Isoprenoid Quinone Composition as a Guide to the Classification of *Listeria*, *Brochothrix*, *Erysipelothrix* and *Caryophanon*

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(Received 28 November 1978)

Representatives of the genera *Brochothrix*, *Caryophanon* and *Listeria* all contained fully unsaturated menaquinones as their sole isoprenoid quinones but *Erysipelothrix rhusiopathiae* contained neither menaquinones nor ubiquinones. Menaquinones with six isoprene units were the major isoprenologues in *Caryophanon tenue* whereas menaquinones with seven isoprene units predominated in *Brochothrix thermosphacta*, *Listeria grayi*, *Listeria monocytogenes* and *Listeria murrayi*. *Listeria denitrificans* had menaquinones with nine isoprene units.

INTRODUCTION

The taxonomic relationships of the genera *Listeria*, *Erysipelothrix* and *Brochothrix* present a number of unresolved problems.

The genera *Listeria* and *Erysipelothrix* were classified in the family Corynebacteriaceae by Breed *et al.* (1957) but subsequent numerical taxonomic studies (Davis *et al.*, 1969; Stuart & Pease, 1972; Jones, 1975 *a*; Wilkinson & Jones, 1977), DNA base ratio determinations (Flossmann & Erler, 1972; Stuart & Welshimer, 1973) and cell wall and peptidoglycan studies (Mann, 1969; Schleifer & Kandler, 1972) have shown that they do not belong to the coryneform group (Keddie, 1978). In the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974), both genera are treated with the genus *Caryophanon* as 'genera of uncertain affiliation' at the end of the section which includes the genus *Lactobacillus*.

The genus *Brochothrix* was proposed by Sneath & Jones (1976) for bacteria previously designated *Microbacterium thermosphactum* (McLean & Sulzbacher, 1953). The relationship of *M. thermosphactum* to *M. lacticum*, the type species of the genus *Microbacterium*, has always been controversial and more recent studies (see Collins-Thompson *et al.*, 1972; Sneath & Jones, 1976) indicate that the two species are sufficiently different to warrant the removal of *M. thermosphactum* from the genus *Microbacterium*.

Erysipelothrix and *Brochothrix* are both monospecific genera but *Listeria* presently contains four species, *L. monocytogenes*, *L. denitrificans*, *L. grayi* and *L. murrayi* (Seeliger

& Welshimer, 1974). While there is general agreement that *L. denitrificans* should be removed from the genus (Stuart & Pease, 1972; Stuart & Welshimer, 1973, 1974; Jones, 1975*a, b*), there is no such agreement on the status of *L. grayi* and *L. murrayi* nor on the degree of their relationship to *L. monocytogenes* (Stuart & Welshimer, 1974; Wilkinson & Jones, 1977).

On the basis of a numerical taxonomic study, Wilkinson & Jones (1977) suggested that the genera *Listeria* (excluding *L. denitrificans*), *Erysipelothrix* and *Brochothrix* should be classified in the family Lactobacillaceae together with the genera *Lactobacillus*, *Gemella* and *Streptococcus*. The main objection to this proposal is that bacteria of the genera *Listeria* and *Brochothrix* possess a haem-containing catalase and a functional cytochrome system (Friedman & Alm, 1962; Davidson & Hartree, 1968; Davidson *et al.*, 1968; Meyer & Jones, 1973; Jones, 1975*a*), whereas members of the genera *Erysipelothrix*, *Gemella*, *Lactobacillus* and *Streptococcus* do not (see Buchanan & Gibbons, 1974). However, Wilkinson & Jones (1977) have discussed the occurrence of haem and non-haem catalases in streptococci and lactobacilli, and functional cytochromes have been reported in streptococci cultured under certain conditions (see Wilkinson & Jones, 1977; Whittenbury, 1978).

Isoprenoid quinones play an important role in bacterial electron transport and possibly oxidative phosphorylation (Brodie & Watanabe, 1966; Redfearn, 1966; Dunphy & Brodie, 1971). In addition, recent studies have indicated the potential value of isoprenoid quinone analyses in the classification of coryneform and related bacteria (see Yamada *et al.*, 1976; Collins *et al.*, 1977, 1979; Minnikin *et al.*, 1978*a, b*). In this study the distribution of isoprenoid quinones has been examined in representatives of the genera *Listeria*, *Brochothrix* and *Erysipelothrix* in an attempt to clarify their taxonomic position. A strain of *Caryophanon tenue* was included only because the genus *Caryophanon* is included in the same section as *Listeria* and *Erysipelothrix* in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974).

METHODS

Cultures and cultivation. Details of the strains and their sources are given in Table 1. Organisms were maintained routinely on nutrient agar (Oxoid) and Dorset egg slopes at room temperature. *Brochothrix*, *Caryophanon* and *Listeria* strains were grown in shake culture at 30 °C for 2 to 3 d in nutrient broth [10 g Neutralised Bacteriological Peptone (Oxoid L34), 8 g Lab Lemco (Oxoid L29) and 5 g NaCl in 1 l distilled water, pH 7.4]. *Erysipelothrix* was grown in the same medium supplemented with glucose (2 mg ml⁻¹) and haematin (5 µg ml⁻¹) in static culture for 2 d at 30 °C. Cultures were checked for purity at maximum growth, killed with formaldehyde (1%, v/v), harvested by centrifuging (4750 *g* for 30 min), washed with distilled water and freeze-dried.

Extraction, purification and analysis of isoprenoid quinones. Isoprenoid quinones were extracted and purified as described by Collins *et al.* (1977) and were further examined by reverse phase partition chromatography using Whatman KC₁₈F reverse phase thin-layer plates (7.6 × 7.6 cm; 200 µm thick) and a polar developing mixture of acetone/water (97:3, v/v), separated components being detected with 254 nm ultraviolet light. Ultraviolet spectra of the quinones were recorded in iso-octane solution on a Perkin-Elmer (Model 137 UV) spectrophotometer. Mass spectra of the isoprenoid quinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 200 to 220 °C.

RESULTS

Neither menaquinones (vitamin K) nor ubiquinones (coenzyme Q) were detected in *E. rhusiopathiae*, NCTC 8163. Components that co-chromatographed with vitamin K₁ were the only isoprenoid quinones detected in representatives of the genera *Brochothrix*, *Caryophanon* and *Listeria*. Ultraviolet spectra of the isoprenoid quinones showed absorption maxima at 242, 248, 260, 270 and 326 nm, in accord with published data for menaquinones (Dunphy & Brodie, 1971).

The mass spectra of all the menaquinone samples showed intense peaks at *m/e* 187 and

Table 1. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from the test strains

The main component in each series is denoted by + + +, any component greater than 50% of the main peak by + +, and all other significant components by +.

Menaquinone isoprenologue m/e		MK-5 512	MK-6 580	MK-7 648	MK-8 716	MK-9 784
<i>Brochothrix thermosphacta</i> ATCC 11509*		+	+	+++	—	—
<i>Brochothrix thermosphacta</i> 24†		+	+	+++	—	—
<i>Brochothrix thermosphacta</i> P2†		+	+	+++	—	—
<i>Caryophanon tenue</i> NCIB 9535		+	+++	—	—	—
<i>Listeria grayi</i> ATCC 19120		+	+	+++	—	—
<i>Listeria grayi</i> ATCC 25400		+	+	+++	—	—
<i>Listeria monocytogenes</i> NCTC 10357*		+	+	+++	—	—
<i>Listeria monocytogenes</i> NCTC 4885		+	+	+++	—	—
<i>Listeria monocytogenes</i> NCTC 4883		+	+	+++	—	—
<i>Listeria murrayi</i> ATCC 25402		+	+	+++	—	—
<i>Listeria murrayi</i> ATCC 25403		+	+	+++	—	—
<i>Listeria denitrificans</i> L26*‡		—	—	+	+	+++

Erysipelothrix rhusiopathiae NCTC 8163* did not contain isoprenoid quinones

* Type strain.

† From Dr G. A. Gardner, Ulster Curer's Association, 2 Greenwood Avenue, Belfast, N. Ireland.

‡ From Pasteur Institute, Paris, France.

225 derived from the naphthaquinone nucleus, as expected from published data (Azerad & Cyrot-Pelletier, 1973). Strong peaks corresponding to molecular ions (M^+) were also observed. Details of the mass spectral analyses are shown in Table 1.

Caryophanon tenue contained unsaturated menaquinones with six isoprene units (abbreviated as MK-6, see *Biochemical Journal*, 1975, **147**, 15–21, for standard nomenclature and abbreviations) as the predominant isoprenologue, although smaller amounts of MK-5 were also detected in the mass spectrum and on reverse phase chromatography. *Brochothrix thermosphacta*, *L. grayi*, *L. monocytogenes* and *L. murrayi* all contained MK-7 as the major isoprenologue, whereas *L. denitrificans* contained MK-9 as the major component.

DISCUSSION

The absence of isoprenoid quinones in *E. rhusiopathiae* distinguishes it from the other bacteria examined and is in accord with the absence of catalase and cytochromes in this taxon (Seeliger, 1974). *Lactobacillus* strains are also generally regarded as being incapable of synthesizing menaquinones (Dunphy & Brodie, 1971).

The presence of MK-6 as the major isoprenologue in *Caryophanon tenue* is of particular interest because such short-chain menaquinones have not, to our knowledge, been noted as major components in other Gram-positive bacteria. Although at present this observation sheds little light on the taxonomic relationships of *Car. tenue*, it could prove of value when more information is available on the menaquinones of other Gram-positive asporogenous rods.

The similarity in the menaquinone profiles of *L. monocytogenes*, *L. grayi* and *L. murrayi* is further evidence of the close relationship between these taxa (Wilkinson & Jones, 1977) but does not resolve the problem of whether *L. grayi* and *L. murrayi* should be reclassified in the genus *Murraya* as proposed by Stuart & Welshimer (1974) or retained in the genus *Listeria* as maintained by Wilkinson & Jones (1977). The recovery of MK-9 as the major isoprenoid quinone in *L. denitrificans* supports the view that this taxon should be removed from the genus *Listeria* (Stuart & Welshimer, 1973; Jones, 1975*a, b*).

The marked distinction between the menaquinone pattern of *B. thermosphacta* and that reported for *M. lacticum* (Collins *et al.*, 1979), the type species of the genus *Microbacterium*, provides additional evidence for the recognition of the new genus *Brochothrix* (Sneath & Jones, 1976). Furthermore, the closely related menaquinone profiles of *B. thermosphacta*, *L. monocytogenes*, *L. grayi* and *L. murrayi* is in accord with the affinity between these taxa as demonstrated by other studies (Wilkinson & Jones, 1977; Collins-Thompson *et al.*, 1972; Stuart & Welshimer, 1973; Schleifer, 1970; Schleifer & Kandler, 1972; Raines *et al.*, 1968; Shaw & Stead, 1970; Tadayon & Carroll, 1971). The menaquinone data together with the presence of predominantly straight-chain and methyl-branched fatty acids within *Brochothrix* and *Listeria* casts serious doubt on the proposal of Wilkinson & Jones (1977) to include these two genera in an extended family Lactobacillaceae. However, further studies are required to clarify the higher taxonomic relationships of the genera *Brochothrix* and *Listeria*.

M. D. C. and D. J. thank the Medical Research Council for support (grant G977/613).

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