Production of Acetoin and Diacetyl by the Genus Salmonella

J. A. GARIBALDI AND H. G. BAYNE

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

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Members (all 42 tested) of the genus Salmonella, although Voges-Proskauer-negative when grown on MR-VP Medium, produce abundant quantities of acetoin or diacetyl, or both, when cultured on a minimal medium containing 1% glucose. The designation that the genus Salmonella does not produce acetoin should be re-examined.

One of the characteristics used to define the genus Salmonella is its inability to produce acetyl-methylcarbinol (acetoin (Bergey's Manual, 7th ed.; 3, 4)). The members of the genus do not produce acetoin under the carefully controlled conditions of the Voges-Proskauer test (3). However, we found that under somewhat different nutritional conditions salmonella produce abundant quantities of acetoin or diacetyl, or both.

MATERIALS AND METHODS

Bacteria. The following species of Salmonella were tested: S. typhimurium LT-2, TM-1, 6087; S. cerro 4837, 6268; S. senftenberg 6101, 4361, S8, 3434-63, 775W; S. milwaukee; S. macellen; S. montevideo; S. urbana; S. memphis; S. albuquerque; S. mission; S. newport; S. bariely; S. heidelberg; S. choleraesuis var. kunzendorf; S. oranienburg; S. worthington; S. bleadon; S. melangrida; S. derby; S. anatum; S. austin; S. tennesse; S. blockley; S. thompson; S. gallinarum; S. pullorum; S. sendai; S. dublin; S1982, S1917, S1547; S729, S280, S2, S1, 3147.

Media. MR-VP Medium (Difco) was prepared as recommended by the manufacturer and was also modified by increasing the glucose concentration to 1% or adjusting the initial pH to 6.0, or both. The minimal medium was composed of K2HPO4, 11 g; (NH4)2SO4, 2.0 g; MgSO4·7H2O, 0.5 g; FeSO4 to give 8 μg of Fe/ml; MnCl2 to give 8 μg of Mn/ml; and ZnSO4 to give 8 μg of Zn/ml; water, 1.0 liter (pH 7.6). Glucose was sterilized separately and added after autoclaving to give a final concentration of 1%. In some tests, this medium was supplemented with yeast extract at levels of 0.3, 0.1, or 0.03%. In others, it was supplemented with the amino acids and vitamins necessary for the growth of S. gallinarum and S. pullorum (10).

The media were inoculated from 15-hr slant cultures, incubated at 30 C on a rotary shaker, and tested after 24 and 48 hr.

Detection and analysis of acetoin or diacetyl or both. Two-milliliter samples of the cultures were tested with the reagents 5% α-naphthol in 95% ethyl alcohol and 0.3% creatine in 40% KOH (3).

Analyses for acetoin and diacetyl were conducted as described by Krampitz (5) on cell-free culture supernatants.

Gas chromatography of diacetyl in culture supernatant. The volatiles from 5 ml of cell-free supernatant of S. typhimurium TM-1, grown on the minimal medium at 30 C for 48 hr, were isolated, trapped, and injected into the gas chromatograph as previously described (7). Helium carrier gas (30 ml/min) was used in the stainless-steel column [outer diameter, 24 ft by 3/4 inch (7.3 m by 0.32 cm)], packed with 5% FFAP on 8% mesh chromosorb G. The column was operated isothermally at 75 C, the flame ionization detector at 250 C, and the injector at 225 C. Toluene was used as an internal standard for the calculation of relative retention times.

RESULTS

MR-VP Medium. The bacteria tested did not give a positive reaction for acetoin or diacetyl, or both, either on the unmodified MR-VP Medium (in which all of the glucose was metabolized) or on the medium modified to a glucose concentration of 1% or an initial pH of 6.0, or both (in which glucose was in excess). The final pH of the cultures after 48 hr of incubation in all cases was approximately 5.0.

Minimal medium. Of the 42 strains grown on minimal medium, 33 gave a positive reaction for acetoin after either 24 or 48 hr of incubation at 30 C. The nine strains giving a negative reaction (strains of S. dublin, S. sendai, S. pullorum, and S. gallinarum) grew sparsely on this minimal medium and are known to have specific growth requirements for vitamins or amino acids, or both (1, 10). In a subsequent experiment when the growth was increased by supplementing this minimal medium with either 0.3, 0.1, or 0.03% yeast.
extract, seven of these nine strains of Salmonella converted glucose to acetoin or diacetyl, or both, to give a positive reaction with the Voges-Proskauer reagents described above. The two salmonellae which still did not produce acetoin in these media were strains of S. pullorum and S. gallinarum.

A positive reaction for acetoin or diacetyl, or both, resulted when these two organisms were cultured on the minimal medium supplemented with the vitamins and amino acids necessary for growth of S. pullorum and S. gallinarum (10).

A gas chromatogram of the volatiles from the cell-free supernatant of a 48-hr culture of S. typhimurium TM-1 showed four well-defined fractions. The fourth fraction had a definite diacetyl-like odor as it emerged from the column, and its relative retention time (0.587) agreed well with that observed for authentic diacetyl (0.585). The level of acetoin-diacetyl in cell-free supernatants of this strain was 28 µg/ml.

DISCUSSION

It is apparent that members of the genus Salmonella have the capability of producing acetoin. It is also obvious that they do not produce amounts detectable with the Voges-Proskauer test under the nutritional conditions of the MR-VP Medium. As discussed by several investigators (6, 8, 11), the initial glucose concentration and the initial pH of the MR-VP Medium greatly affect the production of acetoin by the genus Aerobacter. Our exploratory experiments with these two variables indicate that they are not the factors which prevent the accumulation of acetoin by Salmonella.

The detrimental effect of phosphate on the production of acetoin by sporeformers was described (9). It was recommended that the phosphate be replaced with sodium chloride when the VP test is conducted on the aerobic sporeformers (9). However, in the present study, the minimal medium contains more than twice the concentration of phosphate as the MR-VP Medium without restricting the formation of acetoin by Salmonella.

The nutritional or environmental factors, or both, which prevent the accumulation of acetoin by the genus Salmonella under the conditions of the Voges-Proskauer test, are still to be determined.

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