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Detection of Volatile Sulfide-Producing Bacteria Isolated from Poultry-Processing Plants

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A technique using filter paper strips impregnated with 5-5'dithiobis-nitrobenzoic acid was developed to allow the detection of bacteria (isolated from poultry-processing environs) which produced volatile sulfides (H_2S, CH_3SH, $[CH_3]_2S$). The technique is preferred to conventional methods in that it allows the detection of volatile organic sulfides in addition to hydrogen sulfide.

Volatile sulfides are known to be important components of the spoilage aroma of chill-stored poultry. Freeman et al. (5) detected by GLC-MS hydrogen sulfide, dimethylsulfide, methanethiol, and its oxidation product dimethyldisulfide above chicken breast muscle stored at 2 and 10°C. It is therefore important to detect easily that portion of the flora capable of producing volatile sulfides.

Methods for the detection of sulfide-producing organisms have traditionally relied upon the use of lead or iron salts, which are of limited value in that they detect only hydrogen sulfide (4, 8, 10, 13). Gillespie (7) used 5-5' dithiobis-2-nitrobenzoic acid (DTNB) to detect volatile sulfide-producing strains in spoiling fish, and Sharpe et al. (15) described the use of this reagent to detect methanethiol production by coryneform bacteria isolated from cheese. DTNB has been used in this study to enable detection of volatile sulfide-producing bacteria isolated from chicken carcasses and the environs of poultry-processing plants.

Isolates were obtained from samples taken during hygiene investigations in four poultry-processing plants. Swabs of equipment and plant surfaces and samples from chicken carcasses (breast skin) were taken as described by Patterson (14). Samples were plated on nutrient agar and incubated for 3 days at 22°C. All colonies were picked from a suitable dilution plate (i.e., ca. 50 colonies per plate) inoculated into methionine-cysteine (MC) medium (nutrient broth supplemented with methionine [0.1% wt/vol] and cysteine [0.02% wt/vol]) contained in screw-cap bottles and incubated at 22°C. Volatile sulfides were detected by placing a filter paper strip freshly impregnated with DTNB solution (80 mg/100 ml in tris[hydroxymethyl]aminomethane buffer, pH 8.0) in the headspace above the grown culture. The development of a yellow coloration on the DTNB-impregnated paper was taken as evidence of the presence of volatile sulfides. This was normally evident within 2 h.

Fifty-five volatile sulfide-producing isolates were obtained from chicken carcasses, and 22 strains were recovered from equipment swabs and identified according to the scheme of Shewan et al. (16) as follows: *Pseudomonas* group I (24 strains), *Pseudomonas* group II (9 strains), *Alteromonas putrefaciens* (formerly *Pseudomonas putrefaciens*) (5 strains), flavobacteria (15 strains), and enteric types (24 strains).

All of the isolates produced a yellow coloration on DTNB papers above MC medium, indicating the presence of volatile sulfides. However, some did not show evidence of H_2S production from this medium when tested with lead acetate papers (Table 1). Only one of the nine *Pseudomonas* group II isolates and 17 of 24 *Pseudomonas* group I isolates produced H_2S (9 of the latter gave only a slight reaction with lead acetate paper).

Herbert and Shewan (9) have shown that *Pseudomonas* strains isolated from spoiling fish produce only H_2S from cysteine and methanethiol and possibly dimethylsulfide from methionine. Thus cultures giving a positive result with DTNB only were judged to have attacked only methionine, whereas those which gave positive results on DTNB and lead acetate papers produced H_2S from cysteine and possibly CH_3SH and $(CH_3)_2S$ from methionine. To confirm the latter possibility, washed-cell suspensions were incubated with methionine (0.1% wt/vol; ca. 10^8 cells per ml); all strains gave a positive result when tested with DTNB.

The ability of strains to form and produce volatile sulfides at 5°C was tested in MC medium and with methionine. All of the strains

TABLE 1. *Production of volatile sulfides by various groups of isolates*

Group	No. of strains	No. producing volatile sulfides from MC medium at:		No. producing H_2S from MC medium at:		No. producing volatile sulfides from methionine at:	
		22°C	5°C	22°C	5°C	22°C	5°C
Pseudomonas group I	24	24	18	17	3	24	24
Pseudomonas group II	9	9	9	1	1	9	9
A. putrefaciens	5	5	5	5	5	5	5
Flavobacteria	15	15	15	14	2	15	15
Enteric types	24	24	17	24	19	24	24

were able to grow at this temperature, but several *Pseudomonas* group I isolates and enteric types failed to produce volatile sulfides after growth on MC medium, and weak reactions were recorded for the nine *Pseudomonas* group II strains (Table 1). All strains, however, showed evidence of volatile sulfides from methionine when tested by addition of 0.1 ml of DTNB reagent. The ability to produce H_2S as judged by reaction with lead acetate appeared to be more restricted at this temperature. Fourteen of 17 *Pseudomonas* group I types, 12 of 14 flavobacteria, and 5 of 24 enteric types recorded as positive at 22°C gave negative results at 5°C (Table 1).

The correlation between the detection of sulfide-producing isolates using DTNB and the ability of these strains to produce off-odors on chicken muscle was tested using 19 representative strains. Washed-cell suspensions of these were inoculated onto sterile sections of chicken leg muscle excised as described by McMeekin (12) to give a final cell concentration of ca. 10^4/g. These were incubated at 5°C and examined sensorily for up to 14 days. All strains examined produced sulfide-like odors, and the presence of volatile sulfides in the headspace above the muscle was confirmed by reaction with DTNB.

The test described therefore allows the detection of isolates capable of producing hydrogen sulfide and other volatile sulphides and consequently overcomes the serious limitation of reagents which allow only the detection of H_2S. The value of such a reagent is emphasized by the fact that a number of strains have been detected which produce volatile sulfides but not H_2S at 5°C as judged by reaction with lead acetate.

The volatile sulfide products may be, in part, distinguished by the use of both DTNB and lead acetate reagents and confirmed by the use of specific substrates. It is not possible, without the aid of GLC, to distinguish between CH_3SH and $(CH_3)_2S$. However, this is not a serious disadvantage, as both are products of the metabolism of methionine, and CH_3SH is always the major component (9).

The majority of the organisms detected by the test have previously been reported to produce volatile sulfides during chill storage of flesh foods: *A. putrefaciens* (1, 3–5, 8, 10–12), *Pseudomonas* group I (8, 10, 11), and psychrophilic enterics (6, 10). *Pseudomonas* group II types are normally associated with fruity (2, 8, 10, 11) or "evaporated milk" odors (10) rather than the production of sulfide-like odors. The fact that eight of the nine strains recovered did not produce H_2S suggest that these may not have been detected as volatile sulfide producers by media based on the use of metal salts. Freeman et al. (5) reported the production of volatile sulfides by flavobacteria growing on chicken breast muscle. Although these normally decline in relation to the pseudomonads during storage of chicken muscle (10, 11), they represent the third largest group of isolates recovered and may well be active in the development of odors at least in the incipient stages of spoilage.

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