Arch Microbiol (1984) 137:247–249

Archives of
Microbiology

Fatty acid, menaquinone and polar lipid composition of *Rothia dentocariosa*

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Abstract. The lipid compositions of *Rothia dentocariosa* was investigated. All of the strains tested possessed closely related lipid profiles consisting of predominantly straight-chain saturated and methyl branched long-chain fatty acids, unsaturated menaquinones with seven isoprene units and a polar lipid composition comprising diphosphatidylglycerol, phosphatidylglycerol and a diglycosyldiacylglycerol. The results of the present study indicate *Rothia dentocariosa* is a good and distinct taxon. The lipid data however does not support the classification of *Rothia dentocariosa* in the family Actinomycetaceae.

Key words: *Rothia dentocariosa* – Fatty acids – Menaquinones – Polar lipids – Chemotaxonomy

The genus *Rothia* was created to accommodate organisms previously designated "*Nocardia dentocariosus*" and "*N. salivae*" (Georg and Brown 1967). Although *Rothia dentocariosa* is a relatively well-defined taxon, its affinity to other filamentous actinomycete taxa is controversial. *Rothia dentocariosa* differs from *Nocardia* in having a fermentative metabolism and in failing to grow on inorganic nitrogen sources (Slack and Gerencser 1975). *Rothia* is also differs from nocardiae in possessing a cell wall peptidoglycan based upon lysine (type: Lys-Ala_3) (Schleifer and Kandler 1972). Representatives of the genus *Nocardia* contain a directly cross-linked peptidoglycan based upon *meso*-diaminopimelic acid (Schleifer and Kandler 1972). In the 8th edition of Bergey's Manual of Determinative Bacteriology *Rothia dentocariosa* is included in the family Actinomycetaceae (Slack 1974). Although *Rothia* more closely resembles *Actinomyces* than *Nocardia* in morphology, it differs from the former in being aerobic and in the major end products of glucose metabolism (Slack and Gerencser 1975).

It is now recognized that the results of lipid analyses are of considerable value in the taxonomy of actinomycetes and coryneform bacteria (see Collins et al. 1977; Kroppenstedt and Kutzner 1978; Minnikin et al. 1978; Collins and Jones 1981). Therefore, in the present study the fatty acid, isoprenoid quinone and polar lipid composition of *Rothia dentocariosa* has been examined in an attempt to clarify its relationship with other actinomycete taxa.

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Materials and methods

Cultures and cultivation

The test strains (Table 1) were grown in BM broth (Shah et al. 1976) at 35° C for 3 days. Cultures were checked for purity, harvested by centrifugation, washed with distilled water and freeze-dried.

Extraction and analysis of fatty acid methyl esters

Dry cells (50 mg) were degraded by acid methanolysis and examined by thin-layer and gas liquid chromatography as described previously (Collins and Jones 1980).

Analysis of isoprenoid quinones

Isoprenoid quinones were extracted from dry cells (50 mg) as described by Collins et al. (1977). Purified quinones were analysed by reverse-phase partition high performance liquid chromatography (hplc) using a Laboratory Data Control chromatograph fitted with a Spherisorb ODS (5 μ) column and methanol (1.5 ml/min) as mobile phase. Quinones were monitored at 270 nm and quantitation was achieved using an LDC model 308 computer integrator. Mass spectra of the quinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 160 to 180° C.

Analysis of polar lipids

Free lipids were extracted from dry organisms (50 mg) as described previously (Minnikin et al. 1979). Polar lipids were examined by two dimensional thin-layer chromatography (tlc) using HPTLC Kieselgel $60F_{254}$ (Merck) plates (10 × 10 cm). Chromatograms were developed in the first dimension with chloroform/methanol/water (65:25:4 by vol.) and in the second dimension with chloroform/methanol/acetic acid/water (80:12:15:4 by vol.). Spraying with 10% molybdophosphoric acid in ethanol followed by heating at 140° C for 15 min revealed the presence of all lipids.

Specific spray reagents for lipid phosphate (Dittmer and Lester 1964), α-glycols (Shaw 1968), sugars (α-naphthol, Jacin and Mishkin 1965; anisaldehyde/H_2SO_4, Stahl and Kaltenbach 1961) and free amino groups (ninhydrin) were also used.

Table 1. Percentage fatty acid composition of the test strains

Assignment[a]	*iso*-$C_{12:0}$	$C_{13:0}$	*iso*-$C_{14:0}$	$C_{14:0}$	*iso*-$C_{15:0}$	*anteiso*-$C_{15:0}$	$C_{15:0}$	*iso*-$C_{16:0}$	$C_{16:0}$	*iso*-$C_{17:0}$	*anteiso*-$C_{17:0}$	$C_{18:1}$ ω_9	$C_{18:0}$
Rothia dentocariosa													
NCTC 10917[b]	0.4	2.8	1.2	0.9	2.3	20.4	0.5	19.1	20.8	1.1	29.7	–	0.8
NCTC 10918	0.2	8.6	0.8	0.9	2.4	21.7	–	18.0	18.1	1.2	26.9	0.2	1.0
NCTC 10207	0.5	7.6	1.0	0.8	2.5	20.9	–	17.8	16.8	1.8	28.1	1.0	1.2
R150 (H. N. Shah, dental plaque)	0.4	6.4	0.9	0.8	2.0	19.8	0.4	17.8	16.5	1.6	31.9	0.5	1.0
R22 (H. N. Shah, dental plaque)	0.8	6.6	1.0	0.7	2.5	21.8	0.4	18.0	16.9	1.6	28.2	0.2	1.3
6440 (H. N. Shah, oral cavity)	1.4	8.4	0.2	0.9	1.8	16.1	1.5	17.3	10.9	2.2	35.2	2.4	1.7

[a] Abbreviations for fatty acids are illustrated by the following examples: $C_{15:0}$, pentadecanoic acid; *iso*-$C_{15:0}$, 13-methyltetradecanoic acid; *anteiso*-$C_{15:0}$, 12-methyltetradecanoic acid; $C_{18:1}$, octadecenoic acid

[b] Type strain

Table 2. Percentage menaquinone composition of the test strains

Menaquinone isoprenologue m/e M^+	MK-*6* 580	MK-*7* 648	MK-*8* 716
Rothia dentocariosa			
NCTC 10917	4	95	1
NCTC 10918	6	94	–
NCTC 10207	6	92	2
R150	9	91	–
R22	7	90	3
6440	4	96	–

Results and discussion

Whole organism methanolysates of the test strains showed the presence on tlc of single spots corresponding to non-hydroxylated long-chain fatty acid methyl esters. Mycolic acids were not present. The non-hydroxylated fatty acids were composed of predominantly straight-chain saturated, *iso*- and *anteiso*-methyl branched chain fatty acids. Mono-unsaturated fatty acids were present in only small amounts. The major acids consisted of hexadecanoic, 12-methyltetradecanoic, 14-methylpentadecanoic and 14-methylhexadecanoic acids (Table 1). The detection of major amounts of *iso*- and *anteiso*-methyl branched acids in *R. dentocariosa* indicates this taxon does not belong to the family Actinomycetaceae. Representatives of the genus *Actinomyces* contain predominantly straight-chain saturated, monounsaturated (oleic acid series) and cyclopropane-ring fatty acids (Kroppenstedt and Kutzner 1978).

Components that co-chromatographed with vitamin K were the only isoprenoid quinones detected in the test strains. On examination by ultra-violet spectroscopy the quinones displayed absorption maxima at 242, 248, 260, 270 and 326 nm in accordance with published data for menaquinones (Collins and Jones 1981). The mass spectra of the menaquinone samples showed intense peaks at m/e 187 and 225 derived from the naphthoquinone nucleus (Collins and Jones 1981) with peaks at m/e 648 indicative of unsaturated menaquinones with seven isoprene units (MK-7) (Table 2). The results of mass spectrometry were confirmed by reverse-phase partition hplc. The presence of unsaturated menaquinones in *R. dentocariosa* is particularly distinctive. Actinomycetes generally contain mixtures of partially saturated menaquinones, although unsaturated menaquinones have been reported previously in *Agromyces* (Collins 1982), "*Gordona aurantiaca*" (Goodfellow et al. 1978) and *Intrasporangium calvum* (Collins et al. 1984). The presence of MK-7 in *R. dentocariosa* is incompatible with the classification of this taxon in the family Actinomycetaceae (Slack 1974). Representatives of the genus *Actinomyces* possess hydrogenated menaquinones with ten isoprene units (Collins et al. 1977; Collins and Jones 1981). It is worth noting however that MK-7 has been previously reported in certain 'coryneform' taxa (e.g. *Brevibacterium acetylicum*, *Exiguobacterium*) (Collins and Kroppenstedt 1983; Collins et al. 1983).

Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) were readily identified in the extracts of all the test strains by their chromatographic behaviour and staining properties. In addition, all of the strains possessed a characteristic lipid which gave negative reactions to the lipid phosphate and ninhydrin sprays but positive reactions to the periodate-Schiff and α-naphthol reagents. This unknown glycolipid possessed the chromatographic mobility of a diglycosyldiacylglycerol and gave a green colouration with an anisaldehyde/H_2SO_4 spray indicative of the presence of mannose residues. These findings are consistent with the earlier report of dimannosyldiacylglycerol in *R. dentocariosa* by Pandhi and Hammond (1975). The presence of such a simple polar lipid composition in *R. dentocariosa* was unexpected. Actinomycetes generally exhibit complex polar lipid patterns. Interestingly DPG, PG and dimannosyldiacylglycerol are found in a variety of coryneform taxa (e.g. *Microbacterium*, '*Arthrobacter nicotianae* group of organisms') (Collins and Kroppenstedt 1983; Collins et al. 1983).

The results of the present study indicate *R. dentocariosa* is unrelated to the genus *Actinomyces* despite a superficial morphological resemblance. *Rothia dentocariosa* differs from all species of *Actinomyces*, except *A. bovis*, in lacking ornithine in the cell wall peptidoglycan (Schleifer and Kandler 1972). The detection of major amounts of methyl-branched fatty acids and unsaturated menaquinones in *R. dentocariosa* reinforces this distinction and indicates that this taxon should be removed from the family Actinomycetaceae (Slack 1974). The lipid data however point to a possible relationship with the 'coryneform group of bacteria'. *Rothia dentocarisoa*

resembles the '*Arthrobacter nicotianae* group' of organisms in possessing a relatively high G + C content (ca. 65–69 mol%) (Hammond 1970), cell wall peptidoglycan based upon lysine (Schleifer and Kandler 1972), branched fatty acids, DPG, PG and dimannosyldiacylglycerol (Collins and Kroppenstedt 1983). *Arthrobacter nicotianae* and related organisms however differ from *R. dentocariosa* in possessing MK-*8*/MK-*9* as the major menaquinones (Collins and Kroppenstedt 1983). Similarly *Brevibacterium acetylicum* and *Exiguobacterium aurantiacum* resemble *R. dentocariosa* in possessing lysine in the cell wall peptidoglycan and MK-*7* (Collins and Kroppenstedt 1983; Collins et al. 1983). *Brevibacterium acetylicum* and *E. aurantiacum* however differ from *R. dentocariosa* in possessing relatively low DNA base compositions (ca. 52 to 56 mol% G + C), phosphatidylethanolamine and in lacking dimannosyldiacylglycerol (Collins and Kroppenstedt 1983; Collins et al. 1983). Further studies are necessary to clarify this possible relationship between *R. dentocariosa* and the coryneform group of bacteria.

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Received September 19, 1983/Accepted November 11, 1983