Arch Microbiol (1982) 133:323–329

Archives of
Microbiology

Lipids in the Classification of *Nocardioides*: Reclassification of *Arthrobacter simplex* (Jensen) Lochhead in the Genus *Nocardioides* (Prauser) emend. O'Donnell et al. as *Nocardioides simplex* comb. nov.

Anthony G. O'Donnell[1,2], Michael Goodfellow[2], and David E. Minnikin[1]

[1] Department of Organic Chemistry, The University, Newcastle upon Tyne, NE1 7RU, UK
[2] Department of Microbiology, The University, Newcastle upon Tyne, NE1 7RU, UK

Abstract. Representative strains of *Nocardioides*, *Arthrobacter simplex* and *Arthrobacter tumescens* were degraded by acid methanolysis and the fatty acid esters released examined by thin-layer and gas chromatography. Branched-chain 14-methylpentadecanoic acid (*iso*-16) was the predominant component in all but one of the *Nocardioides* strains. *Arthrobacter simplex* also contained major amounts of this acid whereas *A. tumescens* had only minor amounts. All of the test strains possessed 15 and 17 carbon straight chain acids, tuberculostearic acid (10-methyloctadecanoic acid) and its 17 and 18-carbon homologues. The fatty acid profiles of *Nocardioides* strains lacked 13-methyltetradecanoic and heptadecanoic acids which were both present in *Arthrobacter simplex* and *Arthrobacter tumescens*. The profiles of these latter organisms were quantitatively different from each other. The polar lipids of the test strains all contained diphosphatidylglycerol and phosphatidylglycerol but only *Arthrobacter tumescens* contained phosphatidylinositol and three unidentified polar lipids. *Nocardioides* and *Arthrobacter simplex* strains all contained two very characteristic closely related polar lipids.

All of the test strains contained tetrahydrogenated menaquinones with eight isoprene units as the major isoprenologue. The results of the present study support the integrity of the genus *Nocardioides* and provide a reliable way of distinguishing it from other actinomycetes, such as *Streptomyces*, which also have LL-diaminopimelic acid and glycine in the peptidoglycan. The lipid data, together with results from chemical, genetic and phage host range studies, provide sufficient grounds for the transfer of *Arthrobacter simplex* to *Nocardioides* as *Nocardioides simplex* comb. nov. An emended description of the genus *Nocarioides* is given.

Key words: Chemotaxonomy – Lipids – *Nocardioides* – *Arthrobacter simplex* – *Arthrobacter tumescens* – *Nocardioides simplex* comb. nov.

The genus *Nocardioides* was proposed by Prauser (1976a) for aerobic, Gram-positive, non-acid-fast actinomycetes that formed a mycelium which fragmented to form irregular rod- to coccus-like elements, contained LL-2,6-diaminopimelic acid (LL-DAP) and glycine in the peptidoglycan (wall chemotype I *sensu* Lechevalier and Lechevalier 1970), lacked mycolic acids but were susceptible to phages of a taxon specific set. Initially, the genus contained a single species, *Nocardioides albus*, which was tentatively assigned to the family Streptomycetaceae (Waksman and Henrici 1943) primarily on the basis of wall composition: a second species, *Nocardioides luteus* was subsequently added. *Nocardioides* strains have little DNA in common with the genus *Streptomyces* (Tille et al. 1978) and are not lysed by phages virulent to streptomycetes and related organisms with a wall chemotype I (Prauser and Falta 1968; Prauser 1981; Wellington and Williams 1981). In an extensive numerical phenetic survey of streptomycetes and related strains a representative strain of *N. albus* was recovered on the fringe of an aggregate cluster corresponding to the genus *Streptomyces* (Williams et al. 1981, 1983). Although little is known of the relationships of *Nocardioides* above genus level, *Arthrobacter simplex* and *Nocardioides* strains are both susceptible to two phages (Prauser 1976a, 1981), have LL-DAP and glycine in the peptidoglycan which is of the A3γ type (Schleifer and Kandler 1972; Prauser 1978), and have DNA rich in guanine (G) plus cytosine (C) (Yamada and Komagata 1970; Tille et al. 1978). In addition, reference DNA from *A. simplex* has been reported to have a DNA : DNA homology of 15 to 20% with DNA from *Nocardioides* strains (Prauser 1981).

Chemotaxonomic analyses of lipids have led to significant advances in the systematics of both nocardioform and "coryneform" actinomycetes (Minnikin et al. 1978; Minnikin and Goodfellow 1980), the most productive studies to data have involved the analysis of fatty acids, isoprenoid quinones and polar lipids. Preliminary data also suggest that lipid markers will be of value in establishing relationships between sporoactinomycetes (Minnikin and Goodfellow 1981). In the present study the fatty acid, isoprenoid quinone and polar lipid composition of representative strains of *N. albus* and *N. luteus* were compared with each other and with marker strains of *A. simplex* and *A. tumescens* in an attempt to clarify their taxonomy.

Materials and Methods

Organisms. The test strains (Table 1) were grown in shake flasks for 2 to 7 days at 30° C in modified Sauton's medium (Mordarska et al. 1972). Cultures were checked for purity at maximum growth, killed by shaking with formalin (1%, v/v), separated by centrifugation, washed with distilled water and freeze dried.

Extraction and Analysis of Fatty Acid Methyl Esters. Biomass (50 mg) was examined using the whole-organism acid methanolysis procedure of Minnikin et al. (1980). Analytical thin-layer chromatography (TLC) of methanolysates was perfor-

Offprint requests to: A. G. O'Donnell

Table 1. Percentage fatty acid composition of the test strains

ECL on OV-1[b]		13.7	14.0	14.7	14.7	15.0	15.7	15.8	16.0	16.4	16.7	16.7	16.8	17.0	17.4	17.8	18.0	18.4
ECL on Silar 10C[b]		13.4	14.0	14.4	14.7	15.0	15.4	17.0	16.0	16.0	16.4	16.7	18.0	17.0	17.0	19.0	18.0	18.0
Assignment[c]		i-14	14:0	i-15	ai-15	15:0	i-16	16:1	16:0	t-17	i-17	ai-17	17:1	17:0	t-18	18:1	18:0	t-19
Nocardioides luteus	Source[d]																	
N 947[e]	IMET 7828	0.7	1.0	–	0.3	2.9	22.3	–	11.8	1.7	0.7	2.1	–	13.6	5.0	4.6	15.2	12.7
N 948	IMET 7831	2.7	0.4	–	0.7	1.2	45.0	–	12.9	4.2	0.9	3.4	–	8.8	3.7	0.8	5.7	5.4
N 949	IMET 7830	2.4	0.2	–	0.9	1.0	36.5	–	6.2	7.3	0.8	4.3	–	4.7	10.3	1.7	3.3	13.2
Nocardioides albus																		
N 951	IMET 7802	3.8	0.6	–	3.5	5.4	34.2	–	11.4	1.7	1.3	5.5	–	13.1	4.6	0.3	6.4	2.7
N 952[a]	IMET 7807	0.5	0.6	–	–	2.5	26.6	–	10.5	1.5	0.6	1.3	–	10.8	4.6	5.5	7.5	18.3
N 953	IMET 7812	1.5	0.4	–	2.4	1.1	30.3	–	9.0	2.7	2.9	6.2	–	7.9	4.5	1.4	10.0	9.3
N 954	IMET 7814	1.5	0.5	–	0.4	1.8	36.2	0.1	10.0	2.2	0.6	1.4	–	8.6	3.9	6.4	8.0	16.9
N 955	IMET 7819	0.4	0.7	–	0.4	9.3	11.7	0.1	10.8	0.3	1.4	1.9	–	33.8	3.0	2.3	8.6	2.0
N 956	IMET 7820	1.1	0.9	–	1.2	5.7	23.8	0.2	10.8	1.9	1.9	3.3	–	15.5	4.7	4.0	6.4	6.0
N 957	IMET 7824	1.0	1.2	–	0.2	4.6	25.3	–	16.9	0.8	1.2	1.2	–	15.9	3.0	2.5	11.2	8.3
Arthrobacter simplex																		
N 295[a]	NCIB 8929	tc[f]	tc	2.1	tc	0.7	15.1	–	3.7	1.8	10.2	2.1	5.8	3.0	5.2	17.0	3.9	29.3
Arthrobacter tumescens																		
C 273[a]	NCIB 8914	1.1	3.8	11.4	6.2	5.5	2.4	5.6	11.4	2.9	1.5	3.1	9.0	8.4	1.2	19.6	6.0	0.5

[a] Type strains
[b] *Iso* and *anteiso* fatty acid esters were not separated on the OV-1 columns and therefore had the same equivalent chain length (ECL). On Silar 10C, 16:0 and t-17; 16:1, t-18 and 17:0; 17:1, t-19 and 18:0 co-chromatographed and therefore had the same ECL
[c] Abbreviations for fatty acid methyl esters are explained by the following examples: 15:0, straight chain pentadecanoic acid; *i*-15, 13-methyltetradecanoic acid; *ai*-15, 12-methyltetradecanoic acid; *t*-19, tuberculostearic acid (10-methyloctadecanoic acid); t-18 and t-17, lower homologues of tuberculostearic acid; 16:1, hexadecenoic acid
[d] IMET; Dr. H. Prauser, Zentralinstitut für Mikrobiologie und Therapie, Jena, D.D.R. NCIB; National Collection of Industrial Bacteria, Aberdeen, Scotland, U.K.
[e] Laboratory number
[f] *tc* Trace amounts

med using 10 × 10 cm pieces of aluminium-backed silica gel sheets (Merck 5554) with a developing mixture of petroleum ether (b.p. 60–80° C) – acetone (95:5, v/v) and location of separated components by spraying with 5% ethanolic molybdophosphoric acid and charring at 120° C. Fatty acid methyl esters were purified on 10 × 10 cm pieces of plastic-backed silica gel sheets (Merck 5735) using the same developing mixture. After spraying with 0.01% ethanolic Rhodamine 6G, separated bands were located under ultra violet light (366 nm), cut out and eluted with diethyl ether (2 × 1 ml) using a tube rotator. Any traces of Rhodamine 6G were removed by passing the ethereal extract through a 0.5 cm of neutral alumina (Merck 1097) in a Pasteur pipette. Diethyl ether was removed under a stream of nitrogen.

Gas chromatography of petroleum ether solutions of fatty acid methyl esters was performed using both a stainless steel column (6 m × 2 mm i.d.) packed with polar 10% Silar 10C on 100–120 mesh Gas Chrom Q (Applied Science Laboratories) and a glass column (6 m × 2 mm i.d.) containing non-polar 3% OV-1 on 80–100 mesh Gas Chrom Q (Chrompack Ltd.) in Perkin-Elmer F11 and Pye 104 flame ionization instruments, respectively, with nitrogen as carrier gas. The retention times and relative proportions of the fatty acid methyl esters, expressed as a percentage of the total area, were measured using a Shimadzu CE1B computing integrator. Components were identified by comparison of retention times with those of standard mixtures.

Extraction of Isoprenoid Quinones and Polar Lipids. Isoprenoid quinones and polar lipids were extracted using a recently developed procedure (D. E. Minnikin, A. G. O'Donnell, A. Schaal, G. Alderson, M. Athalye and M. Goodfellow, unpublished results; Embley et al. 1983). Equal volumes (2 ml) of aqueous methanolic NaCl (10 ml 0.3% aqueous NaCl added to 100 ml methanol) and petroleum ether were added to 50 mg biomass in a polytetrafluoroethylene (PTFE) lined capped tube. After mixing on a tube rotator for 15 min and low speed centrifugation the upper layer was removed and the extraction repeated with 1 ml petroleum ether. The combined extracts were evaporated under nitrogen at 37° C to provide crude isoprenoid quinones. The lower layers remaining after isoprenoid quinone extraction were processed using a modification (Card 1973; Minnikin et al. 1979) of the method of Bligh and Dyer (1959) to give a polar lipid extract.

Analysis of Isoprenoid Quinones. Analytical and preparative TLC of isoprenoid quinones were carried out using systems described above for fatty acid methyl esters. Ultra violet light (254 nm) was used for detection in both cases. Purified

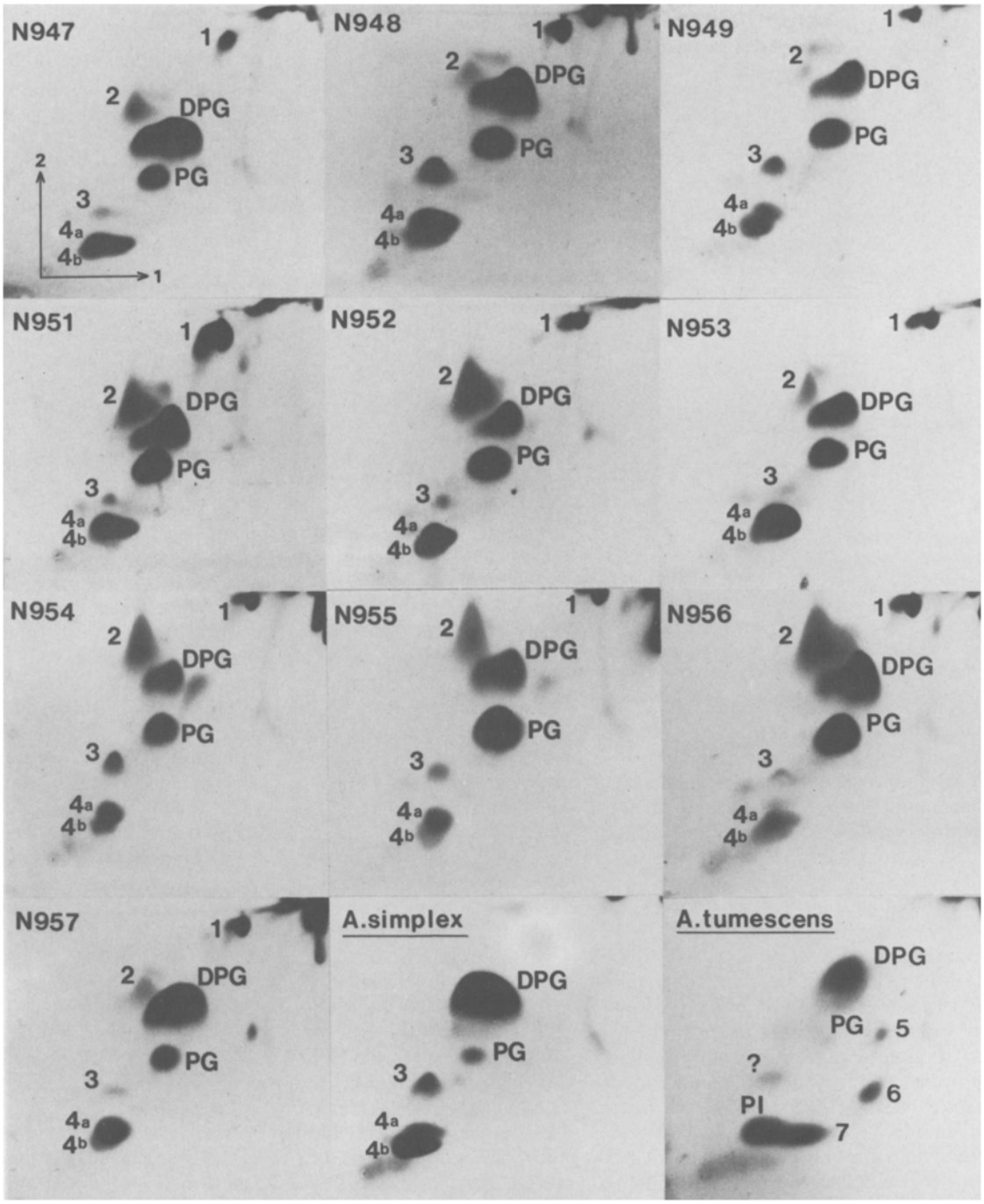

Fig. 1. Two-dimensional thin-layer chromatograms of polar lipids from strains of *Nocardioides* and *Arthrobacter*. Chloroform-methanol-water (65:25:4) by volume was used in the first direction and chloroform-acetic acid-methanol-water (80:18:12:5) by volume in the second direction (Minnikin et al. 1977b). Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol. For the staining reaction of spots 1, 2, 3, 4a, 4b, 5, 6, and 7 see Results

menaquinones were analysed by reverse-phase TLC (Collins et al. 1980) and by mass spectrometry using an A.E.I. MS9 instrument (70 eV, 200 – 220° C).

Analysis of Polar Lipids. Patterns of polar lipids were obtained using 6.6 × 6.6 cm pieces of aluminium-backed silica gel sheets (Merck 5554) and developing solvents as used previously (Fig. 1). All lipids were detected by spraying with 5% ethanolic molybdophosphoric acid followed by charring at 180° C. Specific spray reagents for lipid phosphate (Dittmer and Lester 1964), α-glycols (periodate-Schiff) (Shaw 1968), sugars (α-naphthol) (Jacin and Mishkin 1965) and amino groups [0.2% ninhydrin in water-saturated butanol (Consden and Gordon 1948)] were also used.

Results

Thin-layer chromatographic analysis of whole organism methanolysates of the test strains showed the presence of predominant spots (R_f 0.8) corresponding to non-

Table 2. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from the test strains

Menaquinone isoprenologue		MK8	
Degree of hydrogenation	–	(H_2)	(H_4)
m/e	716	718	720
Nocardioides luteus			
N 947	–	+	+++
N 948	+	+	+++
N 949	–	+	+++
Nocardioides albus			
N 951	+	++	+++
N 952	–	+	+++
N 953	+	+	+++
N 954	–	+	+++
N 955	–	+	+++
N 956	–	+	+++
N 957	–	+	+++
Arthrobacter simplex			
N 295	+	–	+++
Arthrobacter tumescens			
C 273	+	–	+++

Main component denoted by +++, components 50% of main peak by ++ and all other significant components by +

hydroxylated fatty acid methyl esters (Minnikin et al. 1975; 1980; Goodfellow et al. 1976). The extract from *Arthrobacter simplex* also contained, on TLC, a component (R_f 0.2) corresponding to a hydroxy fatty acid methyl ester and lesser amounts of a similar component were observed in the methanolysates of the *Nocardioides* strains but not in that from *A. tumescens*. These hydroxylated components were not studied further.

Results of the gas chromatographic analysis of the non-hydroxylated long chain components are shown in Table 1. All of the strains produced similar fatty acid profiles consisting of *iso*, *anteiso*, straight chain, unsaturated and tuberculostearic (10-methyloctadecanoic) acids. With only one exception the *Nocardioides* strains contained 14-methylpentadecanoic acid (*iso*-16) as the major component. The exception, *N. albus* N955, contained major amounts of heptadecanoic acid. *Arthrobacter simplex* NCIB 8929 also contained major amounts of 14-methylpentadecanoic acid (*iso*-16) whereas *A. tumescens* NCIB 8914 had only small amounts of this acid and large amounts of 13-methylpentadecanoic acid (*iso*-15).

Compounds co-chromatographing with vitamin K were the only isoprenoid quinones detected in the strains of *N. albus* and *N. luteus*. The most intense peaks in the mass spectra of the menaquinones occurred at *m/e* 187 and 225 and were derived from the naphthoquinone nucleus (Azerad and Cyrot-Pelletier 1973). The mass spectra in the high mass region contained strong peaks corresponding to molecular ions (M^+) with smaller peaks at M^+-15 corresponding to the loss of a methyl group from the molecular ion. A summary of the mass spectral data is given in Table 2. All of the test strains contained tetrahydrogenated menaquinones with eight isoprene units, abbreviated as MK-8(H_4), as the major isoprenologue. In addition, several strains were shown to have lesser amounts of MK-8 and MK-8(H_2) (Table 2). Analyses by reverse-phase TLC correlated with the mass spectrometric results.

The results of the two dimensional TLC analyses of polar lipids are shown in Fig. 1. Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) were readily identified in all of the extracts by their chromatographic behaviour and staining properties, the remaining components were not completely characterised. On the basis of their staining properties components 1, 2 and 3 were phospholipids. Components 4a and 4b are unusual lipids and were found in both *Nocardioides* strains and in *Arthrobacter simplex*. These unidentified lipids were phospholipid and α-glycol (periodate-Schiff) positive. *Arthrobacter tumescens* did not have lipids 4a and 4b but contained phosphatidylinositol (PI), a phospholipid (5), which was also ninhydrin positive and two unusual lipids, 6 and 7, which were positive for all the specific spray reagents used (Fig. 1).

Discussion

A similar range of non-hydroxylated fatty acids were found in all the test organisms (Table 1) but qualitative and quantitative differences of possible diagnostic significance were apparent. Unifying factors in the fatty acid profiles are the presence of tuberculostearic acid, with comparable proportions of its homologues having 17 and 18 carbon atoms, and significant amounts of odd-numbered straight-chain acids with 15 and 17 carbons. The profiles of the three strains of *Nocardioides luteus* resemble those of all except one of the *N. albus* strains, 14-methylpentadecanoic (*iso*-16) acid being the major component. *Nocardioides albus* N955 is anomalous in having exceptionally high proportions of pentadecanoic acid at the expense of *iso*-16. The fatty acid profiles of the marker *Arthrobacter simplex* and *A. tumescens* strains were quantitatively different from each other and from the *Nocardioides* strains (Table 1). Qualitatively *A. simplex* and *A. tumescens* also contained *iso*-15 and heptadecenoate (17:1), components absent in *Nocardioides* species. *Arthrobacter tumescens* was the only organism with substantial amounts of hexadecenoate (16:1).

The presence of tuberculostearic acid and its lower homologues in *A. simplex* ("*Corynebacterium*" *simplex*) was first reported by Fujii and Fukui (1969, 1970) and substantiated by Yano et al. (1970, 1971a). In a wider but less detailed study (Bowie et al. 1972), these types of methyl-branched fatty acids were not recognized in representatives of *A. simplex* and *A. tumescens*. Some tentative evidence (Lechevalier et al. 1977) has been presented for the presence of tuberculostearic acid and its lower homologues in *N. albus* IMET 7801 and IMET 7807, the latter being the type strain included in the present study (Table 1). Tuberculostearic acid is found in the majority of the mycolic acid-containing mycobacteria, nocardiae and rhodococci but absent in *Corynebacterium sensu stricto* (Minnikin and Goodfellow 1980; Collins et al. 1982c) with the exception of *Corynebacterium bovis* (Lechevalier et al. 1977; Collins et al. 1982c). Tuberculostearic acid or its lower homologues have also been identified in a variety of actinomycetes lacking mycolic acids (Kroppenstedt and Kutzner 1978; Lechevalier et al. 1977; Minnikin and Goodfellow 1981). Significantly, however, 56 strains of *Streptomyces*, having LL-diaminopimelic acid in their walls, did not contain 10-methylbranched fatty acids (Kroppenstedt and Kutzner 1978).

The presence, in the test strains of fatty acids such as penta- and heptadecanoic and the 18-carbon homologue of

tuberculostearate (t-18) (Table 1) having an odd number of carbons in the main chain requires biosynthetic processes to alter the alkyl chains by a single carbon. Detailed studies by Yano et al. (1971a, b), using *A. simplex* demonstrated that straight-chain acids could be converted via 2-hydroxy acids to acids having one carbon less. A comparable system for reducing the length of both straight-chain und tuberculostearic acids must, therefore, operate in both species of *Nocardioides* and in the marker *A. simplex* and *A. tumescens* strains.

The menaquinone data can also be used to separate *Nocardioides* from *Streptomyces* and other wall chemotype I taxa, strains of which contain major amounts of MK-*9*(H_6) and MK-*9*(H_8) (Alderson et al. 1981; Minnikin and Goodfellow 1981). The presence of MK-*8*(H_4) as the major isoprenologue in *A. simplex* and *A. tumescens* confirmed the results of previous workers (Yamada et al. 1976; Collins et al. 1979) though MK-*8* and MK-*8*(H_2) were the only minor components detected unlike Collins et al. (1979) who reported finding minor amounts of MK-*7*(H_4) in *A. simplex* and *A. tumescens*.

All of the *Nocardioides* strains contained DPG, PG and a number of incompletely characterised phospholipids. This characteristic lipid pattern readily distinguishes *Nocardioides* from *Nocardia* and other mycolic acid containing actinomycetes which contain DPG and characteristic phosphatidylinositol mannosides (PIM's) (Lechevalier et al. 1977; Minnikin et al. 1977a) as well as from *Streptomyces* and related wall chemotype I strains that possess DPG, PE, PI and PIM's (Minnikin and Goodfellow 1976, 1981; Lechevalier et al. 1977). *Arthrobacter simplex* NCIB 8929 contained DPG and PG and also had several uncharacterised lipids in common with the *Nocardioides* strains. Collins et al. (1982b) reported the presence of DPG, PG and two unidentified lipids in two strains of *A. simplex* and considered that the latter might correspond to the hydroxy-acid containing PG found by Yano et al. (1971a, b); the two unidentified lipids correspond to components 4a and 4b (Fig. 1) in the present study. In contrast, the *A. tumescens* strain did not have lipids 4a and 4b but did contain PI and three additional uncharacterised lipids. The presence of PI in *A. tumescens* is in conflict with the report by Komura et al. (1975) who did not find PI but did detect a PIM. The tentative identification by Yanagawa et al. (1972) of substantial proportions of PIM and phosphatidylethanolamine in *A. simplex* ("*Corynebacterium*" *simplex*) also requires confirmation.

The results of the present study help to underline the value of fatty acid, isoprenoid quinone and polar lipid analyses in the classification and identification of actinomycetes. The lipid data highlight the integrity of the genus *Nocardioides* and lend further support for its separation from *Streptomyces* and allied taxa with a wall chemotype I and also from *Nocardia* and related actinomycetes which contain *meso*-diaminopimelic acid, arabinose, galactose and mycolic acids in the cell envelope and form a mycelium that fragments into bacillary and coccoid elements (Prauser 1976a, b, 1981; Tille et al. 1978). Indeed, Prauser and his colleagues placed and retained *Nocardioides* in the family Streptomycetaceae merely to avoid the establishment of a new family for this taxon.

The lipid results provide yet further evidence of an affinity between the genus *Nocardioides* and *A. simplex* (Prauser 1976a, b, 1981). *Arthrobacter simplex* shares a low phenetic similarity with *Arthrobacter sensu stricto* (Bousfield 1972; Jones 1975), has been clearly separated from the latter in wall (Schleifer and Kandler 1972), lipid (Minnikin et al. 1978), DNA base (Keddie and Bousfield 1980) and DNA : DNA pairing studies (Suzuki et al. 1981) and clearly needs to be reclassified. The organism has also been sharply separated from all other actinomycetes, including representatives of most wall chemotype I taxa, in DNA: ribosomal (r)RNA pairing (Fiedler et al. 1981) and 16S rRNA sequencing studies (Stackebrandt and Woese 1981a).

Nocardioides and *A. simplex* are related to one another but have little in common with other actinomycete taxa. They both have a wall chemotype I, a peptidoglycan of the A3γ type (Schleifer and Kandler 1972; Prauser 1978), DNA rich in G plus C (Yamada and Komagata 1970; Prauser 1976a; Tille et al. 1978; Suzuki et al. 1981), about 20% DNA in common (Prauser 1981), show cross-susceptibility to phages (Prauser 1976a; 1981), have tetrahydrogenated menaquinones with eight isoprene units as the major isoprenologue and similar fatty acid and polar lipid profiles. Further, the morphological cycle in *N. albus* can be regarded as a development on the bending type of cell division shown by *A. simplex* (Prauser 1981). On the basis of these data we propose that *Arthrobacter simplex* be transferred to the genus *Nocardioides* as *Nocardioides simplex* Jensen comb. nov.

The inclusion of pleomorphic strains in the genus *Nocardioides* makes the latter heterogeneous with respect to morphology. Other actinomycete taxa such as *Mycobacterium* (Goodfellow and Wayne 1982) and *Rhodococcus* (Goodfellow and Alderson 1977) also accommodate morphologically diverse strains and it has been proposed that the non-mycelial *Corynebacterium pyogenes* be transferred to the genus *Actinomyces* as *Actinomyces pyogenes* comb. nov. (Collins and Jones 1982; Reddy et al. 1982). There is particularly good evidence from 16S rRNA sequencing studies that morphological properties are not always reliable indicators of natural relationships and that the traditional practice of classifying relatively highly differentiated actinomycetes from the morphologically "simple" coryneform bacteria does not always hold good (Stackebrandt et al. 1980; Stackebrandt and Woese 1981b). There are also sufficient grounds for classifying the mycelial-forming genus *Thermoactinomyces* in the family *Bacillaceae* together with other aerobic endospore-forming bacteria (Stackebrandt and Woese 1981b; Collins et al. 1982a). Indeed, it is now evident that the possession of branched hyphae does not automatically place a bacterium with the actinomycetes, conversely the inability of a strain to form branching filaments should not inevitably exclude it from an actinomycete taxon.

Emended descriptions of both the genus *Nocardioides* and the proposed species *N. simplex* are given below.

Emended description of *Nocardioides* Prauser: No. car. di. oi. des M.L. fem. n. *Nocardia* name of a genus in the order Actinomycetales; Gr. n. *idus* form; M.L. mas. n. *Nocardioides Nocardia*-like; intended to refer to the morphological similarity between members of the two genera.

The salient characteristics of the genus based on the original description of Prauser (1970) and our own observations are as follows.

Aerobic, Gram-positive, non-acid-fast actinomycetes which may be pleomorphic or produce a branched primary and secondary mycelium. The hyphae of both the substrate and aerial mycelia fragment into rod- to coccus-like elements which give rise to new mycelia. The primary mycelium on oatmeal agar, yeast extract-malt extract agar and similar rich

media is whitish to faint yellowish. Colonies are pasty with smooth to wrinkled and dull to bright surfaces. The aerial mycelium is thick, dense and chalky. Motile and non-motile strains occur.

The wall peptidoglycan contains alanine, LL-diaminopimelic acid, glutamic acid and glycine and is of the A3γ type (Schleifer and Kandler 1972). Mycolic acids are not present. The long chain fatty acids are primarily of the *iso*, *anteiso*, straight chain, unsaturated and tuberculostearic (10-methyloctadecanoic) acid types. The major respiratory quinones are tetrahydrogenated menaquinones with eight isoprene units. Polar lipid profiles include diphosphatidylglycerol, phosphatidylglycerol and two unusual incompletely characterised lipids which are phospholipid and α-glycol (periodate-Schiff) positive.

Strains are susceptible to phages of a taxon specific set.

The DNA base composition is within the range 66.5 to 72.4 mol % G plus C (Prauser 1976; Keddie and Jones 1981a).

Habitat soil.

The type species is *Nocardioides albus.*

Description of *Nocardioides simplex* comb. nov. simplex. L. adj. *simplex* simple.

The salient characters of this species based upon the description of Keddie (1974) and our own observations are given below. The species description does not include properties characteristic of the genus.

In complex media cells show a change in form during the growth cycle. Older cultures are composed of coccoid cells and very short rods (ca. 0.4–0.5 by 0.5–8.0 µm); large coccoid forms are uncommon. Irregular rods occurring in late lag and exponential phase cultures are ca. 0.4–0.5 by 1.0–3.0 or more µm. When motile, one to four flagella occur in polar or lateral positions.

Nutritionally non-exacting; growth occurs in a suitable mineral salts medium with an ammonium salt or nitrate as sole nitrogen source and glucose as carbon plus energy source. Growth on nutrient agar shows no distinct pigmentation. The type strain is able to use many compounds as sole or principal sources of carbon and energy. These include a very narrow range of carbohydrates and sugar derivatives, a wide range of fatty acids, simple alcohols and amino acids, together with some hydroxy-acids, oxo-acids, amines, pyrimidines and phenol.

Growth occurs at 10 and 37° C; optimum 26–37° C.

The G plus C content of the DNA is 71.7 mole % (Yamada and Komagata 1970; type strain).

The only known source is soil.

Type strain: ATCC 6946.

It is clear that *A. tumescens* should not be classified in *Arthrobacter sensu stricto* (see Keddie and Jones 1981) but it does have chemical, morphological and physiological properties in common with *A. simplex* (Schleifer and Kandler 1972; Minnikin et al. 1978; Collins et al. 1979). The two organisms have, however, been quite sharply distinguished in some numerical phenetic surveys (Davis and Newton 1969; Bousfield 1972; Jones 1975). Detailed analysis of the peptidoglycan composition (Fiedler et al. 1970; Schleifer and Kandler 1972) has shown that *A. simplex* and *A. tumescens* can be separated on the basis of the number of glycine residues forming the interpeptide bridge, *A. simplex* having only one like *Streptomyces* and related organisms with *A. tumescens* having three. Further comparative studies are required to determine the detailed relationship of *A. tumescens* to other actinomycetes which contain LL-DAP and glycine in the peptidoglycan.

Acknowledgements. The authors are indebted to Dr. H. Prauser both for encouragement and the *Nocardioides* strains, to Mr. J. H. Parlett for technical assistance and to the Medical Research Council (Grant G979/134/S) for support.

References

Alderson G, Goodfellow M, Wellington EMH, Williams ST, Minnikin SM, Minnikin DE (1981) Chemical and numerical taxonomy of *Nocardia mediterranea*. Zbl Bakt Suppl 11:39–46

Azerad R, Cyrot-Pelletier MO (1973) Structure and configuration of the polyprenoid side chain of dihydromenaquinones from myco- and corynebacteria. Biochimie 55:591–603

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bousfield IJ (1972) A taxonomic study of some coryneform bacteria. J Gen Microbiol 71:441–455

Bowie IS, Grigor MR, Dunckley GC, Loutit MW, Loutit JS (1972) The DNA base composition and fatty acid constitution of some Gram-positive pleomorphic soil bacteria. Soil Biol Biochem 4:397–412

Card GL (1973) Metabolism of phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin of *Bacillus stearothermophilus*. J Bacteriol 114:1125–1137

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

Collins MD, Shah HN, Minnikin DE (1980) A note on the separation of natural mixtures of bacterial menaquinones using reverse phase thin-layer chromatography. J Appl Bacteriol 48: 277–282

Collins MD, Jones D (1982) Reclassification of *Corynebacterium pyogenes* (Glage) in the genus *Actinomyces*, as *Actinomyces pyogenes* comb. nov. J Gen Microbiol 128:901–903

Collins MD, Mackillop GC, Cross T (1982a) Menaquinone composition of members of the genus *Thermoactinomyces*. FEMS Microbiol Letts 13:151–153

Collins MD, Goodfellow M, Minnikin DE (1982b) Polar lipid composition in the classification of *Arthrobacter* and *Microbacterium*. FEMS Microbiol Lett 15:299–302

Collins MD, Goodfellow M, Minnikin DE (1982c) Fatty acid composition of some mycolic acid containing coryneform bacteria. J. Gen Microbiol 128:2503–2509

Consden R, Gordon AH (1948) Effect of salt on partition chromatograms. Nature 162: 180–181

Davis GHG, Newton KG (1969) Numerical taxonomy of some named coryneform bacteria. J Gen Microbiol 56:195–214

Dittmer JCF, Lester RL (1964) A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. J Lipid Res 5:126–127

Embley TM, Goodfellow M, Minnikin DE, Austin B (1983) Fatty acid, isoprenoid quinone and polar lipid composition in the classification of *Renibacterium salmoninarum*. J Appl Bacteriol (in press)

Fiedler F, Schleifer KH, Cziharz B, Interschick E, Kandler O (1970) Murein types in *Arthrobacter, Brevibacteria, Corynebacteria* and *Microbacteria*. Publ Fac Sci Univ J E Purkyne Brno 47:111–122

Fiedler F, Schäffler MJ, Stackebrandt E (1981) Biochemical and nucleic acid hybridization studies on *Brevibacterium linens* and related strains. Arch Microbiol 129:85–93

Fujii K, Fukui S (1969) Relationship between vitamin B_{12} content and ratio of monounsaturated fatty acids to methyl-branched fatty acids in *Corynebacterium simplex* cells grown on hydrocarbons. FEBS Letts 5:343–346

Fujii K, Fukui S (1970) Hydrocarbon-utilizing microorganism – Relationship of fatty acid composition and biosynthesis to hydrocarbon substrate and to vitamin B_{12} level in *Corynebacterium simplex*. Eur J Biochem 17:552–560

Goodfellow M, Collins MD, Minnikin DE (1976) Thin-layer chromatographic analysis of mycolic acid and other long-chain components

in whole-organism methanolysates of coryneform and related taxa. J Gen Microbiol 96:351–358

Goodfellow M, Alderson G (1977) The actinomycete-genus *Rhodococcus*: a home for the '*rhodochrous*' complex. J Gen Microbiol 100:99–112

Goodfellow M, Wayne LG (1982) Taxonomy and nomenclature. In: Ratledge C, Stanford JL (eds) Biology of the mycobacteria, vol 1. Physiology, identification and classification. Academic Press, London, pp 472–521

Jacin H, Mishkin AR (1965) Separation of carbohydrates on borate-impregnated silica gel G plates. J Chromatogr 18:170–173

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

Keddie RM, Bousfield IJ (1980) Cell wall composition in the classification and identification of coryneform bacteria. In: Goodfellow M, Board RG (eds) Microbiological classification and identification. Academic Press, London, pp 167–188

Keddie RM, Jones D (1981) Saprophytic, aerobic coryneform bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes: A handbook of habitats, isolation and identification of bacteria. Springer, Berlin, pp 1838–1878

Komura I, Yamada K, Otsuka SI, Komagata K (1975) Taxonomic significance of phospholipids in coryneform and nocardioform bacteria. J Gen Appl Microbiol 21:251–261

Kroppenstedt RM, Kutzner HJ (1978) Biochemical taxonomy of some problem actinomycetes. Zbl Bakt Suppl 6:125–133

Lechevalier MP, Lechevalier H (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435–443

Lechevalier MP, De Bièvre C, Lechevalier H (1977) Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochem System Ecol 5:249–260

Minnikin DE, Alshamaony L, Goodfellow M (1975) Differentitation of *Mycobacterium, Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. J Gen Microbiol 88:200–204

Minnikin DE, Goodfellow M (1976) Lipid composition in the classification and identification of nocardiae and related taxa. In: Goodfellow M, Brownell GH, Serrano JA (eds) The biology of the nocardiae. Academic Press, London, pp 160–219

Minnikin DE, Patel PV, Alshamaony L, Goodfellow M (1977a) Polar lipid composition in the classification of *Nocardia* and related bacteria. Int J Syst Bacteriol 27:104–117

Minnikin DE, Pirouz T, Goodfellow M (1977b) Polar lipid composition in the classification of some *Actinomadura* species. Int J Syst Bacteriol 27:118–121

Minnikin DE, Goodfellow M, Collins MD (1978) Lipid composition in the classification and identification of coryneform and related taxa. In: Bousfield IJ, Callely AG (eds) Coryneform bacteria. Academic Press, London, pp 85–160

Minnikin DE, Collins MD, Goodfellow M (1979) Fatty acid and polar lipid composition in the classification of *Cellulomonas, Oerskovia* and related taxa. J Appl Bacteriol 47:87–95

Minnikin DE, Goodfellow M (1980) Lipid composition in the classification and identification of acid-fast bacteria. In: Goodfellow M, Board RG (eds) Microbiological classification and identification. Academic Press, London, pp 189–256

Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M (1980) Thin-layer chromatography of methanolysates of mycolic acid containing bacteria. J Chromatogr 188:221–233

Minnikin DE, Goodfellow M (1981) Lipids in the classification of actinomycetes. Zbl Bakt Suppl 11:99–109

Mordarska H, Mordarski M, Goodfellow M (1972) Chemotaxonomic characters and classification of some nocardioform bacteria. J Gen Microbiol 71:77–86

Prauser H (1976a) *Nocardioides*, a new genus of the order Actinomycetales. Int J Syst Bacteriol 26:58–65

Prauser H (1976b) New nocardioform organisms and their relationship. In: Arai T (ed) Actinomycetes; the boundary microorganisms. University Park Press, Baltimore, pp 193–207

Prauser H (1978) Considerations on taxonomic relations amoung Gram-positive, branching bacteria. Zbl Bakt Suppl 6:3–12

Prauser H (1981) Nocardioform organisms: general characterization and taxonomic relationships. Zbl Bakt Suppl 11:17–24

Prauser H, Falta R (1968) Phagensensibilität, Zellwandzusammensetzung und Taxonomie von Actinomyceten. Z Allg Mikrobiol 8:39–46

Reddy CA, Cornell CP, Frage AM (1982) Transfer of *Corynebacterium pyogenes* (Glage) Eberson to the genus *Actinomyces* as *Actinomyces pyogenes* (Glage) comb. nov. Int J Syst Bacteriol 32:419–429

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

Shaw N (1968) The detection of lipids on thin-layer chromatograms with the periodate Schiff reagent. Biochim Biophys Acta 164:435–436

Stackebrandt E, Lewis BJ, Woese CR (1980) The phylogenetic structure of the coryneform group of bacteria. Zbl Bakt I Abt Orig C1:137–149

Stackebrandt E, Woese CR (1981a) Towards a phylogeny of the actinomycetes and related organisms. Curr Microbiol 5:197–202

Stackebrandt E, Woese CR (1981b) The evolution of prokaryotes. In: Carlile MJ, Collins JF, Moseley BEB (eds) Molecular and cellular aspects of microbial evolution. University Press, Cambridge, pp 1–31

Suzuki K, Kaneko T, Komagata K (1981) Deoxyribonucleic acid homologies among coryneform bacteria. Int J Syst Bacteriol 31:131–136

Tille D, Prauser H, Szyba K, Mordarski M (1978) On the taxonomic position of *Nocardioides albus* Prauser by DNA: DNA-hybridization. Z Allg Mikrobiol 18:459–462

Waksman SA, Henrici AT (1943) The nomenclature and classification of the actinomycetes. J Bacteriol 46:337–341

Wellington EMH, Williams ST (1981) Host ranges of phages isolated to *Streptomyces* and other genera. Zbl Bakt Suppl 11:93–98

Williams ST, Wellington EMH, Goodfellow M, Alderson G, Sackin M, Sneath PHA (1981) The genus *Streptomyces* – a taxonomic enigma. Zbl Bakt Suppl 11:47–57

Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin M (1983) Numerical classification of *Streptomyces* and related genera. J Gen Microbiol (in press)

Yamada K, Komagata K (1970) Taxonomic studies on coryneform bacteria. III. DNA base composition of coryneform bacteria. J Gen Appl Microbiol 16:215–224

Yamada Y, Inouye G, Tahara Y, Kendo K (1976) The menaquinone system in the classification of coryneform and nocardioform bacteria and related organisms. J Gen Appl Microbiol 22:203–214

Yanagawa S, Fujii K, Tanaka A, Fukui S (1972) Lipid composition and localization of 10-methyl branched-chain fatty acids in *Corynebacterium simplex* grown on *n*-alkanes. Agr Biol Chem 36:2123–2128

Yano I, Furukawa Y, Kusunose M (1970) 2-Hydroxy fatty acid-containing phospholipid of *Arthrobacter simplex*. Biochim Biophys Acta 210:105–115

Yano I, Furukawa Y, Kusunose M (1971a) Fatty acid composition of *Arthrobacter simplex* grown on hydrocarbons – Occurrence of α-hydroxy fatty acid. Eur J Biochem 23:220–228

Yano I, Furukawa Y, Kusunose M (1971b) Conversion of α-hydroxypalmitic acid to pentadecanoic acid by resting cells of *Arthrobacter simplex*. J Gen Appl Microbiol 17:429–432

Received October 25, 1982/Accepted December 6, 1982