

# MICROBACTERIUM THERMOSPACTUM, SPEC NOV; A NONHEAT RESISTANT BACTERIUM FROM FRESH PORK SAUSAGE<sup>1</sup>

RUTH A. MCLEAN AND WILLIAM L. SULZBACHER

Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland

Received for publication September 18, 1952

In the course of a study of the microbial flora of fresh pork sausage (Sulzbacher and McLean, 1951) a gram positive rod associated with sausage flavor deterioration was isolated repeatedly. Although this organism tentatively was identified as belonging to the genus *Microbacterium*, it differs sufficiently from the described species of that genus to justify the publication of a complete description together with an explanation of its classification. The name *Microbacterium thermosphactum* is proposed for the organism.

## EXPERIMENTAL METHODS

In addition to the usual morphological and biochemical tests for identifying bacteria, certain reactions of the organism studied were investigated in detail. A description of the methods used in these special studies follows:

**Acid production:** Ten strains, selected at random from the group of isolates studied, were inoculated into separate 250 ml portions of yeast extract, peptone broth containing 5 per cent glucose and 3 per cent calcium carbonate. The cultures were incubated for four weeks, tested for purity microscopically, acidified to pH 1.0 with sulfuric acid, filtered, and steam distilled. Both the distillate and residues were tested for the presence of organic acids (Feigl, 1946). The residues of the steam distillation of three cultures were extracted with ethyl ether for 24 hours in a continuous extractor. Zinc lactate was prepared from the extracts and examined in a manner similar to that described by Pederson, Peterson, and Fred (1926).

**Catalase activity:** Formation of catalase was confirmed by visible reaction with hydrogen peroxide, and reactions on blood agar with benzi-

dine and by Penfold's (1922) method. However, because of the importance of catalase formation in differentiating between the genera *Lactobacillus* and *Microbacterium* a need for more quantitative evidence seemed to be indicated. Washed cells from 18 hour veal infusion agar cultures were suspended in 0.06 M phosphate buffer and their ability to decompose hydrogen peroxide measured in a Warburg manometer by observing the rate of oxygen liberation. The density of the bacterial suspension was determined by direct microscopic counts and catalase capability calculated by the graphical method of Van Schouwenburg (1940).

**Heat resistance:** Thermal resistance was determined by heating suspensions of 48 hour cultures in 0.06 M phosphate buffer in sealed glass tubes immersed in an electrically heated, thermostatically controlled bath. Plate counts were made with veal infusion agar from unheated tubes, and from tubes heated for various lengths of time, and survivor curves were plotted.

## RESULTS AND DESCRIPTION

Forty-six cultures, isolated at various times from sausage and pork trimmings, were studied. Except for the fermentation of arabinose and cellobiose, which were not attacked by two isolates, all showed identical reactions, and they will be treated in the following description as a single species.

**Morphology:** These organisms were gram positive and nonmotile, varying greatly in length from filamentous forms to short rods, but uniformly 0.6  $\mu$  wide. A few gram negative forms were observed even in very vigorously growing 18 hour cultures resulting from 3 consecutive daily transfers. The large bodies shown in figure 1 were masses of gram positive growth occurring between two coils of the filamentous form of the bacterium and supported in a matrix of filaments consisting of gram positive and negative rods

<sup>1</sup> The authors wish to acknowledge the technical assistance of Mrs. Nina B. Hess and the helpful suggestions of Drs. R. N. Doetsch, J. R. Gots, A. P. Hansen, and S. Mudd.

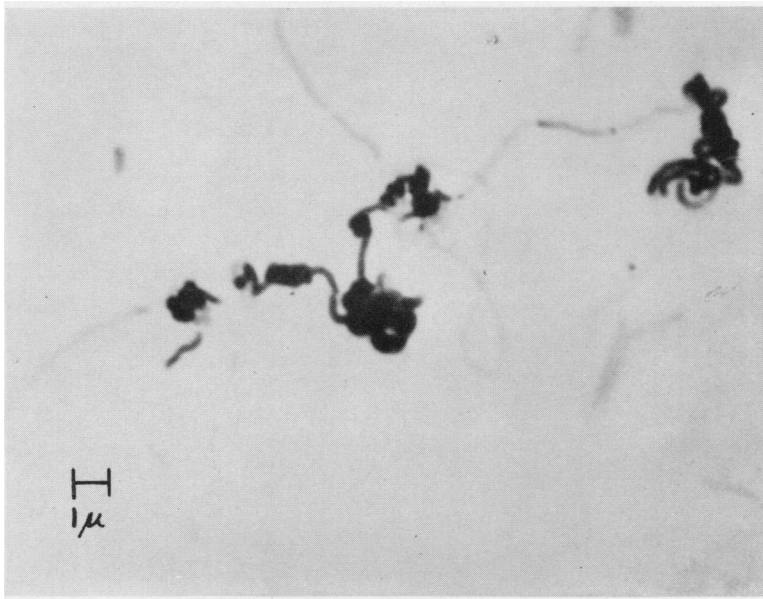


Figure 1. Large bodies of *Microbacterium thermosphactum*. Gram stain from 48 hour veal infusion culture.

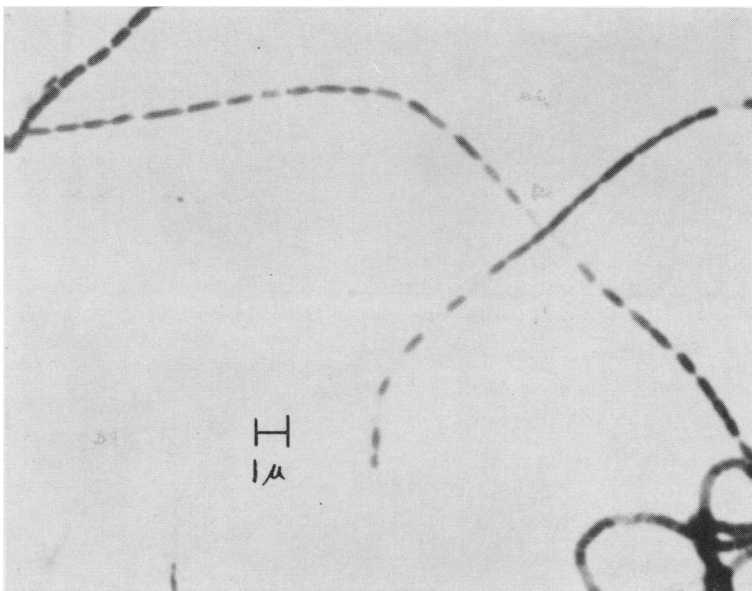


Figure 2. Filaments of gram positive and negative cells seen surrounding large bodies in figure 1.

shown in figure 2. The large bodies were found in a 48 hour veal infusion medium (Difco) culture that had been transferred from a veal infusion culture stored for about 9 months at 9 C. A transfer to a veal infusion agar slant from the culture containing the large bodies produced the

long slender rods shown in figure 3. Veal infusion medium, in which large bodies were observed, is a semisolid medium containing 0.1 per cent agar and provides conditions of lowered oxygen tension. It supported a much heavier growth of these organisms than ordinary liquid medium or

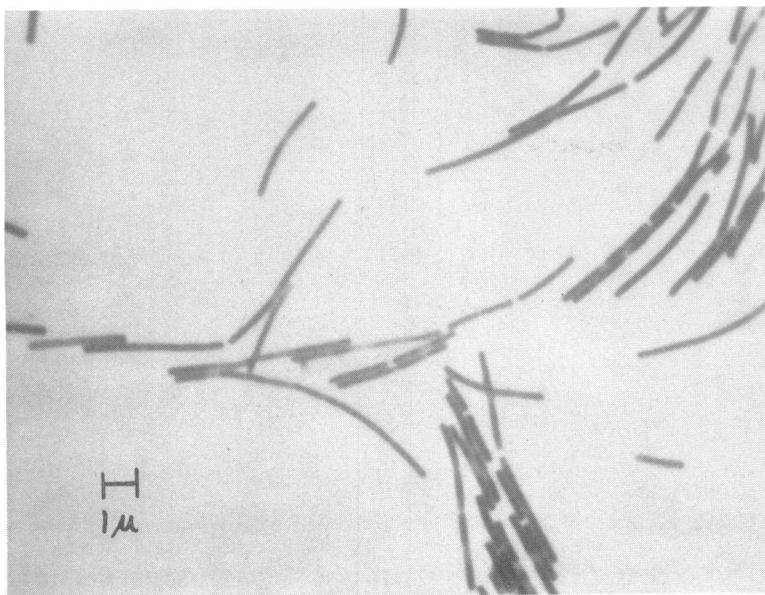


Figure 3. Long rods of *Microbacterium thermosphaerum*. Gram stain from 24 hour veal infusion agar.

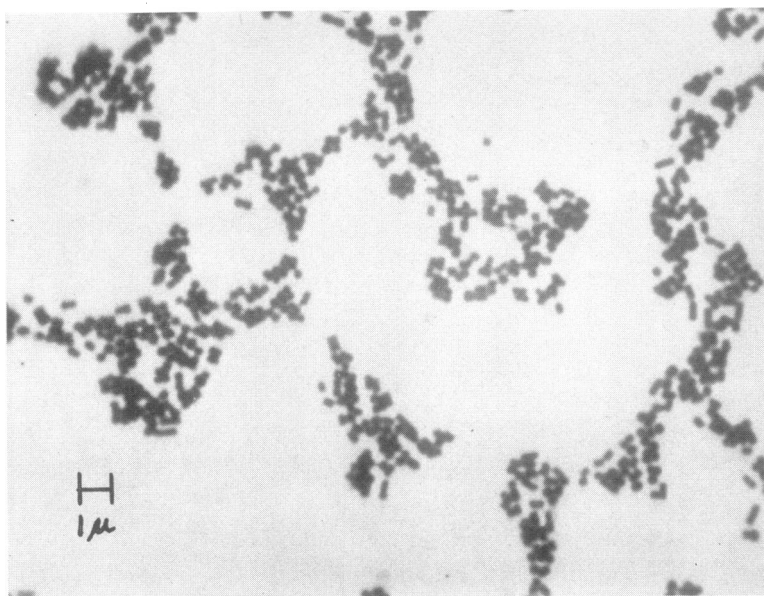


Figure 4. Short rods and coccoid forms of *Microbacterium thermosphaerum*. Carbol fuchsin stain from 24 hour veal infusion agar.

slants prepared from veal infusion medium by the addition of agar. Large bodies have not been observed in surface cultures. The freshly isolated organisms grown as surface cultures usually appear as short rods or even coccoid forms as seen in figure 4. However, nearly all preparations

of the organism studied have shown occasional long cells and filaments like those shown in figures 2 and 3.

The large bodies described were reminiscent of those reported by Johnson and Gray (1949) for *Achromobacter fischeri* and by Klieneberger-

Nobel, Dienes, and other workers for a considerable variety of organisms. A comprehensive review of many of these studies may be found in the recent paper by Dienes and Weinberger (1951). Fragmenting large bodies of *Microbacterium thermosphactum* have been observed, but to date a thorough study of possible L forms has not been made. Further cytological studies of large bodies by methods designed to show nuclear structure are planned.

**Macroscopic growth:** Growth on agar slants was sparse in 24 hours but became somewhat heavier in 2 to 3 days. Growth in 24 hour veal infusion glucose cultures (veal infusion medium plus 1 per cent glucose) was diffuse and heavy at 20 C, while growth in broth cultures was slow and was observed as a sediment at the bottom of the tube with slight turbidity. Blood agar cultures showed no hemolysis. Colonies on nutrient agar were small, round, smooth, and glistening, about 1 to 1.5 mm in diameter. No pigment was formed on any medium tested.

**Temperature relationships:** Optimum growth occurred at from 20 to 22 C. Fairly heavy microscopic growth was observable after 7 days' incubation at 0 to 1 C. No growth occurred at -4 C after 6 weeks, but cultures so incubated remained viable, producing visible growth when shifted to 20 C. No growth occurred at 37 C, and cultures incubated at that temperature for 4 days failed to grow when moved to a lower temperature (20 C). The exact maximum growth temperature was not determined, but in general these reactions were not greatly different from those reported in *Bergey's Manual* (Breed et al., 1948) for *Microbacterium lacticum*.

**Biochemical reactions:** *Microbacterium thermosphactum* did not form H<sub>2</sub>S or indole, did not reduce nitrates, liquefy gelatin, or utilize urea or citrate. Growth in litmus milk produced slight acidity but no coagulation. Acid but no visible gas was formed from arabinose, glucose, lactose, sucrose, maltose, xylose, cellobiose, dulcitol, mannitol, and starch. Tests in Eldridge fermentation tubes showed that carbon dioxide was formed during fermentation. These reactions differ from those reported for members of the same genus *Microbacterium* in *Bergey's Manual* in the formation of acids from arabinose and xylose, while carbon dioxide formation is not reported.

No volatile acids were detected in steam dis-

tillates of the fermented glucose yeast extract broth. Slight acidity of the distillates was found to be due to traces of lactic and sulfuric acid that were carried over during distillation. Lactic acid was the only acid found in the distillation residue in sufficient concentration to be detected. The zinc lactate crystallized from the ether extract of the distillation residue contained, after purification, 13.83 per cent water of crystallization and, at a concentration of 3.5 g per 100 ml, showed an average specific rotation of  $[\alpha]_D^{28} = -6.2$ . When compared with the values of Cori and Cori (1929) the specific rotation indicates that about 80 per cent of the salt was

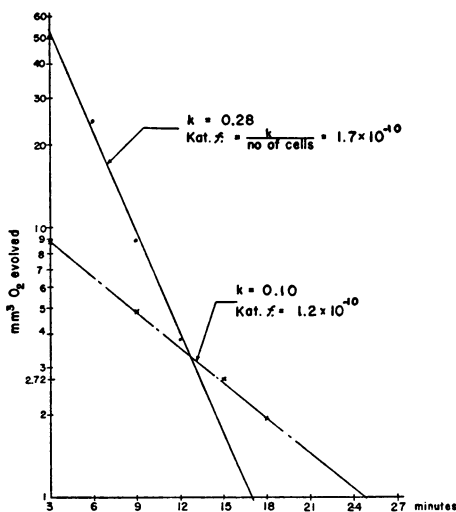


Figure 5. Catalase capability of *Microbacterium thermosphactum*.

optically active while the values for water of crystallization indicate about 83 per cent of the salt to have been active. Since the rotation of the zinc salts of lactic acid is opposite to that of the free acid, the organisms studied must have produced L (+) lactic acid. In this respect they differ from the described species of *Microbacterium* which produce dextro lactic acid.

**Catalase formation:** As indicated under experimental methods above, considerable attention was paid to catalase production by this organism. Figure 5 shows graphically the liberation of oxygen from a hydrogen peroxide solution, and the catalase capability values found agree with those reported by Van Schouwenburg (1940) for other aerobic organisms. These values lend quantitative support to positive observa-

tions of catalase formation on benzidine blood agar and of macroscopically observed decomposition of hydrogen peroxide. Older cultures occasionally failed to show positive qualitative tests for catalase, and quantitative work was done on washed 18 hour cells. Sevag (1933) has pointed out the importance of using young culture for such work.

**Heat resistance:** The known species of *Microbacterium* are described as heat resistant and are capable of withstanding heating at 85 C for 2.5 minutes in the case of *M. lacticum*, and at 71.6 C for 2.5 minutes in the case of *M. flavum*. Figure 6 shows survival curves at 55 C for the least and most resistant of the strains reported

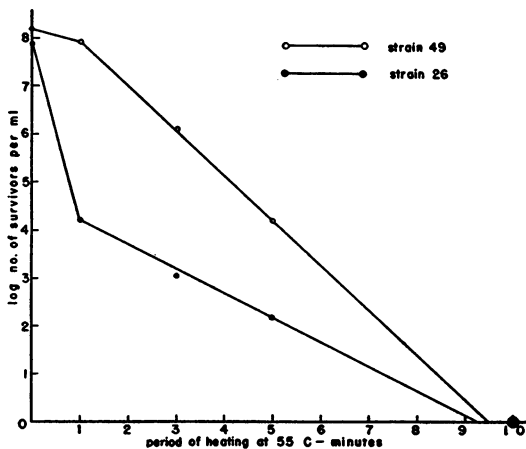


Figure 6. Heat resistance of *Microbacterium thermosphactum*.

on in this study. The points shown are averages of three experiments. In no instance were there survivors after 10 minutes, and the logarithmic numbers of survivors did not depart greatly from linearity except for the first interval. Other tests indicated that the most resistant strain studied could survive heating to 63 C for 3 minutes but not for 5 minutes.

**Habitat:** This organism was cultured from pork trimmings and finished sausage but not from other sausage ingredients. It was isolated occasionally from plant equipment and tables, but it is logical to assume that its occurrence there arose by contamination from pork trimmings since it was isolated repeatedly from previously unopened barrels of trimmings.

#### DISCUSSION

The fermentation reactions and morphology of the organism indicate that it should be classified as a member of the family *Lactobacteriaceae*. In placing an unknown organism in this family as it is defined in *Bergey's Manual* (1948), rods fermenting carbohydrates to produce mainly lactic acid must fall in either the genus *Lactobacillus* or *Microbacterium*. These genera are separated entirely on the basis of catalase production by members of the latter but not of the former. The genus *Microbacterium*, as now recognized, contains 2 species which have been well known for many years. A possible third species *M. liquefaciens* has been shown by Doetsch and Rakosky (1950) practically to be identical with *M. lacticum*. The unknown organism described here certainly falls in the genus *Microbacterium* by reason of its catalase production although in its morphology and low heat resistance it more nearly resembles members of the genus *Lactobacillus*.

H. L. Jensen (1934) considered the members of the genus *Microbacterium* to belong more properly in the genera *Mycobacterium* and *Corynebacterium*. The present authors cannot consider this a reasonable classification for the organism described since it does not show morphological characteristics of either of these groups and displays clearly the biochemical relationships of a lactobacillus.

#### SUMMARY

A gram positive rod isolated from fresh pork sausage was found to produce catalase, ferment carbohydrates with lactic acid as the principal end product, and to have low heat resistance. The organism is classifiable as a member of the genus *Microbacterium* but differs from previously described species of that genus in its source and heat resistance. Transfers from old cultures stored in a refrigerator frequently show large bodies, the significance of which is not fully understood. The name *Microbacterium thermosphactum* spec nov, is proposed.

#### REFERENCES

- BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1948 *Bergey's manual of determinative bacteriology*. 6th Edition. Williams and Wilkins Co., Baltimore, Maryland.

- CORI, C. F., AND CORI, G. T. 1929 Glycogen formation in the liver from D- and L-lactic acid. *J. Biol. Chem.*, **81**, 389-403.
- DIENES, LOUIS, AND WEINBERGER, H. J. 1951 The L forms of bacteria. *Bact. Revs.*, **15**, 245-288.
- DOETSCH, R. N., AND RAKOSKY, J. 1950 Is there a *Microbacterium lacticum*? *Bact. Proc.*, **1950**, 38.
- FEIGL, F. 1946 *Qualitative analysis by spot tests*. Elsevier Publishing Co., New York, N. Y.
- JENSEN, H. L. 1934 Studies on saprophytic Mycobacteria and Corynebacteria. *Proc. Linnean Soc. N. S. Wales*, **59**, 19-61.
- JOHNSON, F. H., AND GRAY, D. H. 1949 Nuclei and large bodies of luminous bacteria in relation to salt concentration, osmotic pressure, temperature, and urethane. *J. Bact.*, **58**, 675-688.
- PEDERSON, C. S., PETERSON, W. H., AND FRED, E. B. 1926 The forms of lactic acid produced by pure and mixed cultures of bacteria. *J. Biol. Chem.*, **68**, 151-164.
- PENFOLD, W. J. 1922 The action of the pneumococcus on aromatic amino bodies. *Med. J. of Australia*, **2**, 120.
- SEVAG, M. G. 1933 Über de Beziehungen Zwischen Enzymatischer Aktivität, Morphologie, und Farbbarkeit von Buttersäurebakterien und über den Mechanismus der Resastmung Sussamenfassung. *Z. Hyg. Infektionskrankh.*, **114**, 756-768.
- SULZBACHER, W. L., AND MCLEAN, RUTH A. 1951 The bacterial flora of fresh pork sausage. *Food Technol.*, **5**, 7-8.
- VAN SCHOUWENBURG, K. L. 1940 On the catalase content of luminous bacteria. *Enzymologia*, **8**, 344-352.