The Distribution of Isoprenoid Quinones in Streptococci of Serological Groups D and N

By M. D. COLLINS AND DOROTHY JONES

Department of Microbiology, School of Medicine and School of Biological Sciences,
University of Leicester, Leicester LE1 7RH

(Received 24 January 1979)

The isoprenoid quinone contents of streptococci of serological groups D and N were investigated. *Streptococcus faecalis*, *S. faecalis* subsp. *liquefaciens* and *S. faecalis* subsp. *zymogenes* strains contained demethylmenaquinones with nine isoprene units as their major isoprenologues. Menaquinones with eight isoprene units predominated in *S. faecium* subsp. *casseliflavus* and *S. faecium* subsp. *mobilis* whereas menaquinones with nine isoprene units constituted the major components in strains of *S. cremoris*, *S. cremoris* subsp. *alactosus*, *S. lactis* and *S. lactis* subsp. *diacetylactis*. Strains of *S. avium*, *S. bovis*, *S. durans*, *S. equinus*, *S. faecium*, *S. raffinolactis* and *S. suis* contained neither menaquinones nor ubiquinones. The isoprenoid quinone data correlate well with other kinds of data on these organisms and are of value in the classification of these bacteria.

INTRODUCTION

Bacteria of the genus *Streptococcus* are conventionally considered to be facultative anaerobes with a preference for anaerobic conditions. They grow aerobically but are unable to synthesize porphyrins and hence cytochromes. However, cytochromes have been demonstrated in strains of *Streptococcus faecalis* grown in media containing haem compounds (Whittenbury, 1964, 1978; Bryan-Jones & Whittenbury, 1969; Sijpesteijn, 1970; Ritchey & Seeley, 1974, 1976; Pritchard & Wimpenny, 1978). A systematic survey of the distribution of 'cytochrome-like respiration' amongst representatives of the genus *Streptococcus* (Ritchey & Seeley, 1976) indicated that this ability was restricted to *S. faecalis* and its subspecies *S. faecalis* subsp. *liquefaciens* and *S. faecalis* subsp. *zymogenes* (serological group D, Lancefield, 1933) and to a few strains designated *Streptococcus lactis* and *S. lactis* subsp. *diacetylactis* (serological group N, Lancefield; Shattock & Mattick, 1943).

Menaquinones [2-methyl-3-(prenyl)_n-1,4-naphthoquinones; I] and ubiquinones [2,3-dimethoxy-5-methyl-6-(prenyl)_n-1,4-benzoquinones; II] are constituents of bacterial plasma membranes (Pennock, 1966) and play an important role in electron transport and possibly oxidative phosphorylation (Brodie & Watanabe, 1966; Redfearn, 1966; Dunphy & Brodie, 1971). Baum & Dolin (1963) isolated a novel naphthoquinone from *S. faecalis* (strain 10C1) which had ultraviolet absorption characteristics distinct from the strict menaquinone family (I). This naphthoquinone was later shown to be a modified menaquinone in which the methyl group at C-2 had been replaced by a hydrogen atom (referred to as demethylmenaquinones: Baum & Dolin, 1965; Dolin & Baum, 1965). *Streptococcus faecalis* strain 10C1 has since been shown to be capable of oxidative phosphorylation (Gallin & Vandemark, 1964) and of forming a functional cytochrome system (Smalley *et al.*, 1968; Ritchey & Seeley, 1976). To our knowledge, however, no data are available on the presence of isoprenoid quinones in any other strain of *S. faecalis* or in any other streptococci of serological groups D and N.

Isoprenoid quinone structural types have been shown to be of taxonomic value in many groups of bacteria (Yamada *et al.*, 1976*a, b*; Collins *et al.*, 1977, 1979*a, b*; Minnikin *et al.*, 1978*a, b*). In view of the unsatisfactory taxonomic state of streptococci of serological groups D and N (for review, see Jones, 1978), we have investigated the type and distribution of isoprenoid quinones amongst representatives of these two serological groups together with some other streptococci and representatives of the genera *Aerococcus*, *Gemella*, *Leuconostoc* and *Pediococcus*.

METHODS

Strains and cultivation. Details of the test strains are shown in Tables 1 to 3. The aerococci, pediococci, leuconostocs and streptococci were grown in shake flasks in nutrient broth no. 2 (Oxoid) supplemented with glucose (2 mg ml⁻¹) and haemin (7.5 µg ml⁻¹) for 2 d at 30 °C. The single strain of *Gemella haemolysans* was grown in shake culture for 3 d at 30 °C in nutrient broth no. 2 supplemented with glucose (2 mg ml⁻¹), haemin (7.5 µg ml⁻¹) and horse serum (1 %, v/v). Cultures were checked for purity at maximum growth, collected by centrifugation, washed with distilled water and freeze-dried.

Extraction and purification of isoprenoid quinones. Dried organisms (250 mg) were examined using the procedure described by Collins *et al.* (1977).

Analysis of isoprenoid quinones. Purified isoprenoid quinones were further examined by reverse phase partition chromatography using Whatman KC₁₈F reverse phase thin-layer plates and a polar developing mixture of acetone/water (97:3, v/v) (Collins *et al.*, 1979*b*). Ultraviolet spectra of the quinones were recorded in iso-octane 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 230 °C. (Detailed mass spectra may be obtained from the authors on request.)

RESULTS

Components that co-chromatographed with vitamin K were the only isoprenoid quinones detected in the extracts of strains of *S. cremoris*, *S. cremoris* subsp. *alactosus*, *S. faecalis*, *S. faecalis* subsp. *liquefaciens*, *S. faecalis* subsp. *zymogenes*, *S. faecium* subsp. *casseliflavus*, *S. faecium* subsp. *mobilis*, *S. lactis* and *S. lactis* subsp. *diacetylactis*. Neither menaquinones (vitamin K) nor ubiquinones (coenzyme Q) were detected in the lipid extracts of the remaining 36 strains of streptococci or in strains of the genera *Aerococcus*, *Gemella*, *Leuconostoc* and *Pediococcus* (Table 1).

The purified isoprenoid quinones from strains of *S. cremoris*, *S. cremoris* subsp. *alactosus*, *S. faecium* subsp. *casseliflavus*, *S. faecium* subsp. *mobilis*, *S. lactis* and *S. lactis* subsp. *diacetylactis* had ultraviolet absorption maxima at 242, 248, 260, 270 and 326 nm. This is in accord with published data for menaquinones (Dunphy & Brodie, 1971). However, the isoprenoid quinones from strains of *S. faecalis*, *S. faecalis* subsp. *liquefaciens* and *S. faecalis* subsp. *zymogenes* had absorption maxima at 243, 248, 254, 263 and 326 nm indicating the presence of demethylmenaquinones (Dunphy & Brodie, 1971). On mass spectrometry, the menaquinones from strains of *S. cremoris*, *S. cremoris* subsp. *alactosus*, *S. faecium* subsp. *casseliflavus*, *S. faecium* subsp. *mobilis*, *S. lactis* and *S. lactis* subsp. *diacetylactis* produced base peaks at *m/e* 225, derived from the naphthoquinone nucleus, and intense peaks at *m/e* 187. The mass spectra of the demethylmenaquinones from *S. faecalis* strains produced intense peaks at *m/e* 211, thereby confirming that the methyl group at C-2 has been replaced

Table 1. *Taxa lacking both menaquinones and ubiquinones*

Strain designation/source	
<i>Aerococcus catalasicus</i> NCIB 9642	<i>S. faecalis</i> var. <i>malodoratus</i> NCDO 846†
<i>A. catalasicus</i> NCIB 9643	<i>S. faecalis</i> var. <i>malodoratus</i> NCDO 847
<i>Aerococcus viridans</i> NCIB 9206	<i>S. faecium</i> NCDO 583†
<i>Gemella haemolysans</i> NCTC 10244	<i>S. faecium</i> NCDO 1228
<i>Leuconostoc dextranicum</i> NCIB 2706	<i>S. faecium</i> ; E. M. Barnes, P/17/8 Type 38
<i>L. mesenteroides</i> NCIB 3351	<i>S. faecium</i> HGH9
<i>Pediococcus acidilactici</i> NCIB 7881†	<i>S. milleri</i> NCTC 10708
<i>P. parvulus</i> NCDO 1250	<i>S. milleri</i> NCTC 10709
<i>P. pentosaceus</i> NCIB 8106†	<i>S. mutans</i> subsp. <i>sobrinus</i> ; R. K. A. Feltham, K697
<i>Streptococcus agalactiae</i> (group B)§ NCDO 1352	<i>S. mutans</i> subsp. <i>sobrinus</i> ; R. K. A. Feltham, K598
<i>S. avium</i> (group Q); R. H. Deibel, SS1†	<i>S. pneumoniae</i> NCTC 7464
<i>S. avium</i> (group Q); R. H. Deibel, CCP1	* <i>S. pneumoniae</i> NCTC 7465
<i>S. avium</i> (group Q); R. H. Deibel, Strep A	<i>S. pyogenes</i> (group A) NCTC 8191
* <i>S. bovis</i> ATCC 9809†	* <i>S. pyogenes</i> (group A) NCTC 8198
<i>S. bovis</i> NCDO 599	* <i>S. raffinolactis</i> NCDO 617‡
<i>S. bovis</i> ; E. M. Barnes, TM/C/33	* <i>S. salivarius</i> (group K) ATCC 9759
* <i>S. durans</i> NCDO 596†	<i>S. sanguis</i> (group H) NCTC 7864
<i>S. durans</i> NCTC 8307	<i>S. suis</i> ; R. K. A. Feltham, K520†
<i>S. durans</i> ; E. M. Barnes, 98D	<i>S. sp.</i> (group E) NCTC 5385
<i>S. dysgalactiae</i> (group C) NCDO 1356	* <i>S. uberis</i> NCTC 3858
<i>S. equi</i> (group C) NCTC 9682	<i>S. uberis</i> NCTC 4674
<i>S. equinus</i> NCDO 1090†	
<i>S. equinus</i> NCDO 1091	
<i>S. equisimilis</i> (group C) NCTC 5371	

* Type strain.

† Taxa which react with group D antiserum (see Jones, 1978; Whittenbury, 1978).

‡ Taxa which react with group N antiserum (see Jones, 1978).

§ Serological group.

|| Sources, other than recognized culture collections, were: E. M. Barnes, Food Research Institute, Colney Lane, Norwich; R. H. Deibel, Department of Bacteriology, University of Wisconsin, Wisconsin, U.S.A.; R. K. A. Feltham, Department of Microbiology, University of Leicester, Leicester.

by a hydrogen atom. The mass spectra of both the menaquinones and demethylmenaquinones in the high mass region contained strong peaks corresponding to molecular ions (M^+). Details of the mass spectral analyses of the isoprenoid quinones are shown in Tables 2 and 3.

Streptococcus faecium subsp. *casseliflavus* and *S. faecium* subsp. *mobilis* strains contained comparable amounts of menaquinones with seven and eight isoprene units (abbreviated as MK-7 and MK-8, respectively). Menaquinones with nine isoprene units predominated in strains of *S. cremoris*, *S. cremoris* subsp. *alactosus*, *S. lactis* and *S. lactis* subsp. *diacetylactis* (Table 2). In contrast, strains of *S. faecalis*, *S. faecalis* subsp. *liquefaciens* and *S. faecalis* subsp. *zymogenes* contained demethylmenaquinones with nine isoprene units (abbreviated DMK-9) as the major isoprenologue, with smaller quantities of DMK-6, DMK-7 and DMK-8 (Table 3). The proportions of the major isoprenologues found by mass spectrometry were confirmed by the results of reverse phase partition chromatography.

DISCUSSION

The recovery of demethylmenaquinones with nine isoprene units from strains of *S. faecalis* confirms the findings of Baum & Dolin (1963, 1965) and is in accord with the presence of a cytochrome-like respiration system. Identical demethylmenaquinone patterns were also observed in strains of *S. faecalis* subsp. *liquefaciens* (non-haemolytic, gelatin liquefied) and *S. faecalis* subsp. *zymogenes* (β -haemolytic, gelatin may or may not be liquefied) (Deibel & Seeley, 1974). Deibel (1964) and Jones *et al.* (1972) have questioned the validity of these

Table 2. *Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from Streptococcus species*

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 <i>m/e</i>	MK-6 580	MK-7 648	MK-8 716	MK-9 784
<i>S. faecium</i> subsp. <i>casseliflavus</i> ATCC 25788†	+	++§	+++	—
<i>S. faecium</i> subsp. <i>casseliflavus</i> ATCC 25789	+	++§	+++	—
<i>S. faecium</i> subsp. <i>mobilis</i> ATCC 14436†	+	++§	+++	—
<i>S. faecium</i> subsp. <i>mobilis</i> NCIB 9645	+	++§	+++	—
<i>S. cremoris</i> NCDO 495‡	+	+	+	+++
* <i>S. cremoris</i> NCDO 607	+	+	+	+++
<i>S. cremoris</i> NCDO 762	+	+	+	+++
<i>S. cremoris</i> NCDO 2006	+	+	+	+++
<i>S. cremoris</i> subsp. <i>alactosus</i> NCDO 1032‡	+	+	+	+++
* <i>S. lactis</i> NCDO 604‡	+	+	+	+++
<i>S. lactis</i> NCIB 10769	+	+	+	+++
<i>S. lactis</i> subsp. <i>diacetyllactis</i> NCDO 176‡	+	+	+	+++
<i>S. lactis</i> subsp. <i>diacetyllactis</i> NCDO 1007	+	+	+	+++
<i>S. lactis</i> subsp. <i>diacetyllactis</i> NCDO 1008	+	+	+	+++
<i>S. lactis</i> subsp. <i>diacetyllactis</i> NCIB 8763	+	+	+	+++
<i>S. lactis</i> subsp. <i>diacetyllactis</i> NCIB 10493	+	+	+	+++

Strains of *S. cremoris*, *S. cremoris* subsp. *alactosus*, *S. lactis* and *S. lactis* subsp. *diacetyllactis* also contained traces of MK-10 (*m/e* 852).

* Type strain.

† Taxa which react with group D antiserum (see Jones, 1978).

‡ Taxa which react with group N antiserum (see Jones, 1978).

§ Intensity very similar to main peak.

Table 3. *Peaks corresponding to molecular ions in the mass spectra of demethylmenaquinones isolated from Streptococcus faecalis*

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 +.

Demethylmenaquinone isoprenologue <i>m/e</i>	DMK-6 566	DMK-7 634	DMK-8 702	DMK-9 770
<i>S. faecalis</i> NCIB 6782†	+	+	++	+++
<i>S. faecalis</i> NCTC 8729	+	+	++	+++
<i>S. faecalis</i> subsp. <i>liquefaciens</i> NCTC 8175†	+	+	++	+++
<i>S. faecalis</i> subsp. <i>zymogenes</i> NCTC 8176†	+	+	++	+++
<i>S. faecalis</i> subsp. <i>zymogenes</i> NCTC 5957	+	+	++	+++

Mass spectroscopy and reverse phase partition chromatography also revealed trace amounts of DMK-10 in the extracts of all the *S. faecalis* strains.

† Taxa which react with group D antiserum (see Jones, 1978).

subspecies and consider all should be included in one species, *S. faecalis*. The present quinone findings add weight to this view. Pette (1955) isolated some atypical *S. faecalis* strains from Gouda cheese and proposed the new variety *S. faecalis* var. *malodoratus*. However, the numerical taxonomic study of Jones *et al.* (1972) indicated no close relationship between these strains and *S. faecalis*. The presence of demethylmenaquinones in strains of *S. faecalis*, *S. faecalis* subsp. *liquefaciens* and *S. faecalis* subsp. *zymogenes* and their absence from *S. faecalis* var. *malodoratus* strains is in accord with the results of the numerical study of Jones *et al.* (1972) and suggests that the latter should be excluded from the species *S. faecalis*.

Streptococcus faecium (Orla-Jensen) is now recognized as a well-defined taxon distinct from *S. faecalis* (see Deibel & Seeley, 1974). The absence of demethylmenaquinones from

strains of the former and their presence in strains of the latter species reinforces this distinction. It is also generally agreed that strains previously called *S. durans* (Sherman & Wing, 1937) should be classified as *S. faecium* (Deibel, 1964; Jones *et al.*, 1972). The lack of isoprenoid quinones in strains of both these taxa does not contradict this view (Table 1).

Strains of *S. faecium* subsp. *casseliflavus* (Mundt & Graham, 1968) and *S. faecium* subsp. *mobilis* (Langston *et al.*, 1960) differ quite markedly in some respects from strains of *S. faecium* (Lund, 1967; Mundt & Graham, 1968; Roop *et al.*, 1974). The detection of major amounts of MK-7 and MK-8 in strains of the first two taxa but not in those of *S. faecium* underlines this distinction and suggests, along with other evidence (see Jones, 1978), that *S. faecium* subsp. *casseliflavus* and *S. faecium* subsp. *mobilis* are probably closely related to each other but are sufficiently distinct from *S. faecium* and *S. faecalis* to be excluded from both these species.

The recovery of menaquinones from strains of *S. lactis* and *S. lactis* subsp. *diacetylactis* is in accord with the reports of cytochrome-mediated electron transport in strains of these taxa when grown in the presence of preformed haem (Sijpesteijn, 1970; Ritchey & Seeley, 1976). *Streptococcus lactis* is now a well described species (see Deibel & Seeley, 1974; Jones, 1978) although the taxonomic status of *S. lactis* subsp. *diacetylactis* (Matuszewski *et al.*, 1936) remains equivocal. *Streptococcus lactis* subsp. *diacetylactis* has the same characteristics as *S. lactis* except that the former metabolizes citrate in the presence of fermentable carbohydrate to form acetoin, diacetyl and CO₂. The identical menaquinone profiles of strains of both *S. lactis* and *S. lactis* subsp. *diacetylactis* underlines the close relationship between these taxa and strengthens the case for reducing *S. lactis* subsp. *diacetylactis* to *S. lactis* (see Jones, 1978).

Cytochrome-mediated electron transport in *S. cremoris* strains has not to our knowledge been described to date. However, the detection of menaquinones in strains of both *S. cremoris* and *S. cremoris* subsp. *alactosus* suggests such an investigation may prove rewarding. *Streptococcus cremoris* and *S. lactis* share many biochemical properties (see Jones, 1978) and preliminary DNA-DNA homology studies (Garvie, 1978) indicate a similarity between these species. It is probable that *S. cremoris* is a variety of *S. lactis* more adapted to a dairy environment (Jones, 1978). The closely related menaquinone profiles of strains of both taxa lend weight to this view.

On the basis of lactate dehydrogenase properties, % G+C determinations and DNA-DNA homology studies, Garvie (1978) concluded that those streptococci for which she proposed the name *S. raffinolactis* are sufficiently distinct from *S. cremoris* and *S. lactis* to warrant separate species status. The apparent absence of menaquinones from strains of *S. raffinolactis* and their presence in the other streptococci of serological group N examined is in accord with this view.

The lack of both menaquinones and ubiquinones in the remaining streptococci examined, i.e. *S. agalactiae*, *S. avium*, *S. bovis*, *S. dysgalactiae*, *S. equi*, *S. equinus*, *S. equisimilis*, *S. milleri*, *S. mutans* subsp. *sobrinus*, *S. pneumoniae*, *S. pyogenes*, *S. salivarius*, *S. sanguis*, *S. suis* and *S. uberis*, is in keeping with the absence of a functional cytochrome-like respiratory system (Ritchey & Seeley, 1976; Whittenbury, 1978).

Members of the genera *Aerococcus* and *Pediococcus* possess many biochemical properties in common with the genus *Streptococcus*. Indeed, the major reason for excluding aerococci and pediococci from the genus *Streptococcus* is the mode of cell division (see Jones, 1978; Whittenbury, 1978). The absence of isoprenoid quinones from aerococci and pediococci reinforces their close relationship to the majority of streptococci as represented by the examples examined here.

Gemella haemolysans was initially classified in the Gram-negative genus *Neisseria*, but subsequent work indicated this species had a Gram-positive wall structure and was probably more closely related to the 'lactic acid' bacteria (Reyn, 1970, 1974; Reyn *et al.*, 1970; Wilkinson & Jones, 1977). In the 8th edition of *Bergey's Manual of Determinative Bacterio-*

logy (Buchanan & Gibbons, 1974), *Gemella haemolysans* is classified in the family Streptococcaceae (Deibel & Seeley, 1974; Reyn, 1974). The lack of detectable isoprenoid quinones in strains of *Gemella haemolysans* does not contradict its classification in the Streptococcaceae and clearly distinguishes it from members of the genus *Neisseria* which contain ubiquinones (coenzyme Q) as their sole isoprenoid quinones (Bishop *et al.*, 1962; Beebe, 1974).

The presence of different types of menaquinones in strains of some species known to react with Lancefield group D and group N antisera, and the apparent absence of menaquinones from others, further emphasizes the physiological heterogeneity of the taxa in these two serological groups. In addition, the data underline the strictures of Jones (1978) concerning the undue weight given in the past to serological grouping in streptococcal classification.

We are grateful to R. H. Deibel, E. M. Barnes and R. K. A. Feltham for generously supplying strains (see Table 1), and to the Department of Organic Chemistry, University of Leicester, for providing mass spectrometry facilities. We thank the Medical Research Council for support (grant G977/613/S).

REFERENCES

- BAUM, R. H. & DOLIN, M. I. (1963). Isolation of a new naphthoquinone from *Streptococcus faecalis* 10C1. *Journal of Biological Chemistry* **238**, PC4109-4111.
- BAUM, R. H. & DOLIN, M. I. (1965). Isolation of 2-solaneyl-1,4-naphthoquinone from *Streptococcus faecalis*, 10C1. *Journal of Biological Chemistry* **240**, 3425-3433.
- BEEBE, J. L. (1974). Lipid composition of *Neisseria catarrhalis*. *Abstracts of the Annual Meeting of the American Society for Microbiology*, p. 45.
- BISHOP, D. H. L., PANDYA, K. P. & KING, H. K. (1962). Ubiquinone and vitamin K in bacteria. *Biochemical Journal* **83**, 606-614.
- BRODIE, A. F. & WATANABE, T. (1966). Mode of action of vitamin K in micro-organisms. *Vitamins and Hormones* **24**, 447-463.
- BRYAN-JONES, D. G. & WHITTENBURY, R. (1969). Haematin-dependent oxidative phosphorylation in *Streptococcus faecalis*. *Journal of General Microbiology* **58**, 247-260.
- BUCHANAN, R. E. & GIBBONS, N. E. (editors) (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore: Williams & Wilkins.
- COLLINS, M. D., PIROUZ, T., GOODFELLOW, M. & MINNIKIN, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *Journal of General Microbiology* **100**, 221-230.
- COLLINS, M. D., GOODFELLOW, M. & MINNIKIN, D. E. (1979a). Isoprenoid quinones in the classification of coryneform and related bacteria. *Journal of General Microbiology* **110**, 127-136.
- COLLINS, M. D., JONES, D., GOODFELLOW, M. & MINNIKIN, D. E. (1979b). Isoprenoid quinone composition as a guide to the classification of *Listeria*, *Brochothrix*, *Erysipelothrix* and *Caryophanon*. *Journal of General Microbiology* **111**, 453-457.
- DEIBEL, R. H. (1964). The group D streptococci. *Bacteriological Reviews* **28**, 330-366.
- DEIBEL, R. H. & SEELEY, H. W. (1974). Streptococcaceae fam. nov. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 490-509. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- DOLIN, M. I. & BAUM, R. H. (1965). Evidence of thioether formation between 2-solaneyl-1,4-naphthoquinone and 2-mercaptoethanol. *Biochemical and Biophysical Research Communications* **18**, 202-205.
- DUNPHY, P. J. & BRODIE, A. F. (1971). The structure and function of quinones in respiratory metabolism. *Methods in Enzymology* **18**, 407-461.
- GALLIN, J. I. & VANDEMARK, P. J. (1964). Evidence for oxidative phosphorylation in *Streptococcus faecalis*. *Biochemical and Biophysical Research Communications* **17**, 630-635.
- GARVIE, E. I. (1978). *Streptococcus raffinolactis* (Orla-Jensen & Hansen); a group N streptococcus found in raw milk. *International Journal of Systematic Bacteriology* **28**, 190-193.
- JONES, D. (1978). Composition and differentiation of the genus *Streptococcus*. In *Streptococci*, pp. 1-49. Edited by F. A. Skinner & L. B. Quesnel. London: Academic Press.
- JONES, D., SACKIN, M. J. & SNEATH, P. H. A. (1972). A numerical taxonomic study of streptococci of serological group D. *Journal of General Microbiology* **72**, 439-450.
- LANCEFIELD, R. C. (1933). A serological differentiation of human and other groups of hemolytic streptococci. *Journal of Experimental Medicine* **57**, 571-595.
- LANGSTON, C. W., GUTTIERREZ, J. & BOUMA, C. (1960). Motile enterococci (*Streptococcus faecium* var. *mobilis* var. N) isolated from grass silage. *Journal of Bacteriology* **80**, 714-718.
- LUND, B. M. (1967). A study of some motile group D streptococci. *Journal of General Microbiology* **49**, 67-80.
- MATUSZEWSKI, T., PIJANOWSKI, E. & SUPINSKA, J. (1936). *Streptococcus diacetilactis* n.sp. and its application to butter making. *Roczników Nauk Rolniczych I Lésnych (Polish Agricultural and Forestry Annual)* **36**, 1-28.

- MINNIKIN, D. E., COLLINS, M. D. & GOODFELLOW, M. (1978*a*). Menaquinone patterns in the classification of nocardioform and related bacteria. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I)*, Supplement 6, 85–90.
- MINNIKIN, D. E., GOODFELLOW, M. & COLLINS, M. D. (1978*b*). Lipid composition in the classification and identification of coryneform and related taxa. In *Coryneform Bacteria*, pp. 85–160. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- MUNDT, J. O. & GRAHAM, W. F. (1968). *Streptococcus faecium* var. *casseliflavus* nov. var. *Journal of Bacteriology* 95, 2005–2009.
- PENNOCK, J. F. (1966). Occurrence of vitamins K and related quinones. *Vitamins and Hormones* 24, 307–329.
- PETTE, J. W. (1955). De vorming van zwavelwaterstof in Goudse Kaas, veroorzaakt door melkzuurbacterien. *Netherlands Milk and Dairy Journal* 10, 291–302.
- PRITCHARD, G. G. & WIMPENNY, J. W. T. (1978). Cytochrome formation, oxygen-induced proton extrusion and respiratory activity in *Streptococcus faecalis* var. *zymogenes* grown in the presence of haematin. *Journal of General Microbiology*, 104, 15–22.
- REDFEARN, E. R. (1966). Mode of action of ubiquinones (coenzyme Q) in electron transport systems. *Vitamins and Hormones* 24, 465–488.
- REYN, A. (1970). Taxonomic position of *Neisseria haemolysans* (Thjotta & Boe, 1938). *International Journal of Systematic Bacteriology* 20, 19–22.
- REYN, A. (1974). *Gemella*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 516–517. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- REYN, A., BIRCH-ANDERSON, A. & BERGER, U. (1970). Fine structure and taxonomic position of *Neisseria haemolysans* (Thjotta & Boe, 1938) or *Gemella haemolysans* (Berger, 1960). *Acta pathologica et microbiologica scandinavica* 78, 375–389.
- RITCHEY, T. W. & SEELEY, H. W. (1974). Cytochromes in *Streptococcus faecalis* var. *zymogenes* grown in haematin-containing medium. *Journal of General Microbiology* 85, 220–228.
- RITCHEY, T. W. & SEELEY, H. W. (1976). Distribution of cytochrome-like respiration in streptococci. *Journal of General Microbiology* 93, 195–203.
- ROOP, D. R., MUNDT, J. O. & RIGGSBY, W. S. (1974). Deoxyribonucleic acid hybridization studies among some strains of Group D and Group N streptococci. *International Journal of Systematic Bacteriology* 24, 330–337.
- SHATTOCK, P. M. F. & MATTICK, A. T. R. (1943). The serological grouping of *Streptococcus lactis* (Group N) and its relationships to *Streptococcus faecalis*. *Journal of Hygiene* 43, 173–188.
- SHERMAN, J. M. & WING, H. U. (1937). *Streptococcus durans* n.sp. *Journal of Dairy Science* 20, 165–167.
- SIJPESTEIJN, A. K. (1970). Induction of cytochrome formation and stimulation of oxidative dissimilation by haemin in *Streptococcus faecalis* and *Leuconostoc mesenteroides*. *Antonie van Leeuwenhoek* 36, 335–348.
- SMALLEY, A. J., JAHRLING, P. & VAN DEMARK, P. J. (1968). Molar growth yields as evidence for oxidative phosphorylation in *Streptococcus faecalis* strain 10C1. *Journal of Bacteriology* 96, 1595–1600.
- WHITTENBURY, R. (1964). Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. *Journal of General Microbiology* 35, 13–26.
- WHITTENBURY, R. (1978). Biochemical characteristics of *Streptococcus* species. In *Streptococci*, pp. 51–69. Edited by F. A. Skinner & L. B. Quesnel. London: Academic Press.
- WILKINSON, B. J. & JONES, D. (1977). A numerical taxonomic survey of *Listeria* and related bacteria. *Journal of General Microbiology* 98, 399–421.
- YAMADA, Y., INOUE, G., TAHARA, Y. & KONDO, K. (1976*a*). The menaquinone system in the classification of coryneform and nocardioform bacteria and related organisms. *Journal of General and Applied Microbiology* 22, 203–214.
- YAMADA, Y., INOUE, G., TAHARA, Y. & KONDO, K. (1976*b*). The menaquinone system in the classification of aerobic Gram-positive cocci in the genera *Micrococcus*, *Staphylococcus*, *Planococcus* and *Sporosarcina*. *Journal of General and Applied Microbiology* 22, 227–236.