The importance of Microbacterium thermosphactum in the microbiology of meats

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INTRODUCTION

When studying the flora of fresh pork sausages, Sulzbacher & McLean (1951) classified 14.9 % of their isolates as *Microbacterium*. Later, McLean & Sulzbacher (1953) proposed the name *Microbacterium thermosphactum* for these non-heat resistant, Gram positive, catalase positive, non-sporing rods, which fermented carbohydrates with lactic acid as the principal end product, which did not produce H_2S, indol, liquefy gelatin, attack urea or nitrate, and were unable to grow on citrate. Since then, these organisms have been isolated by numerous workers from various types of meats such as beef (Rogers & McCleskey, 1957; Wolin, Evans & Niven, 1957; Ayres, 1960; Jaye, Kittaka & Ordal, 1962; Weidemann, 1965; Brownlie, 1966, Barlow & Kitchell, 1966; Davidson, Mobbs & Stubbs, 1968), lamb (Barlow & Kitchell, 1966), pork (Gardner, 1966; Gardner, Carson & Patton, 1966, 1967), chicken (Thornley, 1957), turkey (Barnes & Impey, 1968; Barnes & Shrimpton, 1968), frankfurters (Drake, Evans & Niven, 1958), pork sausages, sausage meat, porkburgers and steakburgers (Gardner, 1966; Dowdell & Board, 1968).

Because of their numerical importance in prepacked pork, a selective medium (STAA) was developed (Gardner, 1966) and when used with comminuted fresh meats, the predominance of *M. thermosphactum* in the flora of such meats was recognised. In our laboratory STAA is routinely used for the enumeration of *M. thermosphactum* and the results of some of this work are presented in this paper.

MATERIAL AND METHODS

Samples. All samples tested were of routine factory production, except for a few samples of spoiled Wiltshire bacon. After collection in sterile containers at the factory, they were transported in a box containing ice to the laboratory, where they were held at 4° and microbiological analyses were carried out within 24 h.

Microbiological analysis. From each sample 5 g were transferred aseptically to a bottle containing 45 ml 0.1 % (w/v) peptone water. The sample was blended for 20 sec using an Ultra-Turrax homogeniser. Serial dilutions were prepared in 0.1 % (w/v) peptone diluents and 0.1 ml aliquots spread over the surface of prepared plates, using the drop and spread technique. For all samples two media were used:

(a) Nutrient agar (% (w/v) peptone (Oxoid, L37) 1.0; Lab Lemco, 0.3; NaCl, 0.5; agar, 1.3; pH 7.4. Sterilised at 15 lbs for 15 min). This medium was used for total bacterial counts of the meat.

(b) STAA (Gardner, 1966). This medium was used as described earlier for the selective enumeration of *M. thermosphactum.*

Colonies were enumerated on both media after incubation at 22° for 48 h.

RESULTS

M. thermosphactum was found in all 123 uncured meat samples tested (Table 1). The flora of 10 % of samples contained < 1 % *M. thermosphactum*; 60 % from 1—50 % *M. thermosphactum* and 24 % from 51—100 % *M. thermosphactum*. 6 % of samples were found to have more than 100 % *M. thermosphactum*, because the count on the selective medium STAA was higher than the total count on nutrient agar. In general these trends were similar for pork sausages, pork mince, beef sausages, beefburgers and pork trimmings such as head meat, minced rinds, bone, pluck and fat trimmings.

Table 1. *Distribution of the levels of* M. thermosphactum *in meat products.*

Product	*No. of samples tested*	*No. of samples in each group* M. thermosphactum (% *of flora*)*				
		Not found	*<1*	*1—50*	*51—100*	*>100*
Pork sausages	29	0	2	16	10	1
Pork mince	41	0	9	24	5	3
Beef sausages	24	0	1	12	8	3
Beefburgeers	18	0	0	14	3	1
Pork trimmings for sausage production	11	0	0	8	3	0
Total fresh meats	123	0	12	74	29	8
Fresh and spoiled Wiltshire bacon	29	16	10	3**	0	0

* $\frac{\text{Count on STAA}}{\text{Count on nutrient agar}} \times 100$

** The flora of these three samples contained, 4, 6 and 14 % *M. thermosphactum.*

In the case of the bacon samples only 3/29 had a level of over 1 % *M. thermosphactum* in the flora, and in 16 samples (55 %) it was not detected at all in the flora; the remainder all had less than 1 %.

There appeared to be no relationship between the total viable count and the percentage of the flora as *M. thermosphactum* (Fig. 1 (a)-(f)). In each product there was a large variation in the level of contamination, but most samples would fal' within the range 500.000 to 50.000.000 organisms/g.

In general *M. thermosphactum* forms a significant part of the flora of comminuted fresh meats, where because of the nature of the raw materials bacterial contamination can be very high.

DISCUSSION

Microbacterium thermosphactum has been found in many meat products

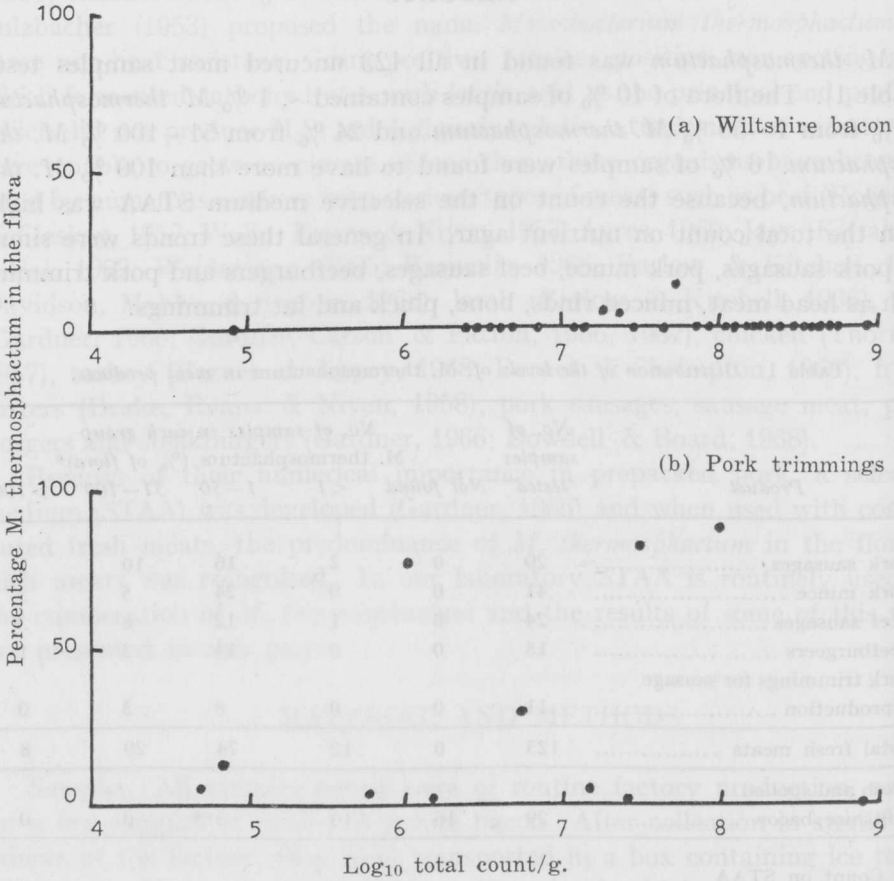
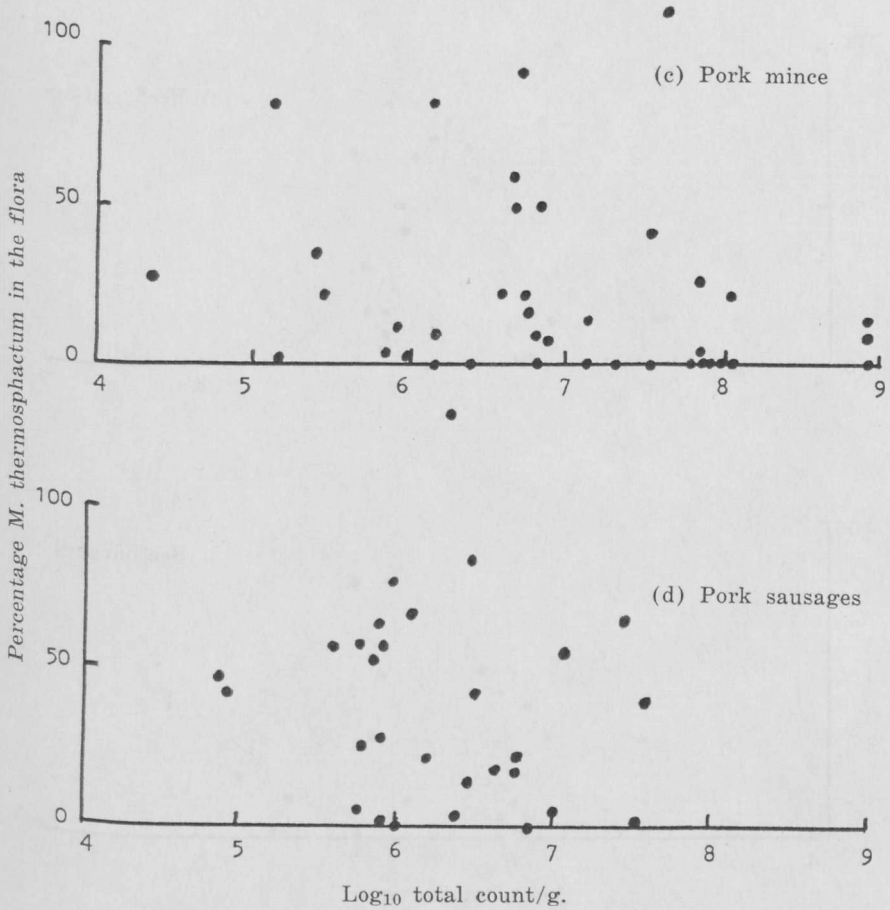


Fig. 1. Relationship between total count and percentage M. thermosphactum in the flora of meat products

and to date there appears to be no information of this organism from any other than a meat environment. The ecological data on this organism will be discussed in relation to the different types of meats.

Poultry. Thornley (1957) examined the effect of radiation on the microbiology of minced chicken meat canned in an atmosphere of N_2 and isolated *M. thermosphactum* from samples irradiated at 17.5×10^4 and 25×10^4 rads and stored at 5° for 21 and 41 days. They were also found in samples irradiated in the frozen state at 25×10^4 rads and stored at 5° for 14 days. Thornley cites the work of Niven & Chesbro, who in 1956 found that a *Microbacterium* species was important in the spoilage of irradiated meat.

Barnes & Impey (1968) examined the composition of the flora isolated from total count plates incubated at 30°, 20° and 1° of an eviscerated packaged turkey carcase stored at 1° for 27 days. From the plates incubated at



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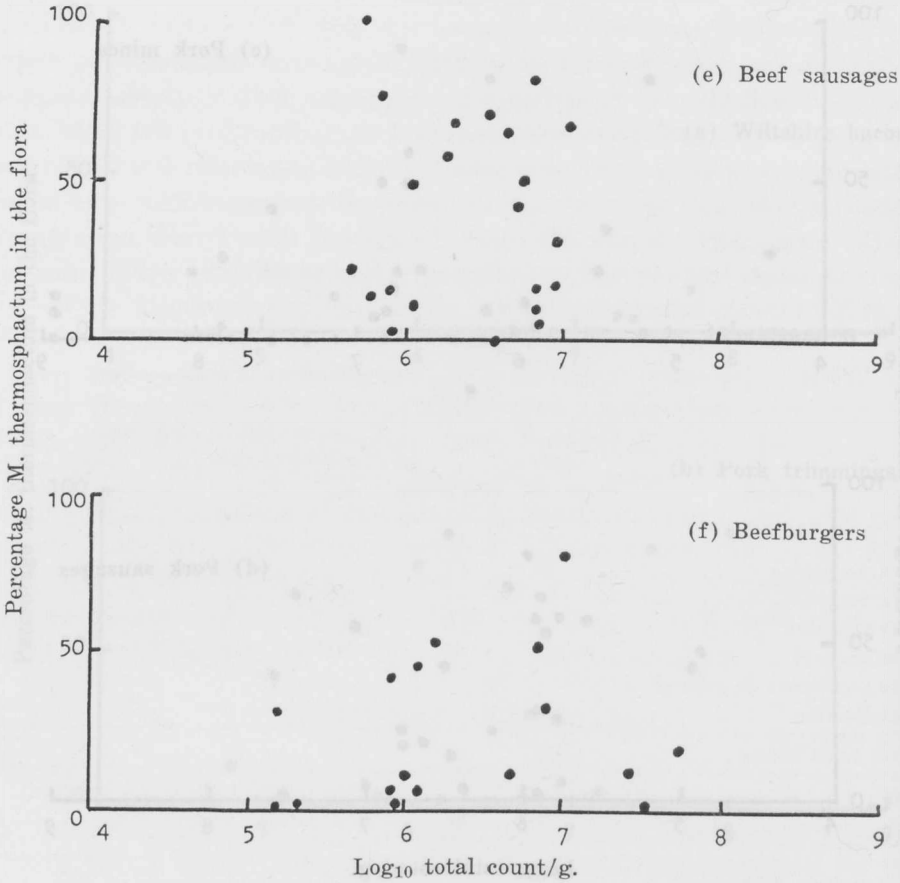
30°, 26 % of the flora was found to be *M. thermosphactum*. This organism, however, was not found on plates incubated at either 20° or 1°. Barnes & Shrimpton (1968) also examined eviscerated turkey carcases packaged in a gas impermeable film and although none were found in the fresh samples, *M. thermosphactum* were isolated from 2 samples held at 1° for 21 days and were found to represent 9 and 40 % of the flora for each sample. They were not found in turkeys stored at 1° for 27 days or 10° for 7 days or 20° for 3 days.

Lamb. There is little information in the literature on the microbiology of lamb, but recently Barlow & Kitchell (1966) found that the spoilage of



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lamb chops is brought about rapidly by *M. thermosphactum*, i.e. within 3 days in a gas permeable film and 6 days in a vacuum pack at 5°.

Pork. When pork fillets were loose wrapped in polythene and held at 7° for 7 days, the spoilage flora was found to contain from 9 to 74 % *M. thermosphactum* (Gardner, 1966). In studies on the flora of prepacked pork stored at 16°, using a number of different wrapping materials, *M. thermosphactum* was found to constitute an important fraction of the flora (Gardner, Carson & Patton, 1966). In subsequent experiments (Gardner, Carson & Patton, 1967) this organism was isolated from the flora of stored packaged pork (Table 2). Also, in 6 samples of fresh pork used in these experiments, *M. thermosphactum* was only found in 1, representing 24 % of the flora.

Table 2. M. thermosphactum *in the flora of packaged and unpackaged pork (Gardner, Carson & Patton, 1967)*

Storage treatment	*Average incidence of* M. thermosphactum (% *of flora*)	
	4 days at 16°	*14 days at 2°*
Film:		
None (aerobic control)	3	2
Gas permeable	10	11
Gas impermeable	18	17

Beef. Wolin, Evans & Niven (1957) noted that irradiated beef stored in cellophane pouches at 2° was spoiled by *M. thermosphactum*, the spoilage being characterized by a sour and flavour. Also of 5 fresh unirradiated beef samples, the flora of 3 was dominated by this organism, and on the other two there were about equal numbers of *M. thermosphactum* and pseudomonads. Ayres (1960) also found *M. thermosphactum* in prepacked beef, and Weidemann (1965) found that the pH of beef muscle surfaces stored under N_2 at 0° fell during storage, caused by the souring action of *M. thermosphactum*. Barlow & Kitchell (1966) found that beef steaks prepacked in a gas permeable film had 12.7 % *M. thermosphactum* in the flora, but when vacuum packed this figure was 8 %. Brownlie (1966) used a strain isolated from »Cryovac» packaged meat in studies of environmental factors which affect *M. thermosphactum*. Davidson, Mobbs & Stubbs (1968) isolated *M. termosphactum* from beef, but did not specify any other detail of the environment and used strains from this source in a study of the properties of these bacteria.

Kirsch, Berry, Baldwin and Foster (1952) isolated numerous organisms from refrigerated ground beef, which were classified as lactobacilli. Their description would suggest that these might have been *M. thermosphactum*,

except that their isolates were catalace negative. In this test these workers incubated strains on slopes of nutrient agar at 30° before testing; such conditions are now known not to favour catalase formation by *M. thermosphactum* (Davidson, Mgbbs & Stubbs, 1968). In minced beef, Rogers & McCleskey (1957) isolated from samples stored for 14 days at 7°, 5 % *M. thermosphactum* from total count plates incubated at 7°. When duplicate plates were incubated at 37°, the incidence increased to 7 %. Jaye, Kittaka & Ordal (1962) found that 13/145 (9 %) of colonies isolated from total count plates of minced beef stored at −1.1° and 3.3° under a gas permeable or gas impermeable film were *M. thermosphactum*. The spoilage in gas permeable packs was regarded as putrefactive, while, that in the gas impermeable packs was of a souring nature.

Fresh sausages and comminuted fresh meats. It was Sulzbacher and McLean (1951) who originally isolated and later described *M. thermosphactum*. This organism was found to represent 14.9 % of the flora of fresh pork sausages. McLean & Sulzbacher (1953) isolated *M. thermosphactum* from pork trimmings and finished sausages, but not from other sausage ingredients. They also isolated it occasionally from plant equipment and tables and assumed that these had been contaminated by the pork trimmings. Drake, Evans & Niven (1958) found that 61/167 (36 %) isolates from cellophane wrapped frankfurters stored at 2° were *M. thermosphactum*. When stored at 10°, 41 strains out of 186 (22 %) were found. *M. thermosphactum* and yeasts were the two most common groups of organism in the surface slime of the frankfurters, the former being more numerous at 2°. Gardner (1966) using a selective medium found that of 5 samples of pork sausages from different manufacturers stored for 7 days at 7°, all contained *M. thermosphactum*, representing from 64—124 % of the total viable count. In the same study isolates were obtained from steakburgers and porkburgers. David (1966) isolated some strains of *M. thermosphactum* from pork sausages stored for 2 days at room temperature, and they have also been reported as the important spoilage bacteria of pork and beef sausages (Dowdell & Board, 1968). The numerical importance of *M. thermosphactum* in the flora of 123 samples of fresh pork and beef sausages, beefburgers, pork mince and pork trimmings for sausage manufacture has been shown in this paper. They were found in all samples and in most formed a large proportion of the total viable count.

Cured meats. M. thermosphactum has rarely been found on cured meats and is not regarded as a spoilage organism. Gardner (1966) detected relatively small numbers in Wiltshire bacon stored for a week at 7°; all 3 samples contained less than 1 %. In an investigation of the bacteriology of baconburgers, a comminuted cured pork product, *M. thermosphactum* was only found in one sample of fresh material. They were not isolated from baconburgers with or without sulphite during storage at 22°, 10° or 5° (Gardner, 1968).

Gardner & Patton (1968) very occasionally found small numbers of *M. thermosphactum* in the flora rind and meat surfaces of matured Wiltshire bacon sides. In the majority (55 %) of samples of fresh and spoiled Wiltshire bacon examined in the present study *M. thermosphactum* was not detected. In only 3 samples were they present in levels over 1 %, i.e. 4, 6 and 14 %. In the remainder the organism was found to represent only a minor proportion of the flora, i.e. less than 1 %. *M. thermosphactum* is markedly sensitive to curing compounds such as salt and sodium nitrite (Brownlie, 1966); 25 strains were unable to grow in broth culture at pH 5.5 in the presence of 200 p of sodium nitrite/m. Baconburgers can contain 300 p sodium nitrite/m in the aqueous phase and the pH is around 6 (Gardner, 1968). Therefore it is not surprising that they are only infrequently found in the flora of cured meats such as Wiltshire bacon.

In conclusion, it would seem that the processing of freash meats, e.q. comminution, packaging in air or inert gases, and irradiation, creates conditions favourable for the multiplication of *M. thermosphactum*. Therefore in routine microbiological analysis of such meats the numbers of these bacteria would be a guide to the levels of potential spoilage organisms. In our experience the selective medium STAA (Gardner, 1966) is a useful tool for this purpose.

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