Prokaryotes (2006) 4:477–491
DOI: 10.1007/0-387-30744-3_12

CHAPTER 1.2.12

The Genus *Brochothrix*

ERKO STACKEBRANDT AND DOROTHY JONES

The genus *Brochothrix* contains Gram-positive, nonsporeforming, nonmotile, catalase-positive, facultatively anaerobic, regular, rod-shaped bacteria that show characteristic changes in cell morphology during growth. The genus was proposed by Sneath and Jones (1976) for some meat spoilage organisms, previously designated "*Microbacterium thermosphactum*" (McLean and Sulzbacher, 1953). The genus *Brochothrix* contains the type species *B. thermosphacta* (see Sneath and Jones, 1986) and *B. campestris* Talon et al. (1988), described for isolates from soil and grass. As most of the scientific interests have focused on *B. thermosphacta* because of its association with off-odor development in meats, especially in prepacked products held at refrigeration temperatures, most of the information on the genus is consequently derived from studies on this species. There is no evidence that any *Brochothrix* strain is pathogenic to humans or animals. Neither species has been exploited in industrial processes.

The type strain of *B. thermosphacta* is ATCC [{www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide=search=11509}{11509}]^T (DSM 20171^T) and that of *B. campestris* is CIP [{www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide=search=10290}{10290}]^T = $S_3{}^T$ (DSM 4712^T).

Phylogeny and Classification

The bacteria were first isolated and described by Sulzbacher and McLean (1951) during studies on pork sausage meat and were later allocated to the genus *Microbacterium* as a new species, *M. thermosphactum* (McLean and Sulzbacher, 1953). Their classification in the genus *Microbacterium* was due largely to the then poor circumscription of that genus (see Keddie and Jones, 1981). McLean and Sulzbacher (1953) noted the marked difference in cell morphology between *M. thermosphactum* and *M. lacticum*, the type species of the genus, and also commented on the close physiological resemblance between *M. thermosphactum* and the lactobacilli. However, at that time, both *Microbacterium* and *Lactobacillus* were classified in the family Lactobacteriaceae, and the main distinction between the two genera was catalase production (Breed et al., 1948), therefore, McLean and Sulzbacher (1953) assigned their catalase-positive isolates to the genus *Microbacterium*.

Later workers confirmed and augmented the differences between *M. thermosphactum* and *M. lacticum*, not only in cell morphology (Davidson et al., 1968b; Jones, 1975) but also in enzymology and protein profiles (Robinson, 1966; Collins-Thompson et al., 1972), in peptidoglycan structure (Schleifer, 1970; Schleifer and Kandler, 1972), and in DNA base composition (Collins-Thompson et al., 1972). In addition, numerical taxonomic studies showed that *M. thermosphactum* strains formed a relatively homogeneous taxon (intra-group similarity greater than 85%) quite distinct from *M. lacticum* (Davis and Newton, 1969a; Davis et al., 1969b; Jones, 1975; Wilkinson and Jones, 1977). The same studies indicated that the closest associates of *M. thermosphactum* were the genera *Listeria* and *Lactobacillus*, and in one study, *Kurthia* (Davis and Newton, 1969a). In none of the studies, however, were the similarities close enough to justify the inclusion of *M. thermosphactum* as a new species in any of these genera. Consequently, Sneath and Jones (1976) concluded that *M. thermosphactum* strains were sufficiently distinct from other Gram-positive bacteria to merit a separate genus and proposed that they be reclassified in a new genus *Brochothrix* as *B. thermosphacta*. These authors were aware that the taxonomic relatedness of *Brochothrix* to other Gram-positive bacteria at the suprageneric level was problematic. After evaluating all the data then available, including the presence of catalase and cytochromes in *B. thermosphacta* (Davidson and Hartree, 1968a; Davidson et al., 1968b) and the reported difference in fatty acid composition between *Brochothrix* and *Lactobacillus* (Shaw and Stead, 1970), they tentatively placed *Brochothrix* in the family Lactobacillaceae (Bucha-

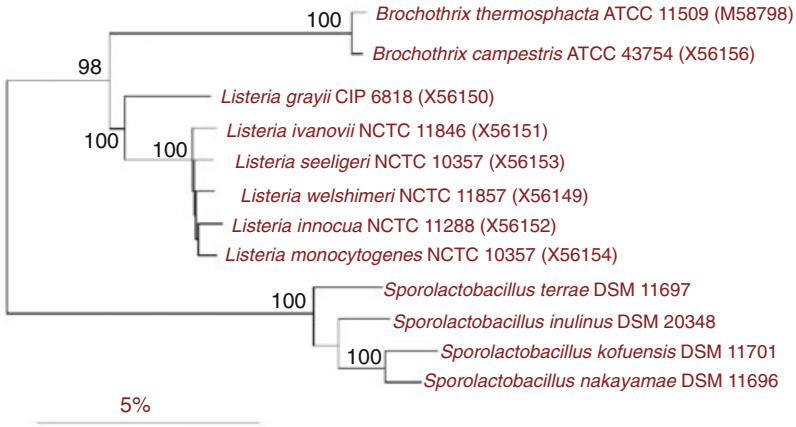


Fig. 1. Dendrogram of 16S rRNA gene similarities (De Soete, 1983) between type strains of species of the genera *Brochothrix*, *Listeria* and *Sporolactobacillus*. Numbers indicate the percentage of bootstrap samplings, derived from 1000 resamplings (Felsenstein, 1993). Bar indicates 5% sequence divergence. Numbers in parentheses are accession numbers of 16S rRNA gene sequences. The sequences of *S. terrae*, *S. kofuensis* and *S. nakayamae* were determined for this revision.

nan and Gibbons, 1974); this family included the genus *Lactobacillus* and three other genera designated as having uncertain affiliation, i.e., *Listeria*, *Erysipelothrix* and *Caryophanon*.

Subsequently, it became apparent that in phenotype, the members of the genus *Brochothrix* more closely resembled those of the genus *Listeria* than those of the genus *Lactobacillus* (see Sneath and Jones, 1986). Both *B. thermosphacta* and *Listeria* spp. possess catalase and cytochromes (Davidson and Hartree, 1968a; Feresu and Jones, 1988). They also contain *meso*-diaminopimelic acid in the cell wall peptidoglycan (Schleifer and Kandler, 1972), possess menaquinones with seven isoprene units (MK-7) as the predominant isoprenoid quinone (Collins et al., 1979; Collins and Jones, 1981; Feresu and Jones, 1988), and contain predominantly methyl-branched chain fatty acids (Shaw, 1974; Feresu and Jones, 1988). This close phenotypic similarity between *B. thermosphacta* and *Listeria* spp. was confirmed by the results of the 16S rRNA oligonucleotide sequencing studies of Ludwig et al. (1984). These studies showed that *B. thermosphacta* and *L. monocytogenes* are phylogenetically very closely related and form one of the several sublines within the *Bacillus-Lactobacillus-Streptococcus* cluster of the clostridial subbranch of the Gram-positive eubacteria (Stackebrandt and Woese, 1981; Collins et al., 1991).

The species *B. campestris* was named by Talon et al. (1988) on the basis of numerical taxonomic and DNA hybridization studies of a number of strains of *Brochothrix* spp. isolated from a variety of sources, including grass and soil.

On the basis of analyses of 16 rRNA gene sequences, *Brochothrix thermosphacta* and *B. campestris* are highly related, sharing 99.3% sequence similarity. Their phylogenetic neighbors are members of the genus *Listeria* (92.8–96.6%) and less closely related members of the genus *Sporolactobacillus* (<92% on the basis of

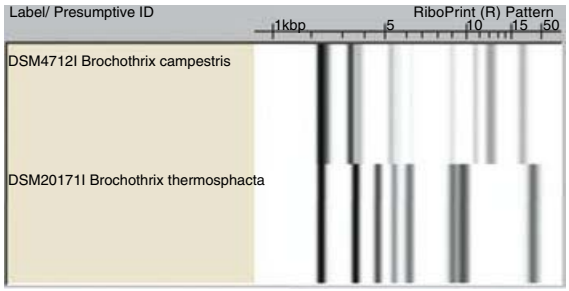


Fig. 2. Diversity of normalized *Eco*RI ribotype patterns found within the two type strains of *Brochothrix* species.

ARB [from Latin arbor, tree] analysis; Ludwig et al., 2004). As depicted in the phylogenetic tree (De Soete, 1983; Fig. 1), the three genera are phylogenetically well separated. The small phylogenetic distance found between the two *Brochothrix* type strains are also seen among some of the *Listeria* type strains, i.e., *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, *L. innocua* and *L. monocytogenes* (>99.1%). The majority of the branching points are supported by high bootstrap values.

As determined by the S1 nuclease method at 60°C, *B. campestris* strains exhibit about 15% DNA similarities to *B. thermosphacta* (Talon et al., 1988), which identifies both strains as sound genomospecies. This is also reflected by the significant difference in the RiboPrint patterns (Fig. 2).

Chemotaxonomy

Other than the presence of *meso*-diaminopimelic acid in the cell wall of *Brochothrix campestris* (Talon et al., 1988) and *B. thermosphacta* (Schleifer, 1970; Schleifer and Kandler, 1972), all the information on chemical composition is based on studies with *B. thermosphacta*. Arabinose and galactose are not present in the cell wall (see

Schleifer and Kandler, 1972), nor are mycolic acids (Minnikin et al., 1978; Feresu and Jones, 1988). The major fatty acid is 12-methyltetradecanoic (*anteiso*-$C_{15:0}$ with substantial amounts of 14-methylhexadecanoic (*anteiso*-$C_{17:0}$) and 13-methyltetradecanoic (*iso*-$C_{15:0}$) acids (Shaw and Stead, 1970; Feresu and Jones, 1988). The major phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The glycolipid fraction contains an acylated glucose and small amounts of a glycosyl diglyceride tentatively identified as dimannosyl diglyceride (Shaw and Stead, 1970; Shaw, 1974). Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) whole-cell protein profiling has been used to characterize *B. thermosphacta* during monitoring changes in populations in chilled vacuum-packed beef (Sakala et al., 2002)

Brochothrix thermosphacta and *B. campestris* contain several membrane-bound respiratory cytochromes, such as aa_3, a_3, *b* and *d* (Gil et al., 1992), but only strains of the former species were subjected to analyses at different growth temperatures. Under moderate aeration, cytochromes *b* and *d* dominate at 30°C, while aa_3 and a_3 are the dominating components when grown at 10°C and 15°C. Reduction of the oxygen content in batch cultures lead to an enhancement of cytochrome *d* and a reduction in *a*-type cytochromes. Cytochrome *c* was missing in both species, which otherwise show similar cytochrome compositions when grown at 10°C.

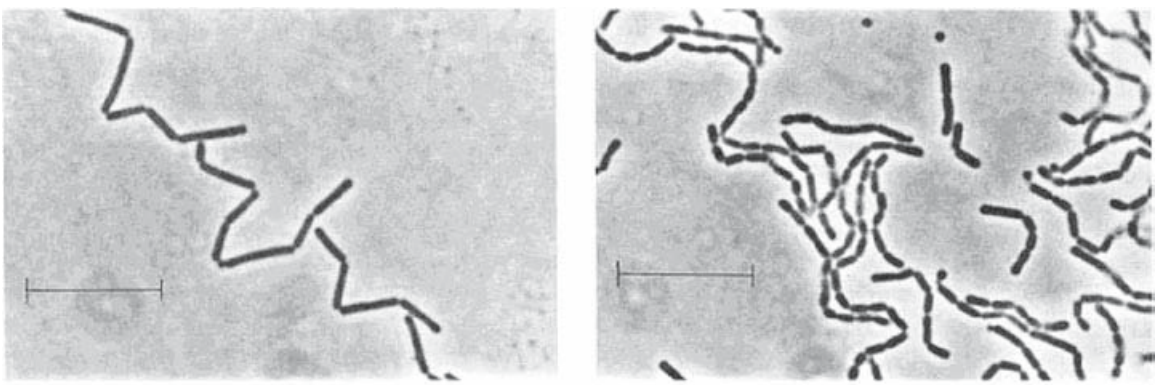
Menaquinones are the sole respiratory quinones; MK-7 is the major component, and MK-6 and MK-5 are minor components (Collins et al., 1979; Collins and Jones, 1981; Feresu and Jones, 1988).

The G+C content of the DNA of *B. thermosphacta* is 36–37 mol% (Collins-Thompson et al., 1972; Collins et al., 1987; Feresu and Jones, 1988) and of *B. campestris*, 38 mol% (Talon et al., 1988).

Characterization

The colonial morphology of *Brochothrix* spp. is not particularly diagnostic. After 24–48 h, the colonies are circular, 0.75–1 mm in diameter, convex with entire margins, and not pigmented. In young cultures of *B. thermosphacta*, two types of colony varying in size and density may be present. These can be so distinct that the culture may appear to be contaminated (see Barlow and Kitchell, 1966). In older cultures of the same species, the edge of the colony breaks up and the center becomes raised to give a "fried egg" appearance. Neither of these two phenomena has been reported for *B. campestris* (Talon et al., 1988). *Brochothrix* spp. are nonhemolytic, but sometimes an area of weak greening is apparent around colonies on blood agar.

Gram-stains on exponential-phase cultures of *B. thermosphacta*, performed on 18–24 h and 2-day cultures grown on nutrient agar such as blood agar base (Columbia or BAB no. 2, Oxoid) or APT medium incubated at 20–25°C, show regular, unbranched rods that occur singly in pairs and short chains and in long, kinked, filamentous-like chains (Fig. 3a) that bend and loop to give characteristic knotted masses. This phenomenon has not yet been reported for *B. campestris*; in this species 24-h cultures are reported to consist of a mixture of long and short rods that usually occur singly or in pairs (Talon et al., 1988). In older cultures of *B. thermosphacta*, the rods give rise to coccoid forms (Fig. 3b) that when subcultured onto a suitable medium develop into rod forms. Both rod and coccal forms are Gram-positive, but a proportion may appear Gram-negative. They are nonmotile and do not form endospores or capsules. Talon et al. (1988) reported that about one-half of the *B. thermosphacta* strains they examined produced slime from sucrose in broth culture.



(a) (b)

Fig. 3. *Brochothrix thermosphacta* isolate, grown on blood agar base no. 2 (Oxoid) at 25°C. (a) After 24 h, showing regular rods in chains. (b) After 48 h, showing development of coccoid forms. Bar = 10 µm.

In MRS medium the type strains of *B. thermosphacta* and *B. campestris* produce acid fermentatively from glucose, ribose, fructose, mannose, salicin, maltose, D-cellobiose, mannitol, trehalose and, weakly, from gluconate (R. Pukall, unpublished observation); acid production from *N*-acetylglucosamine has also been reported; they are methyl-red and Voges-Proskauer positive. Esculin is hydrolyzed. Exogenous citrate and urea are not utilized. Indole and H_2S are not produced, nitrate is not reduced, gelatin is not liquefied, and arginine is not hydrolyzed (McLean and Sulzbacher, 1953; Sneath and Jones, 1976; Wilkinson and Jones, 1977; Talon et al., 1988).

With use of the API ZYM test system (API System, La Balme des Grottes, France), both species are reported to produce the following arylamidases: phenylalanine, histidine, glycyl-phenylalanine, seryl-tyrosine, glutamate, tryptophan, and histidyl-L-phenylalanine (Talon et al., 1988).

In the API esterase tests, Talon et al. (1988) noted that all five strains of *B. campestris*, but only 12 of 165 strains of *B. thermosphacta* tested, hydrolyzed naphthylbutyrate, naphthylvalerate, naphthylcaproate, naphthylcaprylate, naphthylnonanoate and naphthylcaprate.

According to the BIOLOG GP2 substrate panel (R. Pukall, unpublished observation), *B. thermosphacta* DSM [{www.ncbi.nlm.nih.gov/entrez /query.fcgi?db=nucleotide=search=20171}{20171}]^T and *B. campestris* DSM [{www.ncbi.nlm.nih.gov/entrez/query.fcgi? db=nucleotide=search=4712}{4712}]^T utilize dextrin, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, amygdalin, arbutin, D-cellobiose, D-fructose, gentiobiose, D-gluconic acid, μ-D-glucose, *m*-inositol, maltose, maltotriose, D-mannitol, D-mannose, pyruvic acid, palatinose, D-trehalose, thymidine, β-methyl-D-glucoside, D-ribose, salicin, glycerol, inosine and uridine. Neither type strain utilizes α-cyclodextrin, β-cyclodextrin, glycogen, inulin, mannan, Tween 40, Tween 80, L-arabinose, D-arabitol, L-fucose, D-galactose, D-galacturonic acid, α-D-lactose, lactulose, D-melizitose, D-melibiose, D-tagatose, xylitol, D-xylose, acetic acid, α- and β-hydroxy-butyric acid, γ-hydroxy-butyric acid, p-hydroxy-phenyl acetic acid, α-keto-glutaric acid, lactamide, D-lactic acid methyl ester, D- and L-malic acid, mono-methyl succinate, propionic acid, succinamic acid, succinic acid, N-acetyl-L-glutamic acid, L-alaninamide, D- and L- alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, α- and β-methyl-D-galactoside, 3-methyl-glucose, α-methyl-D-glucoside, α-methyl-D-mannoside, D-raffinose, sedoheptulosan, D-sorbitol, stachyose, L-serine, putrescine, 2,3-butandiol, 2'deoxy adenosine, adenosine-5'monophosphate, thymidine-5'monophosphate, uridine-5'monophosphate, fructose-6-phosphate, glucose-1-phosphate, and glucose 6-phosphate.

Both species are susceptible to a wide range of antibiotics. Using antibiotic disks on Mueller-Hinton agar, Talon et al. (1988) reported all strains of *B. thermosphacta* (104 tested) and *B. campestris* (5 tested) were sensitive to novobiocin, tetracycline (except for three strains of *B. thermosphacta*), amikacin, tobramycin, gentamicin, and ampicillin and were resistant to oxacillin, nalidixic acid, and colistin (except the type strain of both species and one other strain of *B. campestris*). A wider range of antibiotics was tested with *B. thermosphacta* (15 strains) on nutrient agar medium by Feresu and Jones (1988).

Further diagnostic information on both species is given in Table 1. Both type strains are suitable reference strains, but it should be noted that *B. campestris* grows with 8% NaCl if incubated for 6 days (Talon et al., 1988).

Sneath and Jones (1976) reported that *B. thermosphacta* does not produce deoxyribonuclease, but later studies have detected both deoxyribonuclease and ribonuclease activity in strains of this species (Wilkinson and Jones, 1977; Feresu and Jones, 1988).

The results of many of the conventional tests can be influenced markedly by the media and methods used. Differences in methodology probably account for some of the discrepancies noted in the literature. Media and methods such as those used by Talon et al. (1988) and Wilkinson and Jones (1977) are suitable for most tests, and incubation should be at 20–25°C.

Differentiation of Brochothrix species

The most useful phenotypic characters for differentiating the two species are growth in the presence of 8 and 10% NaCl, growth on BM medium (Wilkinson and Jones, 1977) containing 0.05% potassium tellurite, hippurate hydrolysis, and production of acid from rhamnose (Table 1).

Molecular Identification

Affiliation of a yet unassigned isolate to the genus is unambiguously done by sequence analysis of the 16S rRNA gene when sequence differences between the type strains of the two species are significant (Stackebrandt and Goebel, 1994). The difference between the type strains of *Brochothrix* is 0.7% only, making it difficult to taxonomically place a new strain. A

Table 1. Additional and differential features of *Brochothrix* spp.

Features	*B. thermosphacta*	*B. campestris*
Growth with		
NaCl, 8% (2 days)	+	–
NaCl, 10% (2–7 days)	d	–
Growth with and reduction of potassium tellurite, 0.05%	+	–
Reduction of tetrazolium, 0.01%	d	+
Slime produced from sucrose	d	–
Resistance to furadoine	d	–
Hydrolysis of:		
Hippurate	–	+
Cellulose	+	ND
Tyrosine	–	ND
Xanthine	–	ND
Production of		
Phosphatase	+	ND
Sulfatase	–	ND
Utilization according to BIOLOG GP2		
Turanose	+	–
α-Keto valeric acid	+	–
L-Lactic acid	+	–
Rhamnose	–	+
Saccharose	+	–
Adenosine	+	–
D,L-α-glycerol-phosphate	+	–
Acid from		
Amygdalin	+	+
Arbutin	d	+
Dulcitol	+	ND
Gentobiose	+	d
Glycerol	–	+
Inositol	+	d
Inulin	d	ND
Lactose	+[a]	ND
Mannitol	+	d
Melezitose	d[a]	–[a]
Melibiose	d[a]	–[a]
Rhamnose	–	+
Sorbitol	d[a]	–[a]
Sorbose	–	ND
Starch	–	d
Sucrose	+	d[a]
Tagatose	+	d
Xylose	d	ND

Symbols and abbreviations: +, 90–100% of strains positive; –, 0–10% of strains negative; d, 11–89% of strains positive; ND, no data.

[a]Type strain negative in MRS. (de Man-Rogosa-Sharpe).

strain branching deeper than the bifurcation of the two type strains but closer to these than to members of the nearest neighboring genus must be taxonomically evaluated by means of genus-specific properties to decide whether it belongs to *Brochothrix* or whether it forms the nucleus of a novel genus.

Riboprints are available for the two type strains (Fig. 2), but patterns from more strains of both species are needed to evaluate the homogeneity of rrn cistron cleaving patterns of these taxa. Oligonucleotide primers targeting variable regions within the 16rRNA gene have been designed for the rapid detection of *Brochothrix* spp. (Grant et al., 1993). When combined with the capture of *Brochothrix* cells using a lectin immobilized on magnetic beads the polymerase chain reaction (PCR) assay targeting DNA from released cells was sufficiently sensitive to detect as low as about 100 colony forming units (CFU) per gram of chopped fresh chicken meat.

A rabbit polyclonal antibody-linked probe allowed detection of 76% of 800 presumptive *B. thermosphacta* isolates from British fresh sausages. Of collection strains investigated, only the type strain NCTC [{www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide=search=10822} {10822}]^T did not react, while one strain each of

Kurthia zopfii and *Staphylococcus aureus* reacted positively, demonstrating the lack of specificity (Stringer et al., 1995). Other detection methods (such as denaturing gradient gel electrophoresis [DGGE; Cocolin et al., 2001], oligonucleotide probes [Kim et al., 1991], and low molecular weight RNA profiles) were able to differentiate lactobacilli and *Listeria* spp. from *Brochothrix* spp. and other meat spoilage organisms (Collins-Thompson et al., 1991).

Differentiation of Brochothrix from Phylogenetically Neighboring Genera

Phenotypic identification of new isolates as *Brochothrix* spp. requires examination of cellular morphology and staining reactions, the maximum growth temperature, and relationships to oxygen and catalase production together with a number of conventional taxonomic tests, such as the ability to produce acid from various sugars. The chemical composition of the organisms also aids the identification of *Brochothrix* spp., but performing the relevant analyses for routine identification is not usually necessary. Features that differentiate the genus from other Gram-positive, nonsporeforming, rod-shaped bacteria are listed in Table 2.

Brochothrix spp. are readily distinguishable from the morphologically similar members of the genus *Kurthia* by examining the oxygen requirements. *Brochothrix* is facultatively anaerobic whereas *Kurthia* is strictly aerobic. In addition, *Brochothrix* spp. produce acid from a wide variety of sugars. Most strains of *Kurthia* are highly motile.

The inability of *Brochothrix* spp. to grow at 35°C distinguishes them from members of the genera *Carnobacterium*, *Erysipelothrix*, *Lactobacillus* and *Listeria*. Although facultatively anaerobic, *Brochothrix* spp. grow best aerobically. This feature, together with their ability to grow on unsupplemented nutrient agar, serves to distinguish *Brochothrix* spp. from members of the genera *Erysipelothrix* and *Lactobacillus* but not from *Carnobacterium* and *Listeria*.

The production of catalase distinguishes *Brochothrix* spp. from the genera *Carnobacterium*, *Erysipelothrix*, and the vast majority of *Lactobacillus* spp., but not from *Listeria*. But as noted previously, care must be taken in examining *Brochothrix* for catalase because its production depends on both the growth medium and the incubation temperature. Growth on APT medium, BAB no. 2 or a similar medium for 24–48 h at 20–25°C is recommended; negative results are very frequently obtained if the bacteria are incubated at 30°C. Although catalase production by *Listeria* spp. is also dependent on the composition of the growth medium (Jones, 1975), all strains of *Listeria* are unequivocally catalase-positive at 35°C if the growth medium is suitable, whereas *Brochothrix* spp. do not grow at this temperature.

The genera *Brochothrix* and *Listeria* share many common characters. Members of both genera are facultatively anaerobic but grow better aerobically; produce acid from a variety of sugars; produce catalase, cytochromes, and menaquinones; possess a cell wall peptidoglycan with *meso*-diaminopimelic acid as the diamino acid; have similar polar lipid and fatty acid profiles and similar G+C content (Table 1). Members of neither genus grow on acetate medium and only poorly on MRS (De Man et al., 1960) medium. In contrast to the comment by Jones (1992), *B. thermosphacta* and *B. campestris* do grow on MRS medium, though the type strain of the latter species grows more slowly. Members of *Listeria* and *Brochothrix* may be distinguished by their morphology, by growth temperature, by motility, and also serologically.

Table 2. Features most useful in differentiating *Brochothrix* from morphologically similar genera.

	Brochothrix	*Listeria*	*Carnobacterium*	*Lactobacillus*	*Erysipelothrix*	*Kurthia*
Growth at 35°C	–	+	+	+	+	+
Motility	–	+	–	–	–	+
Catalase	+	+	–	–	–	+
Facultatively anaerobic	+	+	+	+	+	–
Acid from glucose	+	+	+	+	+	–
H_2S production	–	–	–	–	+	–
Major peptidoglycan diamino acid	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	Lysine or *meso*-A_2pm or ornithine	L-Lysine	L-Lysine
Major menaquinone	MK-7	MK-7	–	–	–	MK-7
Major fatty acid types	S, A, I	S, A, I	S, U, (C)	S, U, (C)	S, A, I, U	S, A, I
G+C mol%	36–38	36–42	33–37	32–53	36–40	36–38

Symbols and abbreviations: +, present; , absent; MK-7, menaquinones with seven isoprene units; S, straight chain saturated; A, *anteiso*-methyl branched; I, *iso*-methyl-branched; U, monounsaturated; and (C), cyclopropane ring fatty acids may be present.

Colonies of the genus *Brochothrix* do not show the blue-green coloration exhibited by *Listeria* spp. when viewed by obliquely transmitted white light. The inability of *Brochothrix* spp. to grow at 35°C and their lack of motility also distinguishes them from the genus *Listeria*. No serological crossreactions have been demonstrated between *B. thermosphacta* and species of the genus *Listeria*.

General Properties

Metabolism and Nutritional Requirements

Brochothrix spp. are aerobic, oxidase negative, catalase positive and facultatively anaerobic (Sneath and Jones, 1986; Talon et al., 1988), but better growth is achieved by *B. thermosphacta* aerobically (see Hitchener et al., 1979 and Gardner, 1981); no such information is yet available for *B. campestris*.

Brochothrix thermosphacta possesses enzymes for both the hexose-monophosphate and Embden-Meyerhof (glycolysis) pathways of glucose catabolism as well as a number of enzymes involved in pyruvate metabolism (Collins-Thompson et al., 1972; Grau, 1983). Fermentative metabolism of glucose always results in the production of L(+) lactic acid, but other end products depend on the growth conditions. McLean and Sulzbacher (1953) found only L(+) lactic acid present in detectable quantities. Davidson et al. (1968b) detected small amounts of acetic and propionic acids in addition to L(+) lactic acid. Hitchener et al. (1979) found that in glucose-limited continuous culture under anaerobic conditions, L-lactate and ethanol were produced in the approximate ratio of 3 : 1. The studies of Grau (1983) showed the major end products of anaerobic glucose fermentation to be primarily L-lactate, acetate, formate and ethanol, but that the ratios of these end products varied with conditions. Both the presence of acetate and formate and a pH below 6 increased L-lactate production from glucose. Of interest in this context is that the growth of *B. thermosphacta* is inhibited at a pH below 5 (Brownlie, 1966). Although McLean and Sulzbacher (1953) reported detectable CO_2 production (in Eldridge fermentation tubes), this has not been confirmed in any subsequent studies, but Hitchener et al. (1979) speculated that CO_2 was a likely end product in their studies on glucose-limited cultures.

The major end products of aerobic metabolism of glucose by *B. thermosphacta* growing on tryptone-based medium, on a minimal defined medium, or on meat are acetoin and acetic, isobutyric, isovaleric (3-methylbutyric), and 2-methylbutyric acids (Dainty and Hibbard, 1980; Dainty and Hibbard, 1983). In the minimal defined medium, glucose is believed to be the source of all the end products (Dainty and Hibbard, 1983); whereas in the complex medium and meat, only acetoin and acetic acid are derived from glucose; and isobutyric, isovaleric, and 2-methylbutyric acids are produced from valine, leucine and isoleucine, respectively (Dainty and Hibbard, 1980; Dainty and Hibbard, 1983). These compounds, or their derivatives, produce the sweet, sickly, malty odors that characterize the growth of *B. thermosphacta* (Dainty et al., 1985). The studies of Grau (1988) indicate that the substrates used by *B. thermosphacta* growing aerobically on meat include glucose, ribose, glycerol, glycerol-3-phosphate, and inosine; of these substrates, only glucose and ribose are metabolized during anaerobic growth.

In cultures of *B. thermosphacta* grown on a tryptone-based medium, enzymes of the tricarboxylic acid (TCA) cycle are almost totally absent (Collins-Thompson et al., 1972). However, Grau (1979) has suggested, on the basis of studies with a defined medium, that the TCA-cycle enzymes may be sufficiently active to provide substrates but not energy for synthesis.

Collins-Thompson et al. (1971) demonstrated the presence of a glycerol ester hydrolase (lipase) in cell suspensions and cell-free extracts of *B. thermosphacta*. The lack of significant lipase activity in supernatant fluids was confirmed by Papon and Talon (1988). This lipase, mostly produced during the logarithmic phase of growth is active on tripropionin, tricaproin, tricaprylin and trilaurin but not on tripalmitin. Because the temperature optimum of the lipase is 35–37°C with little or no activity below 20°C, this enzyme is unlikely to be important in meat stored at refrigeration temperatures; optimal temperatures for growth and lipase production was 24°C. The report that *B. thermosphacta* cultures attack tributyrin (Sutherland et al., 1975) has not been subsequently confirmed (Davis et al., 1969b; Patterson and Gibbs, 1978), and Papon and Talon (1988) could even demonstrate that growth and lipase production is inhibited by tributyrin.

Brochothrix thermosphacta requires cysteine, α-lipoate, nicotinate, pantothenate, *p*-aminobenzoate, biotin, and thiamine for growth (Grau, 1979). Thiamine can fulfil most, but not all, of the yeast extract requirements (Macaskie et al., 1981).

The production of cytochromes and catalase by *B. thermosphacta* depends on both the composition of the growth medium and the temperature of incubation (Davidson and Hartree, 1968a; Davidson et al., 1968b). The latter authors noted that *B. thermosphacta* strains grown on

APT medium (Baltimore Biological Laboratories [BBL], Cockeysville, MD; Evans and Niven, 1951) incubated at 20°C were always catalase-positive but that weak or negative reactions were obtained on HIA (heart infusion agar) medium (Difco, Detroit, MI) incubated at the same temperature The same authors reported that negative results were frequently obtained if the bacteria were grown on either medium at 30°C. Davidson and Hartree (1968a) reported the same effects of growth medium and incubation temperature on the quantitative cytochrome content of the organism. On APT medium incubated at 20°C, *B. thermosphacta* contains cytochromes baa_3 (Davidson and Hartree, 1968a). The temperature effect is difficult to explain; although not the optimum temperature for growth, *B. thermosphacta* grows well at 30°C. The effect of the composition of the culture medium may be related to the iron concentration. Davidson et al. (1968b) noted that APT medium contains added iron (8.0 μg/ml), and more recently both Grau (1979) and Thomson and Collins-Thompson (1986) have noted a high ferric iron requirement for the aerobic growth of *B. thermosphacta* in defined media. More recently it was shown that manganese can partially replace the iron requirement of *B. thermosphacta* under iron-limiting conditions (Thomson and Collins-Thompson, 1988).

Temperature and pH

There is general agreement that the temperature limits of growth are between 0 and 30°C (see Sneath and Jones [1976], Gardner [1981], and Talon et al. [1988]). Limited growth of *Brochothrix thermosphacta* was noted at 35°C and one strain was reported to grow at 37°C and 45°C (Gardner, 1981), but growth above 30°C has been rarely found. *Brochothrix campestris* does not grow at 37°C (Talon et al., 1988). The optimum temperature for growth is 20–25°C (see Gardner, 1981). The heat resistance of *B. thermosphacta* has received much attention because of its former classification in the genus *Microbacterium*, members of which are thermoduric. All workers agree that *B. thermosphacta* does not survive heating at 63°C for 5 min (see Gardner [1981] and Sneath and Jones [1986]).

The optimum pH for growth of *B. thermosphacta* is pH 7.0, but growth occurs within the range pH 5–9 (Brownlie, 1966).

Inhibitory Substances

The ability of *Brochothrix thermosphacta* to grow in the presence of NaCl has been examined by many workers (see Gardner, 1981). All strains grow at 6.5% NaCl and the majority can grow at NaCl concentrations up to 10% (Wilkinson and Jones, 1977; Talon et al., 1988). *Brochothrix campestris* strains do not grow in media with 8% or 10% NaCl and although there is no published information to indicate growth at 6.5% NaCl, circumstantial evidence indicates that *B. campestris* grows with 6.5% NaCl.

Growth of *B. thermosphacta* is inhibited by nitrite, but the degree of inhibition is related to the pH of the medium and incubation temperature (Brownlie, 1966); low pH, low temperature, and high nitrite increase the inhibitory effect. The species does not appear to have a nitrite reductase system (Collins-Thompson and Rodriguez-Lopez, 1980). Dainty and Meredith (1972) found that 200 parts per million (ppm) of nitrite at pH 5.5 stops RNA, DNA, and protein synthesis in *B. thermosphacta* but has no effect on membrane permeability. An interesting study on the combined effects of NaCl, $NaNO_2$, temperature, and pH on the growth of *B. thermosphacta* in broth cultures was done by Roberts et al. (1979). Also, sodium lactate has been used effectively against *B. thermosphacta* (reduction by 4 logs as compared to the control after 14 days; Lemay et al., 2002). Microgard 100, Microgard 300, Alta 2002, and Perlac 1902 had no significant effect on tests using an acidified chicken meat model.

In the United Kingdom, sulfur dioxide is permitted in sausage meat and *B. thermosphacta* tolerates SO_2 up to 500 ppm under both aerobic and anaerobic conditions (Dowdell and Board, 1971).

The studies of Macaskie (1982) indicate that in liquid culture palmitic acid is inhibitory to the growth of *B. thermosphacta*.

In plate overlay assays, triclosan-incorporated plastic (TIP) inhibited, among other Gram-positive and Gram-negative organisms, also *Brochothrix thermosphacta*. When TIP was used to cover irradiated, lean beef surfaces inoculated with various bacteria, only populations of *B. thermosphacta* were reduced (Cutter, 1999).

Brochothrix thermosphacta became the main species responsible for putrefaction when poultry carcasses were decontaminated with AvGuard (or Assur-Rinse in the United States) trisodium phosphate. This treatment preferentially removed pseudomonads with the consequence that *B. thermosphacta* lacked competition for utilization of substrates; as however, growth rate of *B. thermosphacta* was greater than that of *Listeria monocytogenes*, putrefaction would occur before the emergence of large numbers of the latter species (Salvat et al., 1997).

Brochothrix thermosphacta is more resistant to irradiation than common meat spoilage organisms such as *Pseudomonas* (Ouattara et al., 2002)

but are affected by irradiation doses of 0.5 and 2.0 kilogray (kGy; Savvaidis et al., 2002). The species has been frequently isolated from irradiated meat and poultry (see Gardner, 1981).

Serology

There do not appear to have been any systematic serological studies of *Brochothrix* spp. Wilkinson and Jones (1975) did not detect any serological relationship between *B. thermosphacta* and the genera *Erysipelothrix*, *Kurthia* or *Listeria* with antisera raised against representative strains of all four taxa.

Esterase Isoenzymes

Five different esterase isoenzymes have been detected by gel electrophoresis studies of *Brochothrix thermosphacta*. Different combinations of these enzymes resulted in the detection of seven groups among 26 strains examined. There was no correlation between source of isolation and groups based on esterase patterns (G. A. Gardner, personal communication).

Bacteriophages

Greer (1983) isolated bacteriophages active on *Brochothrix thermosphacta* from aqueous extracts of spoiled beef. Both phage plaque size and plating efficiency increased significantly when the incubation temperature was reduced from 25°C to 1°C. The detection of 14 distinct phage lysotypes led Greer (1983) to suggest that phage typing may provide a rapid method of differentiating *B. thermosphacta* strains.

On the basis of their morphology as determined by electron microscopy, Ackermann et al. (1988) grouped these bacteriophages (and two isolated in France from broth cultures of lysogenic *B. thermosphacta* strains) into three viral species of the Myoviridae (species A19) or Siphoviridae (species NF5 and BL3). The bacteriophage species A19 are interesting because of their similarity to some bacteriophage species of the genera *Bacillus*, *Lactobacillus*, *Staphylococcus* and *Streptococcus*. Surprisingly, none of the bacteriophages resembled those of the genus *Listeria* (Ackermann et al., 1988).

Bacteriophages have been used to control *B. thermosphacta* spoilage of pork adipose tissue. In the presence of 10^5 bacteria per cm^2 tissue disk and an equivalent number of phages, phage numbers increase by 1000-fold while bacterial number decreased by a factor of 100. As a result, off-odors were suppressed during refrigeration, allowing increase of the storage life of the adipose tissue (Greer and Dilts, 2002).

Plasmids

Dodd and Waites (1988) detected plasmids in all strains of *Brochothrix thermosphacta* isolated from sausages containing 450 ppm of sulfite, but not in the type strain (NCTC [{www.ncbi.nlm.nih.gov /entrez/query.fcgi?db=nucleotide=search=10082}{10082}]). The plasmids ranged in size from 1600 to 43,000 and plasmid numbers varied from 1 to 8 per cell. Comparison of the plasmid profiles of the isolates showed that fewer profiles were present amongst the strains isolated from sausages stored for various periods at 4°C than were present amongst strains isolated from the same sausages on the day of manufacture. Dodd and Waites (1988) interpreted these observations as indicating that only some strains of *B. thermosphacta* were able to survive the conditions of storage.

Bacteriocins

Brochocin C, produced by *Brochothrix campestris*, was discovered and classified (Siragusa and Cutter, 1993) as a bacteriocin active against *B. thermosphacta*, *Listeria monocytogenes* and several strains of *Carnobacterium*, *Kurthia*, *Lactobacillus*, *Pediococcus*, *Streptococcus* and even endospores of *Clostridium* and *Bacillus* spp. (McCormick et al., 1998). In its activity spectrum, brochocin C is similar to the lantibiotic nisin A. Brochocin C is a nonlantibiotic class II bacteriocin, consisting of two peptides (BrcA and BrcB) that are the result of a Gly-Gly cleavage of a prepeptide. Both peptides are necessary for activity (Garneau et al., 2003). Immunity against the bacteriocin is due to an immunity protein, encoded downstream of the *brcB* gene. In Gram-negative bacteria, the outer membrane acts as a barrier against nisin and brochocin (Gao et al., 1999).

Brochothrix spp, like other Gram-positive relatives, are inhibited by bacteriocins such as the thermosensitive peptide produced by *Streptococcus thermophilus* 580 (Mathot et al., 2003), salivaricin B, excreted by *Lactobacillus salivarius* M7, acidocin B from *Lactobacillus acidophilus* M46 (Ten Brink et al., 1994; Leer et al., 1995), and nisin. Nisin is widely studied as an agent controlling the growth of meat spoilage bacteria (Gill and Holley, 2000; Nattress et al., 2001; Nattress and Baker, 2003). As compared to free nisin, calcium-alginate-bound nisin as well as the meat binding system Fibrimex® plus nisin are more effective inhibitors of *B. thermosphacta* (Cutter and Siragusa, 1996; Cutter and Siragusa, 1998). Nisin-incorporated polymers (polyethylene and polyethylene oxide) also reduce *B. thermosphacta* in experimentally surface-inoculated vacuum packed beef (Cutter et al., 2001).

Habitats

The natural habitat of *Brochothrix* spp. is not known with certainty. This is mainly because for many years after the first reported isolation of the type species, *B. thermosphacta*, from finished pork sausage and its subsequent incrimination as an important psychrotrophic spoilage organism of meat and meat products, most investigators concentrated only on these materials (see Gardner [1981] and Keddie and Jones [1981]). During studies on the development of a selective medium for the enumeration of *B. thermosphacta* from meat sources, Gardner (1966) isolated the organism from soil and feces, but in low numbers. More recently *B. thermosphacta* has been isolated from a variety of sources that include fish and fish products, frozen foods, milk, and cream (see Gardner [1981] and Sneath and Jones [1986]). Both *B. thermosphacta* and *B. campestris* have also been isolated from soil and grass (Talon et al., 1988). To date, soil and grass are the only described habitats of *B. campestris*, but it is possible that this species has been misidentified as *B. thermosphacta* in the past. From the information available it seems probable that *Brochothrix* spp. are widely distributed in the environment and become a prominent part of the flora in habitats that selectively favor their growth.

The turbidimetric method of counting *Brochothrix thermosphacta* correlated well with bacterial numbers determined by plate counts, flow cytometry, and manual counts (by microscope) over a limited range of 10^7–10^9 cells/ml. Flow cytometry and manual counts gave a linear relationship over a wider range of 10^5–10^9 cells/ml. Passage of *B. thermosphacta* cells through the flow cytometer resulted in the breakage of chains into single cells which makes this technique attractive for bacterial enumeration (Rattanasomboon et al., 1999).

Meat and Meat Products

Since *Brochothrix thermosphacta* was first isolated from pork trimmings and finished sausage (Sulzbacher and McLean, 1951), it has been isolated regularly from the same sources and from a variety of meats (including poultry) and meat products (see the detailed list of references in Jones, 1991). An excellent, comprehensive review of the isolation of *B. thermosphacta* from, and its ecological association with, beef, lamb and pork, and cured and uncured products made from these meats and poultry meat was done by Gardner (1981). Most of the recent reports deal with verification of *B. thermosphacta* in meat subjected to different preservation regimes, e.g., Gill and Bryant (1992), Prieto et al. (1993), Kakouri and Nychas (1994), Borch et al. (1996), Jimenez et al. (1997), Lopez-Caballero et al. (1999), Samelis and Georgiadou (2000), Silv et al. (2000), Gill and Badoni (2002), Gonzalez-Rodriguez et al. (2002), Bohaychuk and Greer (2003), Susiluoto et al. (2003), and Greer et al. (2004).

Brochothrix thermosphacta is especially important as a spoilage organism in prepacked meats and meat products stored at chill temperatures (see Gardner, 1981). The conditions prevailing during such storage selectively favor its growth (Gardner et al., 1967). *Brochothrix* spp. grow at temperatures as low as 0°C and under conditions of O_2 depletion and increased CO_2 concentration (Pin et al., 2002). Other factors that inhibit or enhance the growth of *B. thermosphacta* during the storage of particular meats (including poultry) and meat products are reviewed by Gardner (1981). These include the chemical and physical properties of the various meats; temperature of storage; composition of gaseous environment; pH; presence of NaCl, $NaNO_2$ or SO_2; previous heat treatment; and interactions with other spoilage bacteria (see also Nychas et al., 1988).

Unlike proteolytic spoilage bacteria (e.g., *Pseudomonas*), *B. thermosphacta* is usually found only on the meat surface (Gill and Penney, 1977), and in prepacked meats, it grows well at the meat-plastic film interface (Ingram and Dainty, 1971). However, Fournaud et al. (1980) demonstrated histologically that meat spoilage bacteria, including *B. thermosphacta*, can penetrate the deep muscle mass by following the perimysium or endomysium connective tissue. Of interest is their observation that the flora of deep muscle tissue stored aerobically was dominated by *B. thermosphacta* whereas that of vacuum-packed meat was dominated by *Lactobacillus*.

Contamination of meats almost certainly occurs during slaughter and postslaughter procedures. *Brochothrix thermosphacta* has been isolated from the hides of cattle (Mulder, 1978; Newton et al., 1978); cattle hair, rumen contents, floors and equipment in slaughter halls (Patterson and Gibbs, 1978; Talon et al., 1988); sheep wool and sheep feces (Talon et al., 1988); equipment and tables used in the preparation of sausage (McLean and Sulzbacher, 1953); and cooked rind and fat emulsions added to pork sausages (Gardner, 1981).

Fish and Fish Products

In the last 10 years, *Brochothrix thermosphacta* has been isolated from fish sources, but it does not appear to be as economically important; nor does it comprise as large a proportion of the

microflora as it does in meat. Nickelson et al. (1980) isolated *B. thermosphacta* at all stages in the production (whole fish, scaled, beheaded, eviscerated, and minced flesh) of minced fish flesh from nontraditional finfish caught in the Gulf of Mexico, but the proportion of the total microflora rarely exceeded 5%. Lannelongue et al. (1982) isolated *B. thermosphacta* from stored finfish fillets packaged in a CO_2 atmosphere. Again the numbers recovered as a proportion of the total microbial population were small. The species has also been isolated from fish fingers, smoked whiting, frozen coley, and frozen cod (Gardner, 1981). Little information on *Brochothrix* detected on fish has been published in the past 10 years. Investigation of vacuum-packed cold-smoked salmon and trout has indicated a low number of *Brochothrix* spp. (Gonzalez-Rodriguez et al., 2002). These organisms have also been detected in the Mediterranean boque (*Boops boops*) but were found to constitute a population with a density of 2–3 $\log_{10}$ CFU g^{-1}, less than that of pseudomonads (Koutsoumanis and Nychas, 1999).

Brochothrix campestris has not been observed in fish or fish products but prior to the study of Talon et al. (1988), misidentification could have occurred. Talon et al. (1988) did not examine these sources.

Other Sources

Brochothrix thermosphacta has been isolated from soil, grass, hay, and feces but apparently only in low numbers and with the use of selective media. There have also been reports of the isolation of the species from a variety of foods such as frozen peas, frozen runner beans, prepackaged tomato salad, milk, cream, and cottage cheese (Gardner, 1981).

As noted earlier, the only currently known habitats of *B. campestris* are grass and soil (Talon et al., 1988). Inspection of the numbers of isolates of *B. thermosphacta* and *B. campestris* from grass and soil studied by these workers does not indicate that *B. campestris* forms a larger proportion of the microflora of these habitats than does *B. thermosphacta*; no other indication of the relative proportions of the two species in grass and soil samples is reported (Talon et al., 1988).

Neither *B. thermosphacta* nor *B. campestris* appear to have been isolated from clinical sources.

Isolation of Brochothrix

Until recently the methods described for the isolation of *Brochothrix* have been mainly concerned with the isolation of *B. thermosphacta* from meats and meat products. *Brochothrix campestris* grows equally well on the same media. Neither species grows on the acetate medium devised by Rogosa et al. (1951) for the isolation of lactobacilli (see Keddie and Jones [1981] and Talon et al. [1988]). *Brochothrix thermosphacta* (no information is available for *B. campestris*) grows only poorly on the MRS medium of (De Man et al., 1960). Several different media based on peptone, yeast extract, and glucose or glycerol have been used for the isolation of *B. thermosphacta* (Sulzbacher and McLean, 1951; Wolin et al., 1957; Barlow and Kitchell, 1966; Gardner, 1966; Gardner, 1967). More recently, the streptomycin sulfate-thallous acetate-actidione (actidione = cycloheximide) agar medium (STAA) of Gardner (1966) has been used routinely for the selective isolation and enumeration of *B. thermosphacta* from meats, meat products, fish and a variety of other foods (Gardner, 1981). Although extremely useful for such materials, the selectivity of STAA is poor when soils or fecal samples are examined (Gardner, 1981). Talon et al. (1988) used STAA medium supplemented with nalidixic acid and oxacillin for the isolation of *B. thermosphacta* from soil, grass, feces, etc., and for the isolation of *B. campestris* from soil and grass.

Isolation of *Brochothrix thermosphacta* from Meats and Meat Products.

Swabs of various meat surfaces or samples of macerated meat or other materials are usually suspended in 0.1% (w/v) peptone water and shaken vigorously before plating on suitable media (see Gardner et al., 1967). Plates should be incubated at 22°C for up to 5 days, but colonies of *B. thermosphacta* are usually visible within 48 h.

The following media are suitable for the growth of *B. thermosphacta*.

Glycerol Nutrient Agar (Gardner, 1966; Gardner, 1967)

Peptone (Oxoid)	20 g
Yeast extract (Oxoid)	2 g
Glycerol	15 g
K_2HPO_4	1 g
$MgSO_4 \cdot 7H_2O$	1 g
Agar (Oxoid no. 3)	13 g

Dissolve ingredients and dilute up to 1 liter with distilled water. Adjust pH to 7.0 and autoclave at 121°C for 15 min.

Glucose Nutrient Agar (Sulzbacher and McLean, 1951; Wolin et al., 1957)

Tryptone (Difco)	10 g
Yeast extract (Difco)	5 g
K_2HPO_4	5 g
NaCl	5 g
Glucose	5 g
Agar	15 g

Dissolve ingredients and dilute up to 1 liter with distilled water. Adjust pH to 7.0 and autoclave at 121°C for 15 min.

Medium STAA for Selective Isolation of *Brochothrix thermosphacta* (Gardner, 1966)

Peptone (Oxoid)	20 g
Yeast extract (Oxoid)	2 g
Glycerol	15 g
K_2HPO_4	1 g
$MgSO_4 \cdot 7H_2O$	1 g
Agar (Oxoid no. 3)	13 g

Dissolve ingredients and dilute up to 1 liter with distilled water. Adjust pH to 7.0 and autoclave at 121°C for 15 min. To this sterile, molten medium, add the following solutions (prepared in sterile, distilled water) to give the final concentrations indicated; streptomycin sulfate (Glaxo), 500 µg/ml; cycloheximide (Upjohn), 50 µg/ml; and thallous acetate, 50 µg/ml).

After incubation of appropriate samples on this medium at 20–22°C for 48 h, a large majority of the colonies are *B. thermosphacta*; the exceptions are a few pseudomonads.

Modified Medium STAA for Selective Isolation of *Brochothrix* spp. from Grass, Soil, Feces, and Similar Material (Talon et al., 1988)

Prepare as for STAA medium but supplement with nalidixic acid (15 µg/ml) and oxacillin (5 µg/ml).

After incubation of appropriate samples on this medium at 20–22°C for 48 h, a large majority of the colonies are *Brochothrix* spp.

Enrichment of *Brochothrix* spp.

Enrichment of *Brochothrix* spp. is not usually performed, but holding meat or meat product samples under gas-permeable film at temperatures below 10°C can act as enrichment. Similar enrichment for other materials, grass or soil, could be useful but apparently has not been attempted.

Preservation of Cultures

Cultures may be preserved for short periods (6 months to 1 year) in nutrient agar (plus 0.1% glucose) stabs in screw-capped bottles stored in the dark at room or refrigeration temperature. Longer-term preservation (over 10 years with *Brochothrix thermosphacta*) may be achieved by freezing on glass beads at –60 to –70°C (Jones et al., 1984). Cultures may also be preserved by freeze drying (lyophilization).

Acknowledgments. G. Gehrich-Schröter and R. Pukall are thanked for providing results of BIOLOG GP2 substrate utilization, acid formation from substrates in MRS medium, and riboprinting.

Literature Cited

Ackermann, H. W., G. G. Greer, and J. Rocourt. 1988. Morphology of Brochothrix thermosphacta phages. Microbios 56:19–26.

Barlow J., and A. G. Kitchell. 1966. A note on the spoilage of prepacked lamb chops by Microbacterium thermosphactum. J. Appl. Bacteriol. 29:185–188.

Bohaychuk, V. M., and G. G. Greer. 2003. Bacteriology and storage life of moisture-enhanced pork. J. Food Prot. 66:293–299.

Borch, E., M. L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and cured meat products. Intl. J. Food Microbiol. 33:103–120.

Breed, R. S., E. G. D. Murray, and A. P. Hitchens (Eds.). 1948. Bergey's Manual of Determinative Bacteriology, 6th ed. Williams & Wilkins. Baltimore, MD.

Brownlie, L. E. 1966. Effects of some environmental factors on psychrophilic microbacteria. J. Appl. Bacteriol. 29:447–454.

Buchanan, R. E., and N. E. Gibbons (Eds.). 1974. Bergey's Manual of Determinative Bacteriology, 8th ed. Williams & Wilkins. Baltimore, MD.

Cocolin, L., M. Manzano, C. Cantoni, and G. Comi. 2001. Denaturing gradient gel electrophoresis analysis of the 16S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. Appl. Environ. Microbiol. 67:5113–5121.

Collins, M. D., M. Goodfellow, and D. E. Minnikin. 1979. Isoprenoid quinones in the classification of coryneform and related bacteria. J. Gen. Microbiol. 110:127–136.

Collins, M. D., and D. Jones. 1981. The distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol. Rev. 45:316–354.

Collins, M. D., J. A. E. Farrow, B. A. Phillips, S. Feresu, and D. Jones. 1987. Classification of Lactobacillis divergens, Lactobacillus piscicola, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus Carnobacterium. Intl. J. Syst. Bacteriol. 37:310–316.

Collins, M. D., S. Wallbanks, D. J. Lane, J. Shah, R. Nietupski, J. Smida, M. Dorsch, and E. Stackebrandt. 1991. Phylogenetic analysis of the genus Listeria based on reverse transcriptase sequencing of 16S rRNA. Intl. J. Syst. Bacteriol. 41:240–246.

Collins-Thompson, D. L., T. Sorhaug, L. D. Witter, and Z. J. Ordal. 1971. Glycerol ester hydrolase activity in Microbacterium thermosphactum. Appl. Microbiol. 21:9–12.

Collins-Thompson, D. L., T. Sorhaug, L. D. Witter, and Z. J. Ordal. 1972. Taxonomic consideration of Microbacterium lacticum, Microbacterium flavum and Microbacterium thermosphactum. Intl. J. Syst. Bacteriol. 22:65–72.

Collins-Thompson, D. L., and G. Rodriguez-Lopez. 1980. Influence of sodium nitrite, temperature and lactic acid bacteria on the growth of Brochothrix thermosphacta under anaerobic conditions. Can. J. Microbiol. 26:1416–1421.

Collins-Thompson, D. L., P. J. Slade, and M. Goethals. 1991. Use of low molecular mass RNA profiles to identify lactic acid bacteria and related organisms associated with foods. Intl. J. Food Microbiol. 14:135–143.

Cutter, C. N., and G. R. Siragusa. 1996. Reduction of Brochothrix thermophacta on beef surfaces following immobilization of nisin in calcium alginate gels. Lett. Appl. Microbiol. 23:9–12.

Cutter, C. N., and G. R. Siragusa. 1998. Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. Lett. Appl. Microbiol. 27:19–23.

Cutter, C. N. 1999. The effectiveness of triclosan-incorporated plastic against bacteria on beef surfaces. J. Food Prot. 62:474–479.

Cutter, C. N., J. L. Willett, and G. R. Siragusa. 2001. Improved antimicrobial activity of nisin-incorporated polymer films by formulation change and addition of food grade chelator. Lett. Appl. Microbiol. 33:325–328.

Dainty, R. H., and G. C. Meredith. 1972. Mechanisms of inhibition of growth of bacteria by nitrite. Meat Research Institute Annual Report 1972–73. HMSO. London, UK. 82.

Dainty, R. H., and C. M. Hibbard. 1980. Aerobic metabolism of Brochothrix thermosphacta growing on meat surfaces and in laboratory media. J. Appl. Bacteriol. 48:387–396.

Dainty, R. H., and C. M. Hibbard. 1983. Precursors of the major end-products of aerobic metabolism of Brochothrix thermosphacta. J. Appl. Bacteriol. 55:127–133.

Dainty, R. H., R. A. Edwards, and C. M. Hibbard. 1985. Time course of volatile compound formation during refrigerated storage of naturally contaminated beef in air. J. Appl. Bacteriol. 59:303–309.

Davidson, C. M., and E. F. Hartree. 1968a. Cytochrome as a guide to classifying bacteria: Taxonomy of Microbacterium thermosphactum. Nature 220:502–504.

Davidson, C. M., P. Mobbs, and J. M. Stubbs. 1968b. Some morphological and physiological properties of Microbacterium thermosphactum. J. Appl. Bacteriol. 31:551–559.

Davis, G. H. G., and K. G. Newton. 1969a. Numerical taxonomy of some named coryneform bacteria. J. Gen. Microbiol. 56:195–214.

Davis, G. H. G., L. Fomin, E. Wilson, and K. G. Newton. 1969b. Numerical taxonomy of Listeria, streptococci and possibly related bacteria. J. Gen. Microbiol. 57:333–348.

De Man, J. C., M. Rogosa, and M. E. Sharpe. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23:130–135.

DeSoete, G. 1983. A least squares algorithm for fitting additive trees to proximity data. Psychrometrika 48:621–626.

Dodd, C. E. R., and W. Waites. 1988. The use of plasmid profiling to determine strains of Brochothrix thermosphacta important in spoilage of sausages. J. Appl. Bacteriol. 65:XV.

Dowdell, M. J., and R. G. Board. 1971. The microbial associations in British fresh sausages. J. Appl. Bacteriol. 34:317–337.

Evans, J. B., and C. F. Niven Jr. 1951. Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. J. Bacteriol. 62:599–603.

Felsenstein, J. 1993. PHYLIP (Phylogenetic Inference Package) Version 3.5.1. Department of Genetics, University of Washington. Seattle, WA.

Feresu, S. B., and D. Jones. 1988. Taxonomic studies on Brochothrix, Erysipelothrix, Listeria and atypical lactobacilli. J. Gen. Microbiol. 134:1165–1183.

Fournaud, J., T. Degas, O. Schmitt, and J. Sechet. 1980. Pénétration des bactéries dans la viande. Proceedings of the 26th European Meeting of Meat Research Workers. Colorado Springs, CO. 2:268–271.

Gao, Y., M. J. van Belkum, and M. E. Stiles. 1999. The outer membrane of Gram-negative bacteria inhibits antibacterial activity of brochocin-C. Appl. Environ. Microbiol. 65:4329–4333.

Gardner, G. A. 1966. A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. J. Appl. Bacteriol. 29:455–460.

Gardner, G. A., A. Carson, and J. Patton. 1967. Bacteriology of prepacked pork with reference to the gas composition within the pack. J. Appl. Bacteriol. 30:321–333.

Gardner, G. A. 1981. Brochothrix thermosphacta (Microbacterium thermosphactum) in the spoilage of meats: A review. *In:* T. A. Roberts, G. A. Hobbs, J. H. B. Christian, and N. Skovgaard (Eds.) Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press. London, UK. 139–173.

Garneau, S., C. A. Ference, M. J. van Belkum, M. E. Stiles, and J. C. Vederas. 2003. Purification and characterization of brochocin A and brochocin B(10-43), a functional fragment generated by heterologous expression in Carnobacterium piscicola. Appl. Environ. Microbiol. 69:1352–1358.

Gil, A., R. G. Kroll, and R. K. Poole. 1992. The cytochrome composition of the meat spoilage bacterium Brochothrix thermophacta: Identification of cytochrome a3- and d-type terminal oxidases under various conditions. Arch. Microbiol. 158:226–233.

Gill, C. O., and N. Penney. 1977. Penetration of bacteria into meat. Appl. Environ. Microbiol. 33:1284–1286.

Gill, C. O., and J. Bryant. 1992. The contamination of pork with spoilage bacteria during commercial dressing, chilling and cutting of pig carcasses. Intl. J. Food Microbiol. 16:51–62.

Gill, A. O., and R. A. Holley. 2000. Surface application of lysozyme, nisin, and EDTA to inhibit spoilage and pathogenic bacteria on ham and bologna. Food Prot. 63:1338–1346.

Gill, C. O., and M. Badoni. 2002. Microbiological and organoleptic qualities of vacuum-packaged ground beef prepared from pasteurized manufacturing beef. Intl. J. Food Microbiol. 74:111–118.

Gonzalez-Rodriguez, M. N., J. J. Sanz, J. A. Santos, A. Otero, and M. L. Garcia-Lopez. 2002. Numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish at the retail level. Intl. J. Food Microbiol. 77:61–66.

Grant, K. A., J. H. Dickinson, M. J. Payne, S. Campbell, M. D. Collins, and R. G. Kroll. 1993. Use of the polymerase chain reaction and 16S rRNA sequences for the rapid detection of Brochothrix spp. in foods. J. Appl. Bacteriol. 74:260–267.

Grau, F. H. 1979. Nutritional requirements of Microbacterium thermosphactum. Appl. Environ. Microbiol. 38:818–820.

Grau, F. H. 1983. End products of glucose fermentation by Brochothrix thermosphacta. Appl. Environ. Microbiol. 45:84–90.

Grau, F. H. 1988. Substrates used by Brochothrix thermosphacta when growing on meat. J. Food Prot. 51:639–642.

Greer, G. G. 1983. Psychrotrophic Brochothrix thermosphacta bacteriophages isolated from beef. Appl. Environ. Microbiol. 46:245–251.

Greer, G. G., and B. D. Dilts. 2002. Control of Brochothrix thermophacta spoilage of pork adipose tissue using bacteriophages. J. Food Prot. 65:861–863.

Greer, G. G., F. Nattress, B. Dilts, and L. Baker. 2004. Bacterial contamination of recirculating brine used in the commercial production of moisture-enhanced pork. J. Food Prot. 67:185–188.

Hitchener, B. J., A. F. Egan, and P. J. Rogers. 1979. Energetics of Microbacterium thermosphactum in glucose-limited continuous culture. Appl. Environ. Microbiol. 37:1047–1052.

Ingram, M., and R. H. Dainty. 1971. Changes caused by microbes in spoilage of meats. J. Appl. Bacteriol. 34:21–39.

Jimenez, S. M., M. S. Salsi, M. C. Tiburzi, R. C. Rafaghelli, M. A. Tessi, and V. R. Coutaz. 1997. Spoilage microflora in fresh chicken breast stored at 4 degrees C: Influence of packaging methods. J. Appl. Microbiol. 83:613–618.

Jones, D. 1975. A numerical taxonomic study of coryneform and related bacteria. J. Gen. Microbiol. 87:52–96.

Jones, D., P. A. Pell, and P. H. A. Sneath. 1984. Maintenance of bacteria on glass beads at –60°C to –76°C. *In:* B. E. Kirsop and J. J. S. Snell (Eds.) Maintenance of Microorganisms: A Manual of Laboratory Methods. Academic Press. London, UK. 35–40.

Jones, D. 1991. The genus Brochothrix. *In:* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (Eds.) The Prokaryotes. Springer-Verlag. New York, NY. 1617–1628.

Kakouri, A., and G. J. Nychas. 1994. Storage of poultry meat under modified atmospheres or vacuum packs: Possible role of microbial metabolites as indicator of spoilage. J. Appl. Bacteriol. 76:163–172.

Keddie, R. M., and D. Jones. 1981. Saprophytic, aerobic coryneform bacteria. *In:* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (Eds.) The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Springer-Verlag. Berlin, Germany. 1838–1878.

Kim, C., B. Swaminathan, P. K. Cassaday, L. W. Mayer, and B. P. Holloway. 1991. Rapid confirmation of Listeria monocytogenes isolated from foods by a colony blot assay using a digoxigenin-labeled synthetic oligonucleotide probe. Appl. Environ. Microbiol. 57:1609–1614.

Koutsoumanis, K., and G. J. Nychas. 1999. Chemical and sensory changes associated with microbial flora of Mediterranean boque (Boops boops) stored aerobically at 0, 3, 7, and 10 degrees C. Appl. Environ. Microbiol. 65:698–706.

Lannelongue, M., M. O. Hanna, G. Finne, R. Nickelson, and C. Vanderzant. 1982. Storage characteristics of fin-fish fillets (Archosargus probatocephalus) packaged in modified gas atmospheres containing carbon dioxide. J. Food Prot. 45:440–444.

Leer, R. J., J. M. van der Vossen, M. van Giezen, J. M. van Noort, and P. H. Pouwels. 1995. Genetic analysis of acidocin B, a novel bacteriocin produced by Lactobacillus acidophilus. Microbiology 141:1629–1635.

Lemay, M. J., J. Choquette, P. J. Delaquis, G. Claude, N. Rodrigue, and L. Saucier. 2002. Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. Intl. J. Food Microbiol. 78:217–226.

Lopez-Caballero, M. E., J. Carballo, and F. Jimenez-Colmenero. 1999. Microbiological changes in pressurized, prepackaged sliced cooked ham. J. Food Prot. 62:1411–1415.

Ludwig, W., K.-H. Schleifer, and E. Stackebrandt. 1984. 16S rRNA analysis of Listeria monocytogenes and Brochothrix thermosphacta. FEMS Microbiol. Lett. 25:199–204.

Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, I. Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Förster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R. Lüßmann, M. May, B Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K.-H. Schleifer. 2004. ARB: A software environment for sequence data. Nucl. Acids Res. 32:1363–1371.

Macaskie, L. E., R. H. Dainty, and P. J. F. Henderson. 1981. The role of thiamin as a factor for the growth of Brochothrix thermosphacta. J. Appl. Bacteriol. 50:267–273.

Macaskie, L. E. 1982. Inhibition of growth of Brochothrix thermosphacta by palmitic acid. J. Appl. Bacteriol. 52:339–343.

Mathot, A. G., E. Beliard, and D. Thuault. 2003. Streptococcus thermophilus 580 produces a bacteriocin potentially suitable for inhibition of Clostridium tyrobutyricum in hard cheese. J. Dairy Sci. 86:3068–3074.

McCormick, J. K., A. Poon, M. Sailer, Y. Gao, K. L. Roy, L. M. McMullen, J. C. Vederas, M. E. Stiles, and M. J. van Belkum. 1998. Genetic characterization and heterologous expression of brochocin-C, an antibotulinal, two-peptide bacteriocin produced by Brochothrix campestris ATCC 43754. Appl. Environ. Microbiol. 64:4757–4766.

McLean, R. A., and W. L. Sulzbacher. 1953. Microbacterium thermosphactum, spec. nov., a nonheat resistant bacterium from fresh pork sausage. J. Bacteriol. 65:428–433.

Minnikin, D. E., M. Goodfellow, and M. D. Collins. 1978. Lipid composition in the classification and identification of coryneform and related taxa. *In:* I. J. Bousfield and A. G. Callely (Eds.) Coryneform bacteria. Special Publications of the Society for General Microbiology. Academic Press. London, UK. 85–160.

Mulder, S. J. 1978. Microbacterium thermosphactum: Spoilage indicator of beef. Proceedings of the 24th European Meeting of Meat Research Workers. Kulmbach, Germany. 1.

Nattress, F. M., C. K. Yost, and L. P. Baker. 2001. Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. Intl. J. Food Microbiol. 70:111–119.

Nattress, F. M., and L. P. Baker. 2003. Effects of treatment with lysozyme and nisin on the microflora and sensory properties of commercial pork. Intl. J. Food Microbiol. 85:259–267.

Newton, K. G., J. C. L. Harrison, and A. M. Wauters. 1978. Sources of psychrotrophic bacteria on meat at the abattoir. J. Appl. Bacteriol. 45:75–82 .

Nickelson, R., G. Finne, M. O. Hanna, and C. Vanderzant. 1980. Minced fish flesh from non-traditional Gulf of Mexico finfish species: Bacteriology. J. Food Sci. 45:1321–1326.

Nychas, G. J., V. M. Dillon, and R. G. Board. 1988. Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. Biotechnol. Appl. Biochem. 10:203–231.

Ouattara, B., M. Giroux, W. Smoragiewicz, L. Saucier, and M. Lacroix. 2002. Combined effect of gamma irradiation, ascorbic acid, and edible coating on the improvement of microbial and biochemical characteristics of ground beef. J. Food Prot. 65:981–987.

Papon, M., and R. Talon. 1988. Factors affecting growth and lipase production by meat lactobacilli strains and Brochothrix thermosphacta. J. Appl. Bacteriol. 64:107–115.

Patterson, J. T., and P. A. Gibbs. 1978. Sources and properties of some organisms isolated in two abattoirs. Meat Sci. 2:263–273.

Pin, C., G. D. Garcia de Fernando, and J. A. Ordonez. 2002. Effect of modified atmosphere composition on the metabolism of glucose by Brochothrix thermophacta. Appl. Environ. Microbiol. 68:4441–4447.

Prieto, M., M. L. Garcia-Lopez, M. R. Garcia-Armesto, A. Otero, T. M. Lopez, and B. Moreno. 1993. Factors affecting spoilage microflora succession on lamb carcasses at refrigeration temperatures. J. Appl. Bacteriol. 74:521–525.

Rattanasomboon, N., S. R. Bellara, C. L. Harding, P. J. Fryer, C. R. Thomas, M. Al-Rubeai, and C. M. McFarlane.

1999. Growth and enumeration of the meat spoilage bacterium Brochothrix thermophacta. Intl. J. Food Microbiol. 51:145–158.

Roberts, T. A., C. R. Britton, and N. N. Shroff. 1979. The effect of pH, water activity, sodium nitrite and incubation temperature on growth of bacteria isolated from meats. *In:* B. Jarvis, J. H. B. Christian, and H. D. Michener (Eds.) Food Microbiology and Technology. Medecina Viva. Parma, Italy. 57–71.

Robinson, K. 1966. Some observations on the taxonomy of the genus Microbacterium. II: Cell wall analysis, gel electrophoresis and serology. J. Appl. Bacteriol. 29:616–624 .

Rogosa, M., J. A. Mitchell, and R. F. Wiseman. 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. 62:132–133 .

Sakala, R. M., H. Hayashidani, Y. Kato, T. Hirata, Y. Makino, A. Fukushima, T. Yamada, C. Kaneuchi, and M. Ogawa. 2002. Change in the composition of the microflora on vacuum-packaged beef during chiller storage. Intl. J. Food Microbiol. 74:87–99.

Salvat, G., P. Coppen, J. C. Allo, S. Fenner, M. J. Laisney, M. T. Toquin, F. Humbert, and P. Colin. 1997. Effects of AvGard treatment on the microbiological flora of poultry carcases. Br. Poultry Sci. 38:489–498.

Samelis, J., and K. G. Georgiadou. 2000. The microbial association of Greek taverna sausage stored at 4 and 10 degrees C in air, vacuum or 100% carbon dioxide, and its spoilage potential. J. Appl. Microbiol. 88:58–68.

Savvaidis, I. N., P. Skandamis, K. A. Riganakos, N. Panagiotakis, and M. G. Kontominas. 2002. Control of natural microbial flora and Listeria monocytogenes in vacuum-packaged trout at 4 and 10 degrees C using irradiation. J. Food Prot. 65:515–522.

Schleifer, K.-H. 1970. Die Mureintypen in der Gattung Microbacterium. Archiv. Mikrobiologie 71:271–282 .

Schleifer, K.-H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. 36:407–477.

Shaw, N., and D. Stead. 1970. A study of the lipid composition of Microbacterium thermosphactum as a guide to its taxonomy. J. Appl. Bacteriol. 33:470–473.

Shaw, N. 1974. Lipid composition as a guide to the classification of bacteria. Adv. Appl. Microbiol. 17:63–108.

Silv, C. F., R. F. Schwan, E. S. Sousa Dias, and A. E. Wheals. 2000. Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. Intl. J. Food Microbiol. 60:251–260.

Siragusa, G. R., and C. N. Cutter. 1993. Brochocin-C, a new bacteriocin produced by Brochothrix campestris. Appl. Environ. Microbiol. 59:2326–2328.

Sneath, P. H. A., and D. Jones. 1976. Brochothrix, a new genus tentatively placed in the family Lactobacillaceae. Intl. J. Syst. Bacteriol. 26:102–104.

Sneath, P. H. A., and D. Jones. 1986. The genus Brochothrix. *In:* P. H. A. Sneath, N. S., Mair, and M. E. Sharpe (Eds.) Bergey's Manual of Systematic Bacteriology. Williams & Wilkins. Baltimore, MD. 2:1249–1253.

Stackebrandt, E., and C. R. Woese. 1981. The evolution of prokaryotes. *In:* Carlile, M. J., J. F. Collins, and B. E. B. Moseley (Eds.) Molecular and Cellular Aspects of Evolution. Cambridge University Press. Cambridge, UK. Society for General Microbiology, Symposium 32:1–31.

Stackebrandt, E., and B. M. Goebel. 1994. A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Intl. J. Syst. Bacteriol. 44:846–849.

Stringer, S. C., B. J. Chaffey, C. E. Dodd, M. R. Morgan, and W. M. Waites. 1995. Specific antibody-mediated detection of Brochothrix thermophacta in situ in British fresh sausage. J. Appl. Bacteriol. 78:335–340.

Sulzbacher, W. L., and R. A. McLean. 1951. The bacterial flora of fresh pork sausage. Food Technol. 5:7–8.

Susiluoto, T., H. Korkeala, and K. J. Bjorkroth. 2003. Leuconostoc gasicomitatum is the dominating lactic acid bacterium in retail modified-atmosphere-packaged inated broiler meat strips on sell-by-day. Intl. J. Food Microbiol. 80:89–97.

Sutherland, J. P., J. T. Patterson, P. A. Gibbs, and J. G. Murray. 1975. Some metabolic and biochemical characteristics of representative microbial isolates from vacuum-packaged beef. J. Appl. Bacteriol. 39:239–249.

Talon, R., P. A. D. Grimont, F. Grimont, F. Gasser, and J. M. Boeufgras. 1988. Brochothrix campestris sp. nov. Intl. J. Syst. Bacteriol. 38:99–102.

Ten Brink, B., M. Minekus, J. M. van der Vossen, R. J. Leer, and J. H. Huis in't Veld. 1994. Antimicrobial activity of lactobacilli: preliminary characterization and optimization of production of acidocin B, a new bacteriocin produced by Lactobacillus acidophilus M46. J. Appl. Bacteriol. 77:140–148.

Thomson, I. Q., and D. L. Collins-Thompson. 1986. Iron requirement of Brochothrix thermosphacta. Intl. J. Food Microbiol. 3:299–309.

Thomson, I. Q., and D. L. Collins-Thompson. 1988. Secondary role of manganese during iron deprivation of Brochothrix thermosphacta. System. Appl. Microbiol. 10:195–199.

Wilkinson, B. J., and D. Jones. 1975. Some serological studies on Listeria and possibly related bacteria. *In:* M. Woodbine (Ed.) Problems of Listeriosis. Leicester University Press. Leicester, UK. 251–261.

Wilkinson, B. J., and D. Jones. 1977. A numerical taxonomic survey of Listeria and related bacteria. J. Gen. Microbiol. 98:399–421.

Wolin, E. F., J. B. Evans, and C. F. Niven. 1957. The microbiology of fresh and irradiated beef. Food Res. 22:682–686.