Menaquinone Composition in the Classification of *Streptomyces* and Other Sporactinomycetes

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The menaquinones of 59 actinomycetes representing *Streptomyces*, *Streptoverticillium* and related taxa were examined by mass spectrometry and the results compared with those of an earlier numerical phenetic survey. The *Streptomyces* and *Streptoverticillium* strains contained complex mixtures of partially saturated menaquinones with nine isoprene units with the hexa- and/or octahydrogenated components predominating. The detection of essentially similar menaquinones in representatives of the genera *Chainia*, *Elytrosporangium*, *Kitasatoa* and *Microellobosporia* is consistent with the suggestion that these taxa be reduced to synonyms of the genus *Streptomyces*. The recovery of markedly different menaquinones from representatives of *Nocardiopsis dassonvillei* and *Saccharopolyspora hirsuta*, together with chemical data from previous studies, clearly show that these taxa should not be associated with the *Streptomyces* cluster-group, contrary to the results of the numerical phenetic survey. Streptomycetes can also be distinguished from other actinomycetes including those strains of *Nocardia* lacking mycolic acids. The results show that menaquinone composition is of value in both the classification and the identification of streptomycetes. Indeed, the present data suggest that the minimum description of the genus *Streptomyces* should include information on menaquinone composition.

INTRODUCTION

The genus *Streptomyces* currently contains aerobic, Gram-positive, highly oxidative actinomycetes which usually form a stable substrate mycelium with aerial mycelium bearing long chains of conidia. Streptomycetes have a wall chemotype I, that is, they contain LL-diaminopimelic acid and glycine but no characteristic sugar in the peptidoglycan (Lechevalier & Lechevalier, 1970). The confused state of streptomycete systematics (Sneath, 1970; Kutzner, 1981) has made it difficult to choose representative strains for taxonomic studies (Ridell & Williams, 1983; Gładek *et al.*, 1985), while it can be difficult to distinguish *Streptomyces* from related wall chemotype I taxa and from *Actinomadura*, *Nocardiopsis* and *Nocardia* strains that lack mycolic acids (Gordon & Horan, 1968; Lacey *et al.*, 1978; Goodfellow & Pirouz, 1982; Goodfellow & Haynes, 1984; Goodfellow & Minnikin, 1984). However, in a recent numerical phenetic study of *Streptomyces* and related genera (Williams *et al.*, 1983) most of the 394 *Streptomyces* type cultures fell into an aggregate taxon, cluster-group A, that encompassed 19 major, 14 minor and 18 single-member clusters. Some streptomycete taxa, notably *S. clavuligerus*, *S. fradiae*, *S. lavendulae* and *S. rimosus*, were recovered in distinct but less stable cluster-groups while *S. lavendulae* and related organisms not only consistently fell outside the *Streptomyces* cluster-group but shared a relatively high overall similarity to the *Kitasatoa* and *Streptoverticillium* strains in cluster-group F.

Isoprenoid quinones are characteristic components of most bacterial membranes and those from actinomycetes are exclusively menaquinones, that is, 2-methyl-3-polypropenyl-1,4-naphthoquinones (Collins & Jones, 1981; Collins *et al.*, 1985). The taxonomic value of menaquinones lies

in the variation in the length and degree of unsaturation of the C₃ isoprenyl side-chain. Much of the data on the menaquinone composition of actinomycetes has come from analyses of actinobacteria and nocardioform actinomycetes (Goodfellow & Cross, 1984; Collins *et al.*, 1985). In contrast, sporoactinomycetes have received somewhat less attention (Lester & Crane, 1959; Campbell *et al.*, 1971; Dunphy *et al.*, 1971; Batrakov *et al.*, 1976; Collins *et al.*, 1977, 1980, 1982, 1984, 1985; Yamada *et al.*, 1977a; Fischer *et al.*, 1983; Athalye *et al.*, 1984). It was therefore decided to determine the menaquinone composition of judiciously chosen representatives of the taxa defined by Williams *et al.* (1983) in order to evaluate the integrity of the phenetic groups and the potential value of menaquinones in the circumscription of the genus *Streptomyces* and its differentiation from related taxa.

METHODS

Cultures. The test strains (Tables 1 and 2) were stored on yeast/malt extract agar slants (Pridham *et al.*, 1956–1957) both at room temperature and at –25 °C, and on slopes of modified Bennett's agar (Jones, 1949) with glucose replaced by glycerol, at 4 °C. In addition, spore or mycelial fragment suspensions were stored in glycerol (20%, v/v) at –20 °C (Wellington & Williams, 1978). Detailed histories of the test strains can be found elsewhere (Williams *et al.*, 1983). Binomials in inverted commas are not on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and have not been validly published since 1 January 1980.

Growth conditions. The strains were grown in shake flasks of modified Sauton's broth (Mordarska *et al.*, 1972) for 5 to 7 d at 30 °C. At maximum growth, cultures were checked for purity, killed with formaldehyde (1%, v/v), harvested by centrifugation, washed twice with distilled water and freeze-dried.

Extraction and analysis of isoprenoid quinones. Dried organisms (about 100 mg) were extracted by a recently developed procedure involving shaking with a biphasic mixture of equal amounts of methanol/0.3% aqueous sodium chloride (10:1, v/v) and petroleum ether (b.p. 60–80 °C) followed by preparative thin layer chromatography (TLC) (Athalye *et al.*, 1984; Minnikin *et al.*, 1984). Reverse phase TLC was done according to Collins *et al.* (1980). Mass spectra of the purified menaquinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 200 to 220 °C.

RESULTS AND DISCUSSION

The lipid extracts of all of the test strains contained menaquinones which co-chromatographed with standards of vitamin K. The most intense peaks in the mass spectra occurred at *m/z* 187 and 225 and were derived from the naphthoquinone nucleus. The mass spectra of the menaquinone samples 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 ions. The results of the mass spectral analyses are shown in Tables 1 and 2, the patterns of representative strains being checked by reverse phase TLC.

Without exception, all of the representative strains of *Streptomyces* from cluster-group A contained complex mixtures of partially saturated menaquinones with nine isoprene units (Table 1). The detection of major amounts of hexa- and octahydrogenated menaquinones with nine isoprene units, abbreviated as MK-9(H₆) and MK-9(H₈), in the cluster-group A strains is in good agreement with reports of similar menaquinone profiles in *Streptomyces albus* (Campbell *et al.*, 1971; Collins *et al.*, 1984), *S. fradiae* (Collins *et al.*, 1985), *S. griseochromogenes* (Collins, 1982; Collins *et al.*, 1985), *S. olivaceus* (Batrakov *et al.*, 1976; Batrakov & Bergelson, 1978), *S. platensis* (Collins *et al.*, 1980, 1985), *S. rimosus* (Collins *et al.*, 1985), *S. somaliensis* (Collins *et al.*, 1977; Athalye *et al.*, 1984), *S. xantholyticus* (Collins *et al.*, 1985), *S. viridochromogenes* (Collins *et al.*, 1984) and *Streptomyces* spp. (Collins *et al.*, 1977; Dunphy *et al.*, 1971; Phillips *et al.*, 1971). The presence of essentially similar menaquinone profiles in *S. clavuligerus*, *S. lavendulae* and *S. rimosus*, and in all but one of the streptomycetes which fell outside the *Streptomyces* cluster-group (Williams *et al.*, 1983) is not unexpected as all of the excluded organisms exhibit properties characteristic of typical members of the genus *Streptomyces* (Lechevalier & Lechevalier, 1970; Shirling & Gottlieb, 1968a, b, 1969, 1972; Pridham & Lyons, 1969). In addition, the *S. lavendulae* strain is lysed by phage virulent for *Streptomyces* (Prauser, 1976a) and has been recovered in a rRNA homology group corresponding to the genus *Streptomyces* (Gładek *et al.*, 1985). The menaquinone data lend support to the view (Williams *et al.*, 1983) that

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Table 1. Peaks corresponding to molecular ions in the mass spectra of menaquinones of *Streptomyces* and related taxa

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

Strain	Cluster*	Cluster group*	MK-8										MK-9					
			(H ₂)	(H ₄)	(H ₆)	(H ₈)	(—)	(H ₂)	(H ₄)	(H ₆)	(H ₈)	(H ₁₀)	(—)	(H ₂)	(H ₄)	(H ₆)	(H ₈)	(H ₁₀)
			m/z ... 718	720	722	724	784	786	788	790	792	794						
<i>Streptomyces albidoflavus</i> ISP 5455†	1A	A	—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. griseus</i> ISP 5236†	1B		—	—	—	—	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. olivaceus</i> ISP 5072†	1C		—	—	—	—	—	—	+	+	+	—	—	—	+	+	+	—
' <i>S. scabies</i> ' ISP 5078	3		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. umbrinus</i> ISP 5278†	5		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. spectabilis</i> ISP 5512†	10		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. griseorubens</i> ISP 5160†	12		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. albus</i> ISP 5313†	16		—	—	—	—	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. chartreusis</i> ISP 5085†	18		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. griseochromogenes</i> ISP 5499†	18		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>Elytrosporangium brasiliensis</i> M-086†	20		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. platensis</i> ISP 5041†	29		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. hygroscopicus</i> N736	32		—	—	—	—	—	—	+	+	+	—	—	—	+	+	+	—
' <i>S. thermoflavus</i> ' ISP 5574	36		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. hirsutus</i> ISP 5095†	37		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>Chainia antibiotica</i> ISP 5269†	40		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. albofaciens</i> ISP 5268†	42	B	—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. anandii</i> ISP 5535†	42		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
' <i>S. flavofungini</i> ' ISP 5366	42		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. rimosus</i> ISP 5260†	42		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—
<i>S. amakusaensis</i> ISP 5219†	SMC		—	—	+	+	—	—	+	+	+	—	—	—	+	+	+	—

Table 2. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from strains of *Actinomadura*, *Nocardia*, *Nocardioopsis*, *Saccharopolyspora* and related taxa

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

Strain	Cluster*	Cluster group*	m/z ...	MK-6					MK-7					MK-8					MK-9					MK-10				
				(H ₄)	(—)	(H ₄)	(—)	(H ₂)	(H ₂)	(H ₄)	(H ₄)	(H ₆)	(H ₈)	(H ₂)	(H ₂)	(H ₄)	(H ₄)	(H ₆)	(H ₆)	(H ₈)	(H ₁₀)	(—)	(H ₂)	(H ₄)	(H ₆)			
<i>Nocardioopsis dassonvillei</i> A14†	4	A		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>Nocardia</i> ' <i>aerocolonigenes</i> ISP 5034	12			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Saccharopolyspora hirsuta</i> N745†	41			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Actinomadura madurae</i> A16†	50	E		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>A. madurae</i> A17	50			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>A. verrucosospora</i> A4†	50			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Nocardia asteroides</i> N317†	52			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>N.</i> ' <i>orientalis</i> ISP 5040†	52			++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>N.</i> ' <i>mediterranei</i> N730	53			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>N.</i> ' <i>mediterranei</i> N733	53			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>N.</i> ' <i>mediterranei</i> ISP 5501†	53			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>Streptomyces</i> ' <i>tolypophorus</i> N737†	53	I		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>Nocardia</i> ' <i>autotrophica</i> ISP 5011†	69			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>N.</i> ' <i>salmonicida</i> ISP 5472	69			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Actinomadura pelletieri</i> A19†	73	J		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
' <i>Nocardia capreola</i> ' ISP 5225	SMC		SMCG		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

* Taxa defined by Williams *et al.* (1983); SMC, single-member cluster; SMCG, single-member cluster-group.

† Type strains.

1977; Goodfellow *et al.*, 1982). Indeed, it seems likely that the association of *Nocardioides albus*, *Nocardioopsis dassonvillei* and *Saccharopolyspora hirsuta* with *Streptomyces* (Williams *et al.*, 1983) is not firmly based. Thus, while *Nocardioides* strains have a wall chemotype I, they are resistant to phage virulent to *Streptomyces* (Prauser 1976*a, b*; Wellington & Williams, 1978) and have been distinguished from the latter in DNA : DNA pairing (Tille *et al.*, 1978) and lipid analyses (Lechevalier *et al.*, 1977; O'Donnell *et al.*, 1982; Collins *et al.*, 1983). Similarly, *Nocardioopsis dassonvillei* differs from *Streptomyces* in its wall chemotype (Lechevalier & Lechevalier, 1970), resistance to streptomycete phages (Wellington & Williams, 1981), rRNA oligonucleotide sequences (Stackebrandt & Woese, 1981) as well as on the basis of menaquinone composition. In addition to menaquinone content, *Saccharopolyspora hirsuta* can be separated from *Streptomyces* by wall chemotype (Lacey & Goodfellow, 1975) and its resistance to phages virulent for streptomycetes.

The present study has confirmed and extended our knowledge on the menaquinone composition of sporoactinomycetes with a wall chemotype I and has provided further evidence of the value of these compounds in the systematics of actinomycetes. In a narrower context, the menaquinone profiles have helped to clarify some of the apparent anomalies in the recent numerical phenetic classification of *Streptomyces* and related taxa (Williams *et al.*, 1983). The menaquinone data also support the integrity of the genus *Streptomyces* and help to distinguish it from actinomycete taxa including *Kineosporia*, *Nocardioides*, *Nocardioopsis*, *Saccharopolyspora* and sundry *Nocardia* strains lacking mycolic acids. Despite improvements in streptomycete systematics there is still a dearth of good characters for the description of the genus. The present findings raise the question as to whether data on menaquinone composition should form part of the minimum description of the genus *Streptomyces*. Mass spectrometric analyses, as used in the present study, provide precise information concerning the overall molecular composition of the principal menaquinone components. Complementary analyses using high performance liquid chromatography provide quantitative profiles (Kroppenstedt, 1982, 1985; Collins *et al.*, 1984) which allow minor components to be observed more clearly and the significance of variations in the proportions of the isoprenologues to be evaluated. It would be of value to apply this latter technique to a similar number of streptomycetes and related taxa but studies should also be done to assess the stability of such menaquinone profiles under varying culture conditions.

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