Polar Lipid and Isoprenoid Quinone Composition in the Classification of *Staphylococcus*

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Representatives of 13 species of *Staphylococcus* were examined using a small-scale procedure for the sequential extraction of isoprenoid quinones and polar lipids. Menaquinones were the only isoprenoid quinones found in the 77 test strains which were divided into three groups based upon the predominant isoprenologue detected: (i) *S. hyicus* subsp. *hyicus*, *S. sciuri* subsp. *lentus* and *S. sciuri* subsp. *sciuri* contained unsaturated menaquinones with six isoprene units; (ii) *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. hyicus* subsp. *chromogenes*, *S. intermedius*, *S. saprophyticus*, *S. simulans*, *S. warneri* and *S. xylosus* contained unsaturated menaquinones with seven isoprene units and (iii) *S. aureus* contained unsaturated menaquinones with eight isoprene units and varying amounts of the corresponding lower isoprenologue. All of the organisms contained very similar polar lipid patterns consisting of diphosphatidylglycerol, phosphatidylglycerol, β -gentiobiosyl diacylglycerol and a number of glycolipids and phospholipids. One of the glycolipids was chromatographically indistinguishable from β -gentiotriosyl diacylglycerol. Lysylphosphatidylglycerol was a major component in *S. aureus* and *S. intermedius* but was usually present in minor amounts in the coagulase-negative strains. The polar lipid data underline the homogeneity of the genus *Staphylococcus* and distinguish staphylococci from aerobic, Gram-positive cocci and from the phylogenetically related aerobic, endospore-forming bacteria. Menaquinone composition can also be used to separate staphylococci from other aerobic, Gram-positive cocci.

INTRODUCTION

Staphylococci form a well-defined taxon (Kloos, 1980; Kloos & Schleifer, 1981) that is phylogenetically closer to *Bacillus* than to *Micrococcus* (Stackebrandt & Woese, 1979, 1981; Mordarski *et al.*, 1981*a*). The genus *Staphylococcus* has been the subject of marked taxonomic revision since the last edition of *Bergey's Manual of Determinative Bacteriology* (Baird-Parker, 1974) and currently contains 16 species described primarily on the basis of biochemical, chemical, morphological and physiological properties (Skerman *et al.*, 1980; Kilpper-Bälz & Schleifer, 1981; Schleifer & Fischer, 1982; Schleifer *et al.*, 1982; Kloos & Schleifer, 1983). The status of most of the recently described species is supported by DNA–DNA pairing and protein homology data (Meyer & Schleifer, 1978; Schleifer *et al.*, 1979, 1982; Goodfellow *et al.*, 1980; Kilpper-Bälz & Schleifer, 1981; Kloos & Wolfshohl, 1982; Schleifer & Fischer, 1982; Kloos & Schleifer, 1983) and simplified schemes have been recommended for the identification of human *Staphylococcus* species (Kloos & Schleifer, 1975; Kloos & Wolfshohl, 1982). A number of workers have questioned the integrity of both recently described and well-established staphylococcal species (Feltham, 1979; Hájek & Schindler, 1981; Mordarski *et al.*, 1981*b*), others the

Abbreviations: MK, menaquinone; TSB, Trypticase Soy Broth.

reliability of tests currently employed for the identification of unknown strains (Heddaeus *et al.*, 1978, 1981; Marples, 1981; Feltham & Sneath, 1982) and there is evidence that additional centres of variation exist within the genus (Weckbach & Langlois, 1976; Feltham, 1979; Kloos & Wolfshohl, 1982; Goodfellow *et al.*, 1983; Schumacher-Perdreau *et al.*, 1983).

Lipid markers are being increasingly used for both the classification and identification of Gram-positive bacteria (see Lechevalier, 1977; Minnikin *et al.*, 1978; Minnikin & Goodfellow, 1980, 1981); especially valuable data have been derived from the analysis of fatty acids (Bousfield *et al.*, 1983; Collins *et al.*, 1982*a*), isoprenoid quinones (see Collins & Jones, 1981) and polar lipids (Lechevalier *et al.*, 1977; Minnikin *et al.*, 1978; Collins *et al.*, 1982*b*). Staphylococci contain qualitatively similar fatty acid profiles consisting of major amounts of *iso*- and *anteiso*-methyl branched components (Durham & Kloos, 1978; Sincoweay *et al.*, 1981) and have unsaturated menaquinones (MK) as their sole respiratory isoprenoid quinone (see Collins & Jones, 1981). *Staphylococcus aureus* strains contain menaquinones with eight isoprene units, abbreviated as MK-8, as their main component, although MK-7 is also present in substantial proportions (Cawthorne *et al.*, 1967; Frerman & White, 1967; Jeffries *et al.*, 1967*a,b*, 1969; Hammond & White, 1969; Jeffries, 1969; Yamada *et al.*, 1976; Collins, 1981). Little is known about the distribution of menaquinones amongst coagulase-negative staphylococci, though there is evidence of two distinct menaquinone patterns each of which can be distinguished readily from the profile characteristic for *S. aureus* (Bishop *et al.*, 1962; Jeffries *et al.*, 1967*b*, 1969; Jeffries, 1969; Kushwaha & Kates, 1976; Yamada *et al.*, 1976; Collins *et al.*, 1980; Collins, 1981).

Polar lipid analyses have been restricted to *S. aureus* (Macfarlane, 1962; Houtsmuller & van Deenen, 1965; White & Frerman, 1967; Brundish & Baddiley, 1968; Gould & Lennarz, 1970; Joyce *et al.*, 1970; Short & White, 1970; Rédei *et al.*, 1971; Komura *et al.*, 1975) and *S. epidermidis* strains (Komaratat & Kates, 1975; Komura *et al.*, 1975) and in most of these investigations the emphasis has been either on the effect of growth conditions on polar lipid composition or on the characterization of specific phospholipid fractions. *Staphylococcus aureus* and *S. epidermidis* contain major amounts of diphosphatidylglycerol and phosphatidylglycerol but the former also possesses lysylphosphatidylglycerol, diglucosyldiacylglycerol and occasionally a glycopospholipid and the latter phosphatidic acid, phosphatidyl diglycosyldiglyceride and unknown phospholipids.

In the present study, representatives of 13 species of *Staphylococcus* were examined to determine the value of menaquinones and polar lipids in their classification.

METHODS

Cultures. The test strains (Table 1) were maintained on nutrient agar (Oxoid) supplemented with glucose (2%, w/v), and as frozen glycerol suspensions at -25 °C (Wellington & Williams, 1979). More detailed information on the strain histories can be found elsewhere (Goodfellow *et al.*, 1983).

Cultivation. *Staphylococcus aureus* SCH103, the *S. hyicus* and coagulase-negative strains were grown overnight at 35 °C in 2 ml Trypticase Soy Broth supplemented with glucose (0.25%, w/v, TSB; Difco). Samples (0.05 ml) of these cultures were used to seed 50 ml TSB in flasks which were shaken on a rotary shaker at 200 r.p.m., 35 °C. The OD of cultures was checked after 6 h using two different wavelengths, and when OD₆₀₀ reached 2.5 or OD₄₅₀ reached 3.5, 6 ml of each culture was transferred to 1 litre conical flasks containing 300 ml TSB to give approximately 10⁸ c.f.u. per 50 ml. Inoculated flasks were shaken in an orbital incubator (150 r.p.m.) at 35 °C for 24 h. The cultures were then checked for purity, killed by the addition of 1 ml formalin (40%, v/v), harvested by centrifugation at 3000 g for 20 min, washed twice with 67 mM-Sorenson phosphate buffer at pH 7.0 and freeze-dried. The remaining *S. aureus* strains and all of the *S. intermedius* strains were grown on sheep blood agar (5%, v/v) at 37 °C for 24 h and kept for 2 d at ambient temperature in daylight; harvested biomass was washed twice with normal saline and freeze-dried.

Extraction of isoprenoid quinones and polar lipids. Isoprenoid quinones and polar lipids were extracted using a recently developed procedure (Minnikin *et al.*, 1984). Equal volumes (2 ml) of aqueous methanolic NaCl (10 ml 0.3%, w/v, aqueous NaCl added to 100 ml methanol) and petroleum ether (b.p. 60–80 °C) were added to 100 mg biomass in polytetrafluoroethylene-lined capped tubes and the contents mixed on a tube rotator for 15 min. The tubes were then centrifuged at low speed for 5 min, the upper layer removed and the extraction repeated with 1 ml petroleum ether. The combined extracts were evaporated under nitrogen at about 37 °C to provide crude isoprenoid quinones. The residual aqueous layers remaining after isoprenoid quinone extraction were treated

Table 1. Designation and source of test strains

Laboratory number	Strain designation/Source†
SCH103*	<i>Staphylococcus aureus</i> NCTC 8532; isolated from human pleural fluid
SCH113–SCH115	<i>S. aureus</i> biotype A; V. Hájek, Microbiological Institute, Palacky University, Olomouc, Czechoslovakia, B13, B31, B42; patients with bronchopneumonia complicating influenza infection
SCH116–SCH118	<i>S. aureus</i> biotype B; V. Hájek, V1, V5, V15; slaughtered pigs
SCH119–SCH121	<i>S. aureus</i> biotype B; V. Hájek, S26, S41, S49; chickens with septicaemia
SCH122–SCH124	<i>S. aureus</i> biotype C; V. Hájek, M1, M39, M85; cows with mastitis
SCH125–SCH127	<i>S. aureus</i> biotype C; V. Hájek, R18, R28, R31; sheep
SCH128–SCH130	<i>S. aureus</i> biotype D; V. Hájek, Z10 (CCM5771), Z17, Z20; hares
SCH104*	<i>Staphylococcus capitis</i> ; NCTC 11045
SCH92, SCH93	<i>S. capitis</i> ; G. Pulverer, Institute of Hygiene, University of Cologne, Cologne, FRG, 152, 203
SCH105*	<i>Staphylococcus cohnii</i> NCTC 11041; isolated from human skin
SCH25, SCH30, SCH32, SCH37	<i>S. cohnii</i> ; G. Pulverer, 182, 202, 216, 251; cow's milk
SCH106*	<i>Staphylococcus epidermidis</i> NCTC 11047
SCH107*	<i>Staphylococcus haemolyticus</i> NCTC 11042
SCH108*	<i>Staphylococcus hominis</i> NCTC 11320
SCH98, SCH99	<i>S. hominis</i> ; G. Pulverer, 263, 100
SCH167*, SCH164	<i>Staphylococcus hyicus</i> subsp. <i>chromogenes</i> ; S. Rowinski, Polish Collection of Microorganisms, Institute of Immunology and Experimental Therapy, Wrocław, Poland, SH220 (NCTC 10530), SH208; pigs
SCH170	<i>S. hyicus</i> subsp. <i>chromogenes</i> ; S. Rowinski, SH226; cow, Belgium
SCH157*	<i>Staphylococcus hyicus</i> subsp. <i>hyicus</i> ; S. Rowinski, SH30 (NCTC 10350); isolated from swine
SCH154, SCH161	<i>S. hyicus</i> subsp. <i>hyicus</i> ; S. Rowinski, SH59, SH170; isolated from swine, Belgium and England, respectively
SCH131–SCH133	<i>Staphylococcus intermedius</i> biotype E; V. Hájek, P13 (CCM 5738), P15, P71; dogs
SCH134–SCH136	<i>S. intermedius</i> biotype E; V. Hájek, K2, K20, K35; horses
SCH137–SCH139	<i>S. intermedius</i> biotype E; V. Hájek, L91, L103, L105; minks
SCH140*–SCH142	<i>S. intermedius</i> biotype F; V. Hájek, H11 (NCTC 11048), H12, H17; pigeons
SCH94*, SCH95	<i>Staphylococcus saprophyticus</i> ; G. Pulverer, 075, 247
SCH89*	<i>Staphylococcus sciuri</i> subsp. <i>lentus</i> DSM 20352; isolated from udder of goat
SCH90	<i>S. sciuri</i> subsp. <i>lentus</i> CCM 3472
SCH91*	<i>Staphylococcus sciuri</i> subsp. <i>sciuri</i> DSM 20345; isolated from skin of eastern gray squirrel
SCH110*	<i>Staphylococcus simulans</i> NCTC 11046
SCH4, SCH6, SCH9, SCH96, SCH97, SCH221	<i>S. simulans</i> ; G. Pulverer, 28, 37, 70, 119, 166, 41
SCH111*	<i>Staphylococcus warneri</i> ; NCTC 11044
SCH102	<i>S. warneri</i> ; G. Pulverer, 101
SCH112*	<i>Staphylococcus xylosus</i> NCTC 11043; isolated from human skin
SCH12, SCH16, SCH31	<i>S. xylosus</i> ; G. Pulverer, 83, 103, 212
SCH1, SCH41, SCH46	<i>Staphylococcus</i> sp.; G. Pulverer, 16, 298, 349, cluster 11A‡
SCH5, SCH38, SCH43	<i>Staphylococcus</i> sp.; G. Pulverer, 35, 261, 326, cluster 11B‡

* Type strain.

† CCM, Czechoslovak Collection of Micro-organisms, Brno, Czechoslovakia; DSM, Deutsche Sammlung von Mikroorganismen, Teilsammlung Darmstadt, 61 Darmstadt, FRG; NCTC, National Collection of Type Cultures, London, UK.

‡ Goodfellow *et al.* (1983).

using a modification (Card, 1973; Minnikin *et al.*, 1979, 1984) of the method of Bligh & Dyer (1959) to give a polar lipid extract which was stored at -20°C .

Analysis of isoprenoid quinones. Crude isoprenoid quinone samples were dissolved in petroleum ether, applied as 0.5 cm bands to 10×10 cm pieces of plastic-backed silica gel sheets (Merck 5735), and developed with petroleum ether/acetone (96:4, v/v) in the first direction and petroleum ether/toluene (50:50, v/v) in the second direction. Separated menaquinones were detected with short-wave UV light (254 nm) and menaquinone bands cut out and extracted by shaking with diethyl ether (1 ml) for 15 min. The extraction was repeated with a further 1 ml diethyl ether, the combined extracts passed through a Pasteur pipette plugged with cotton wool prewashed with diethyl ether, the filtrate evaporated to dryness using nitrogen (around 37°C) and stored in the dark at -20°C . Purified

menaquinones were analysed by mass spectroscopy on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 170–220 °C, and by reverse-phase TLC (Collins *et al.*, 1980).

Analysis of polar lipids. Patterns of polar lipids were obtained by spotting samples onto 6.6 × 6.6 cm pieces of aluminium-backed silica gel sheets (Merck 5554) and developing with chloroform/methanol/water (65:25:4, by vol.) in the first direction and chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) in the second direction. All lipids were detected by spraying with 5% ethanolic molybdophosphoric acid followed by charring at 180 °C. Additional preparations were examined using specific spray reagents for amino groups (0.2% ninhydrin in water-saturated butanol) (Consden & Gordon, 1948), α -glycols (periodate-Schiff) (Shaw, 1968), lipid phosphate (Dittmer & Lester, 1964) and sugars (α -naphthol) (Jacin & Mishkin, 1965).

RESULTS

Compounds that co-chromatographed with vitamin K₁ were the only isoprenoid quinones detected by TLC in extracts of the 74 strains. Purification of the menaquinones, using a two-dimensional TLC system, was necessary to avoid spurious peaks in mass spectrometry. The most intense peaks in the mass spectra of the purified menaquinones occurred at *m/e* 187 and 225 and were derived from the naphthoquinone nucleus (Azerad & Cyrot-Pelletier, 1973). All of the mass spectra in the high mass region contained strong peaks corresponding to molecular ions (*M*⁺) and in some cases smaller peaks at *M*⁺ – 15 corresponded to the loss of a methyl group from the molecular ion. The results of the mass spectral analyses of the menaquinones shown in Tables 2 and 3, were confirmed by reverse phase TLC.

The strains of *S. intermedius* contained unsaturated menaquinones with seven isoprene units as the only component; in contrast the *S. aureus* strains possessed major amounts of unsaturated menaquinones with eight isoprene units and varying amounts of MK-7 (Table 2). Some of the *S. aureus* strains contained approximately equal amounts of MK-7 and MK-8 but only the type

Table 2. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from representatives of *Staphylococcus aureus* (biotypes A–D) and *Staphylococcus intermedius* (biotypes E and F)

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

Strain	Menaquinone isoprenologue . . . MK-6 Biotype* <i>m/e</i> . . . 580	MK-7 648	MK-8 716
SCH103		+	+
SCH113	A	–	+
SCH114		–	+
SCH115		–	+
SCH116		–	+
SCH117		–	+
SCH118	B	–	+
SCH119		–	+
SCH120		–	+
SCH121		–	+
SCH122		–	+
SCH123	C	–	+
SCH124		–	+
SCH125		–	+
SCH126		–	+
SCH127		–	+
SCH128	D	–	+
SCH129		–	+
SCH130		–	+
Strains†	E	+	+
Strains‡	F	+	–

* Biotypes recognised by Hájek & Marsalek (1971, 1976).

† Strains SCH131, SCH132, SCH133, SCH134, SCH135, SCH136, SCH137, SCH138, SCH139 and SCH139 (duplicate).

‡ Strains SCH140, SCH140 (duplicate), SCH141 and SCH142.

Table 3. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from representative strains of coagulase-negative *Staphylococcus* species

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

Strain	Menaquinone isoprenologue			
	m/e	... MK-6 ... 580	MK-7 648	MK-8 716
<i>Staphylococcus capitis</i>	SCH92	—	+++	—
	SCH93	—	+++	—
	SCH104	—	+++	—
<i>Staphylococcus cohnii</i>	SCH25	—	+++	—
	SCH30	—	+++	—
	SCH32	—	+++	—
	SCH37	—	+++	—
	SCH105	—	+++	—
<i>Staphylococcus epidermidis</i>	SCH106	+	+++	+
<i>Staphylococcus haemolyticus</i>	SCH107	—	+++	—
<i>Staphylococcus hominis</i>	SCH98	—	+++	—
	SCH99	—	+++	+
	SCH108	—	+++	+
<i>Staphylococcus hyicus</i> subsp. <i>chromogenes</i>	SCH164	+	+++	+
	SCH167	+	+++	—
	SCH170	+	+++	+
<i>Staphylococcus hyicus</i> subsp. <i>hyicus</i>	SCH154	+++	+	—
	SCH157	+++	+	—
	SCH161	+++	+	—
<i>Staphylococcus saprophyticus</i>	SCH94	—	+++	—
	SCH95	—	+++	—
<i>Staphylococcus sciuri</i> subsp. <i>lentus</i>	SCH89	+++	—	—
	•SCH89	+++	—	—
	SCH90	+++	—	—
<i>Staphylococcus sciuri</i> subsp. <i>sciuri</i>	SCH91	+++	—	—
	•SCH91	+++	—	—
<i>Staphylococcus simulans</i>	SCH4	+	+++	—
	SCH6	+	+++	—
	SCH9	+	+++	—
	SCH96	+	+++	—
	SCH110	—	+++	—
	SCH221	+	+++	—
<i>Staphylococcus warneri</i>	SCH102	—	+++	—
	SCH111	—	+++	—
<i>Staphylococcus xylosus</i>	SCH12	—	+++	—
	SCH16	—	+++	+
	SCH31	—	+++	—
	SCH112	—	+++	—
<i>Staphylococcus</i> sp.	SCH1	—	+++	+
	SCH5	—	+++	+
	SCH38	—	+++	+
	SCH41	—	+++	+
	SCH43	—	+++	+
	SCH46	+	+++	+

• Duplicate cultures.

strain had MK-6 as a minor component. Analyses by reverse phase TLC correlated with these mass spectral data. The remaining staphylococci fell into two broad groups on the basis of menaquinone composition. Thus, *S. sciuri* and *S. hyicus* subsp. *hyicus* had major amounts of MK-6, and in the second group MK-7 was the major isoprenologue. It was also possible to distinguish between *S. sciuri* and *S. hyicus* subsp. *hyicus* as only the latter had minor amounts of MK-7. Similarly, all of the representatives of *S. capitis*, *S. cohnii*, *S. haemolyticus*, *S. saprophyticus* and *S.*

warneri, and all but one of the *S. xylosus* strains contained MK-7 as the only component whereas *S. hominis* and strains received as *Staphylococcus* sp. also had minor amounts of MK-8. Further, all but one of the *S. simulans* strains possessed small amounts of MK-6, whereas *S. epidermidis* SCH106 and two of the three strains of *S. hyicus* subsp. *chromogenes* contain minor amounts of both MK-6 and MK-8.

The results of the two-dimensional TLC analyses of the polar lipids of representative staphylococci are shown in Fig. 1; all 74 strains gave similar polar lipid patterns. The major

components co-chromatographed with and had the staining properties of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and β -gentiobiosyl diacylglycerol (G_1). Lysylphosphatidylglycerol (LPG) was a major component in all of the coagulase-positive strains and was also present in the coagulase-negative strains but usually in minor amounts. The strains also contained a number of unknown phospholipids (P) and glycolipids (G) (Fig. 1). The polar glycolipid (G_2) (Fig. 1) corresponded in chromatographic behaviour to a β -gentiotriosyl diacylglycerol from the lipids of *Bacillus* species, previously observed as an unidentified trace component in the lipids of *S. aureus* DSM 346 (Fischer *et al.*, 1978).

DISCUSSION

It is evident from the present study that qualitative polar lipid analyses are of little value in the subgeneric classification of *Staphylococcus* as all of the *S. aureus*, *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. hyicus*, *S. intermedius*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, *S. warneri* and *S. xylosus* strains contained DPG, PG, LPG, a glycolipid chromatographically indistinguishable from the β -gentiobiosyl diacylglycerol of *S. aureus* (Shaw, 1975; Fischer *et al.*, 1978) and several unknown glyco- and phospholipids. An essentially similar polar lipid pattern has been reported for *S. (Micrococcus) caseolyticus* (Whiteside *et al.*, 1971). In contrast to nearly all of the *S. aureus* and *S. intermedius* strains, most of the coagulase-negative cultures contained only traces of LPG, indeed, the lipid could not always be detected on the chromatograms. *Staphylococcus aureus* SCH103 grown on TSB, gave the same polar lipid pattern as the *S. aureus* strains grown on sheep blood agar.

The polar lipid patterns of staphylococci distinguish them from other aerobic, Gram-positive cocci, and from aerobic, endospore-forming bacteria. Thus, *Bacillus*, *Planococcus* and *Sporosarcina* strains typically contain phosphatidylethanolamine as well as phosphatidylglycerol, and diphosphatidylglycerol (Komura *et al.*, 1975; Minnikin & Goodfellow, 1981), and *Micrococcus luteus* has phosphatidylglycerol, phosphatidylinositol and a dimannosyl diacylglycerol (Whiteside *et al.*, 1971; De Siervo & Salton, 1971; Shaw, 1975). It is interesting that staphylococci and aerobic, endospore-forming bacilli have the same (1 $\rightarrow$ 6)- β -linked diglucosyl (gentiobiosyl) diacylglycerol given their close phylogenetic relationship (Stackebrandt & Woese, 1979, 1981). In contrast, micrococci have a different glycolipid based on mannose but streptococci may also contain a diglycosyl diacylglycerol but with a (1 $\rightarrow$ 2)- α -linkage (Shaw, 1975; Lechevalier, 1977).

The menaquinone data extend and confirm earlier investigations notably that of Collins (1981) who assigned a small number of staphylococci to three groups on the basis of whether they contained MK-6, MK-7 or MK-8 as the predominant isoprenologue. In the present study, the *S. sciuri* and *S. hyicus* subsp. *hyicus* strains contained major amounts of MK-6, the *S. aureus* strains major proportions of MK-8 and *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. intermedius*, *S. saprophyticus*, *S. simulans*, *S. warneri* and *S. xylosus* had MK-7 as the predominant isoprenologue. The presence of unsaturated menaquinones distinguishes staphylococci from *M. luteus* and related strains which contain dihydrogenated menaquinones with

Fig. 1. Two-dimensional TLC of the polar lipids of the type strains of (a) *S. aureus* SCH103, (b) *S. capitis* SCH104, (c) *S. cohnii* SCH25, (d) *S. epidermidis* SCH106, (e) *S. haemolyticus* SCH107, (f) *S. hominis* SCH108, (g) *S. hyicus* subsp. *chromogenes* SCH167, (h) *S. hyicus* subsp. *hyicus* SCH157, (i) *S. intermedius* SCH140, (j) *S. saprophyticus* SCH94, (k) *S. sciuri* subsp. *lentus* SCH89, (l) *S. sciuri* subsp. *sciuri* SCH91, (m) *S. simulans* SCH110, (n) *S. warneri* SCH111 and (o) *S. xylosus* SCH112. Chloroform/methanol/water (65:25:4, by vol.) was used in the first direction followed by chloroform/acetic acid/methanol/water (85:15:12:4, by vol.) in the second direction. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; LPG, lysylphosphatidylglycerol; G_1 , gentiobiosyl diacylglycerol; G_2 , possible gentiotriosyl diacylglycerol; G, unknown glycolipid; P, unknown phospholipid; A, positive to ninhydrin; B, positive to periodate-Schiff.

Photocopies of the polar lipid patterns of the remaining strains have been deposited with the British Lending Library, Boston Spa LS23 7BQ, UK as Supplementary Publication no. 28017. Copies may be obtained from the BLL on demand. Where possible, requests should be accompanied by prepaid coupons (held by many university and technical libraries and by the British Council).

seven, eight or nine isoprene units (Jeffries *et al.*, 1967*b*, 1969; Jeffries, 1969; Yamada *et al.*, 1976). Similarly, staphylococci can be separated from those streptococci that contain major amounts of MK-9 or demethyl-menaquinones with nine isoprene units and from *Aerococcus*, *Gemella*, *Leuconostoc*, *Pediococcus* and most *Streptococcus* strains which lack isoprenoid quinones (Collins & Jones, 1981). They cannot, however, be distinguished from *Bacillus*, *Sporolactobacillus* and *Sporosarcina* strains which have MK-7 as the major isoprenologue (Minnikin & Goodfellow, 1981) nor from *Streptococcus faecium* strains containing major amounts of MK-8 (Collins & Jones, 1981).

The detection of major amounts of MK-8 and substantial proportions of MK-7 in *S. aureus* strains is in agreement with previous studies (Cawthorne *et al.*, 1967; Jeffries *et al.*, 1967*b*, 1969; Jeffries, 1969; Yamada *et al.*, 1976; Collins, 1981); some of these workers reported the presence of minor amounts of MK-6, others MK-9. It is known that the relative amounts of such isoprenologues vary between strains (Jeffries *et al.*, 1969). It is possible that the presence or absence of such components is influenced by the growth regime, since in the present study MK-9 was not detected, but traces of MK-6 were found in the *S. aureus* strain grown on TSB. The presence of only major amounts of MK-7 in the *S. intermedius* strains distinguishes them from *S. aureus* and provides further support for assigning coagulase-positive staphylococci to two species. Thus, representatives of *S. aureus* and *S. intermedius* have few DNA sequences in common (Meyer & Schleifer, 1978), can be separated readily from one another and from *S. hyicus* in numerical phenetic surveys (Hájek & Schindler, 1981; Goodfellow *et al.*, 1983), show differences in wall composition (Schleifer & Kandler, 1972; Hájek, 1976; Schleifer *et al.*, 1976), in the configuration of lactic acid produced from glucose under anaerobic conditions, in esterase pattern and in the occurrence of fructose-1,6-diphosphate-activated L-lactate dehydrogenase (Schleifer *et al.*, 1976). The inability of Feltham (1979) to separate *S. intermedius* and *S. hyicus* strains can probably be attributed to an overreliance placed on biochemical properties in his numerical phenetic study.

The assignment of coagulase-negative staphylococci to two broad groups on the basis of menaquinone composition is in agreement with earlier preliminary studies (Jeffries *et al.*, 1967*b*, 1969; Jeffries, 1969; Kushwaha & Kates, 1976; Yamada *et al.*, 1976; Collins *et al.*, 1980; Collins, 1981). It is especially interesting that the two subspecies of *S. sciuri* are characterized by unsaturated menaquinones with six isoprene units, and that menaquinone data provide some support for the continued recognition of the two subspecies of *S. hyicus* (Devriese *et al.*, 1978). However, further studies on additional strains are needed to determine whether menaquinone analysis can be used to distinguish between the two subspecies of *S. hyicus*; Hájek & Schindler (1981) considered that the latter merited species status. Additional studies are also needed to see whether coagulase-negative species containing major amounts of MK-7 can be distinguished on the basis of the minor amounts of menaquinones they contain.

The present results are in good agreement with preliminary studies which showed the value of polar lipids and isoprenoid quinones in staphylococcal systematics (Komura *et al.*, 1975; Collins & Jones, 1981). The polar lipid data help underline the homogeneity of the genus *Staphylococcus*, but further studies are needed to evaluate the full potential of menaquinone analyses in the identification of staphylococcal species. Both the polar lipid and menaquinone results argue against the suggestion (Feltham, 1979) that *S. sciuri* be moved to a new genus *Cellobiosococcus*. DNA homology and catalase immunological studies also support the continued recognition of *S. sciuri* (Kloos, 1980).

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