uninoculated sterile breast muscle held at 2 to 6°C for 14 days. Acetone, toluene, methyl thiolacetate, dimethyl disulfide, dimethyl trisulfide, 2-nonanone, and 1-undecene were detected when *P. fluorescens* culture 060 was grown on either substrate. The compound 1-undecene was also detected on uninoculated TSY agar (9). However, a number of different compounds were also detected when each culture was allowed to grow on the two different substrates. This appears to support the finding of Bohannon et al. (1) that the chromatographic profile obtained for an organism is also dependent upon the growth environment.

In the present study and in the work of Lee et al. (9), it was found that the chromatograms were unique for each culture growing on the same substrate. In the present study, the only compound that all three *P. fluorescens* cultures produced that the one *P. putida* did not produce was 1-undecene. The one compound that the *P. putida* produced that the three *P. fluorescens* did not produce was carbon disulfide. The limited number of *P. fluorescens* and *P. putida* cultures whose volatiles have been identified on chicken breast muscle prevents any general conclusion at present on the use of specific volatile compounds produced to detect different species of microorganisms growing in a mixed flora on chicken muscle.

The association of volatile sulfides with the spoilage of meat, fish, and poultry is well documented (4, 5, 11, 12). In an earlier study by Freeman et al. (4) where radiation-sterilized chicken breasts were used as a substrate, in addition to a different sample collection technique and different GC-MS conditions, dimethyl sulfide, dimethyl disulfide, and methyl thiolacetate were detected from *P. fluorescens* culture 060. In the same study, the only sulfur compound detected for *P. putida* culture 014 was dimethyl disulfide. In the present study, methanethiol, dimethyl disulfide, and methyl thiolacetate were found in both culture 060 and culture 014. Dimethyl sulfide was still not detected in culture 014, but was again detected in culture 060.

The identification of volatiles produced by pure cultures and a mixed flora of spoilage organisms growing on meat, fish, and poultry may help to chemically define spoilage and to detect specific microorganisms, but the substrate used, as well as sampling techniques and GC-MS conditions, must be considered before general conclusions are drawn.

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