NOTE

Polyamine profiles within genera of the class *Actinobacteria* with LL-diaminopimelic acid in the peptidoglycanHans-Jürgen Busse^{1†} and Peter Schumann²

Author for correspondence: Hans-Jürgen Busse. Tel: +43 1 25077 2128. Fax: +43 1 25077 2190.
e-mail: Hans-Juergen.Busse@vu-wien.ac.at

¹ Institute of Microbiology and Genetics, University of Vienna, A-1030 Vienna, Austria

² DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, D-07745 Jena, Germany

Polyamine patterns of coryne- and nocardioform representatives of the class *Actinobacteria* with LL-diaminopimelic acid in the peptidoglycan, comprising strains of the genera *Aeromicrobium*, *Nocardioides*, *Intrasporangium*, *Terrabacter*, *Terracoccus*, *Propioniferax*, *Friedmanniella*, *Microlunatus*, *Luteococcus* and *Sporichthya*, were analysed. The different polyamine patterns were in good agreement with the phylogenetic heterogeneity within this group of actinomycetes. Strains of the closely related genera *Nocardioides* and *Aeromicrobium* were characterized by the presence of cadaverine. The second cluster, consisting of the type strains of the species *Friedmanniella antarctica*, *Propioniferax innocua*, *Microlunatus phosphovorus* and *Luteococcus japonicus*, displayed as a common feature the presence of the two predominant compounds spermidine and spermine. The presence of putrescine was common to the type strains of the species *Intrasporangium calvum*, *Terrabacter tumescens* and *Terracoccus luteus*. *Sporichthya polymorpha*, which is a representative of a separate line of descent, displayed spermidine as the predominant polyamine. These data indicate that polyamine patterns are suitable for the classification of actinomycetes with LL-diaminopimelic acid in the peptidoglycan.

Keywords: polyamine patterns, chemotaxonomic classification, LL-diaminopimelic-acid-containing *Actinobacteria*

Coryneform and nocardioform actinomycetes with LL-diaminopimelic acid (LL-A₂pm) as diagnostic diamino acid of their peptidoglycan are a biotechnologically interesting group of metabolically versatile bacteria. Representatives of this group have, for example, the capacity to accumulate polyphosphate (Nakamura *et al.*, 1995), to produce the antibiotic erythromycin (Miller *et al.*, 1991) and to transform steroids (Hayakawa & Kurokawa, 1963; Nobile *et al.*, 1955). Organisms with LL-A₂pm occupy different phylogenetic lineages which represent five families within the class *Actinobacteria*, as revealed by 16S rRNA sequence analyses (Prauser *et al.*, 1997; Stackebrandt *et al.*, 1997). The sporoactinomycetes *Streptomyces* (*Streptomycetaceae*) (Stackebrandt *et al.*, 1991) and *Sporichthya* (*Sporichthyaceae*) (Rainey *et al.*, 1993) are

distinctly separated from the three clusters of coryneform and nocardioform actinomycetes, which comprise the following genera: (i) *Nocardioides* and *Aeromicrobium* (Collins *et al.*, 1994; Collins & Stackebrandt, 1989; Miller *et al.*, 1991; O'Donnell *et al.*, 1982; Tamura & Yokota, 1994) (*Nocardioidaceae*); (ii) *Terrabacter* (Collins *et al.*, 1989), *Terracoccus* (Prauser *et al.*, 1997) and *Intrasporangium* (Kalakoutskii, 1989) (*Intrasporangiaceae*); (iii) *Propioniferax* (Yokota *et al.*, 1994), *Luteococcus* (Tamura *et al.*, 1994), *Microlunatus* (Nakamura *et al.*, 1995) and *Friedmanniella* (Schumann *et al.*, 1997) (*Propionibacteriaceae*). These genera are distinguished from each other by their chemotaxonomic characteristics. The genera *Nocardioides*, *Aeromicrobium*, *Luteococcus* and *Propioniferax* share the peptidoglycan type A3 γ (Schleifer & Kandler, 1972), with a single glycine residue as interpeptide bridge. The genera *Terrabacter*, *Terracoccus* and *Intrasporangium* are characterized by the cross-linkage of the A3 γ -type peptidoglycan via a triglycine-interpeptide bridge and

[†]Present address: Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A-1210 Wien, Austria.

Abbreviation: LL-A₂pm, LL-diaminopimelic acid.

by a glycine residue bound to the α -carboxyl group of D-glutamic acid at position 2 of the peptide subunit. The genera *Microtholunatus* and *Friedmanniella* are distinguished by the rare peptidoglycan type A3 γ ' (Schleifer & Seidl, 1985), with glycine at position 1 of the peptide subunit. The genera *Nocardioides*, *Terrabacter* and *Terracoccus* have MK-8(H₄) as their major isoprenoid quinone, the genus *Intrasporangium* shows MK-8, whereas the menaquinone MK-9(H₄) is common to all other coryne- and nocardioform actinomycetes with LL-A₂pm. Fatty acid- and polar lipid patterns are useful tools for the further differentiation of this group of organisms (Schumann *et al.*, 1997).

Recently, the suitability of polyamine patterns for classification of coryneform actinobacteria has been demonstrated (Altenburger *et al.*, 1997; Hamana, 1995, 1996). However, these studies did not include representatives of the class *Actinobacteria* with LL-A₂pm or the data are difficult to interpret due to nonstandardized growth conditions. Thus, we analysed the polyamine composition of coryne- and nocardioform actinomycetes with LL-A₂pm to estimate the value of this approach for classification of members of this phylogenetically heterogeneous group (Schumann *et al.*, 1997).

Strains analysed are listed in Table 1. Biomass for polyamine analyses was obtained by submerged cultivation of the strains in R medium (Yamada & Komagata, 1972) or polyamine-free glucose/asparagine medium (MM) (Lindenbein, 1952) at 28 °C using a horizontal shaker run at 220 r.p.m. Cells were harvested in the exponential phase of growth when 70% of the maximal optical density at 578 nm was reached. Because of the tendency to aggregation of *Luteococcus japonicus* IFO 12422^T and *Propioniferax innocua* NCTC 11082^T, their cultivation times were estimated. *Sporichthya polymorpha* IMET 7840^T and *Friedmanniella antarctica* AA-1042^T did not grow in submerged cultures, therefore cells were harvested from R agar slant cultures. Polyamine extractions and analyses were performed as described recently (Altenburger *et al.*, 1997).

The analysis of the polyamine patterns of coryne- and nocardioform actinobacteria with LL-A₂pm revealed the presence of at least four different profiles (Table 1). The majority of strains, which belong to the genus *Nocardioides*, were characterized by the presence of high concentrations of the diamines putrescine and cadaverine. The similarity in the polyamine patterns is in agreement with the phylogenetic homogeneity of the species of the genus *Nocardioides* (Collins *et al.*, 1994). *Nocardioides simplex* IMET 10283 and *Nocardioides plantarum* NCIMB 12834^T were found to contain relatively low polyamine concentrations and displayed only cadaverine as the predominant diamine. The relatively high amounts of spermine were in the same range as found in the other strains of the genus *Nocardioides* and it is most likely that this tetraamine is taken up from the growth medium as was previously supposed for members of the genera *Clavibacter* and

Corynebacterium (Altenburger *et al.*, 1997). When the *Nocardioides albus* strains IMET 7807^T and IMET 7815 were grown on a mineral medium (Lindenbein, 1952), cadaverine was detected as the predominant compound. The high concentration of putrescine in the majority of strains of the genus *Nocardioides* when grown in R medium, in addition to cadaverine, appears to be useful for differentiation from the genus *Aeromicrobium*. The two species *Aeromicrobium fastidiosum* and *Aeromicrobium erythreum* are phylogenetically associated with the genus *Nocardioides* (Tamura & Yokota, 1994) and are included in the family *Nocardioideaceae* (Stackebrandt *et al.*, 1997). The polyamine patterns of these two species were similar to members of the genus *Nocardioides* in containing significant concentrations of cadaverine, which distinguished *Aeromicrobium fastidiosum* IMET 7849^T and *Aeromicrobium erythreum* NRRL B-3381^T from the other LL-A₂pm-containing actinobacteria. However, the two species did not display identical polyamine patterns. *Aeromicrobium fastidiosum* IMET 7849^T contained a rather low concentration of polyamines and spermine was found to be the predominant compound, whereas *Aeromicrobium erythreum* NRRL B-3381^T was characterized by the second major compound, spermidine.

The second polyamine similarity cluster consisted of the phylogenetically closely related species *Propioniferax innocua*, *Microtholunatus phosphovorus* and *Luteococcus japonicus*, which are included in the family *Propionibacteriaceae*, and *Friedmanniella antarctica* (Schumann *et al.*, 1997). Due to its phylogenetic position and similar chemotaxonomic characteristics *Friedmanniella antarctica* also belongs to the family *Propionibacteriaceae* (Schumann *et al.*, 1997; Stackebrandt *et al.*, 1997). The type strains of these four species, NCTC 11082^T, JCM 9379^T, IFO 12422^T and AA-1042^T, respectively, contained polyamine patterns in which spermidine and spermine were the predominant polyamines. Recently, six species of the genus *Propionibacterium*, including the LL-A₂pm-containing species *Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium jensenii*, *Propionibacterium lymphophilum*, *Propionibacterium propionicum* and *Propionibacterium thoenii*, have been shown to contain similar polyamine patterns (Hamana, 1995). All these six species contained spermidine and spermine as the predominant compound in the polyamine pattern, demonstrating the close phylogenetic relatedness to the genera *Propioniferax*, *Luteococcus*, *Friedmanniella* and *Microtholunatus* (Nakamura *et al.*, 1995; Schumann *et al.*, 1997; Tamura *et al.*, 1994; Yokota *et al.*, 1994). Based on the data shown, as well as already published data on species of the genus *Propionibacterium*, it appears that members of the family *Propionibacteriaceae* (Stackebrandt *et al.*, 1997) are characterized by the presence of the major compounds spermidine and spermine in their polyamine patterns.

Intrasporangium calvum IMET 7816^T and *Terrabacter*

Table 1. Polyamine patterns of coryneform and nocardioform actinomycetes with ω -A₂pm as diagnostic diamino acid of their peptidoglycan

Organism*	Growth medium†	Polyamine content [μ mol (g dry wt) ⁻¹ ‡]						
		DAP	PUT	CAD	SPD	HSPD	SPM	TYR
<i>Intrasporangium calvum</i> IMET 7816 ^T (= 7 KIP ^T , DSM 43043 ^T)	R	0.17 (4.3)	2.02 (51.7)	0.30 (7.6)	1.03 (26.9)	0.05 (1.3)	0.31 (7.9)	0.03 (0.8)
<i>Terrabacter tumescens</i> IMET 10431 ^T (= NCIB 8914 ^T , DSM 20308 ^T)	R	–	1.35 (84.9)	Tr	0.04 (2.5)	Tr	0.20 (12.6)	–
<i>Terracoccus luteus</i> IMET 7848 ^T (= DSM 44267 ^T)	R	0.07 (1.2)	2.04 (30.0)	0.55 (8.1)	1.98 (29.1)	–	2.16 (31.8)	Tr
<i>Janibacter limosus</i> DSM 11140 ^T (IFO 16128 = HKI 83 ^T)	R	0.07 (2.7)	1.36 (52.3)	0.73 (28.1)	0.06 (2.3)	0.05 (1.9)	0.24 (9.2)	0.09 (3.5)
<i>Janibacter limosus</i> DSM 11141 (IFO 16128 ^T = HKI 84)	R	0.02 (0.7)	1.14 (39.6)	0.84 (29.2)	0.50 (17.4)	–	0.25 (8.7)	0.13 (4.5)
<i>Sanguibacter keddietii</i> DSM 10542 ^T (= ATCC 51767 ^T = NCFB 3025 ^T)	R	0.01 (1.5)	0.06 (9.0)	0.07 (10.4)	0.12 (17.9)	0.01 (1.5)	0.40 (59.7)	Tr
<i>Sanguibacter suarezii</i> DSM 10543 ^T (= NCFB 3023)	R	0.03 (7.0)	0.02 (4.7)	0.02 (4.7)	0.05 (11.6)	–	0.31 (72.1)	Tr
<i>Nocardioides albus</i> IMET 7807 ^T (= DSM 43109 ^T)	R	0.08 (0.8)	5.23 (49.6)	5.16 (49.0)	Tr	–	Tr	0.07 (0.7)
<i>Nocardioides albus</i> IMET 7807 ^T (= DSM 43109 ^T)	MM	–	0.04 (7.8)	0.45 (88.2)	0.02 (3.9)	–	–	–
<i>Nocardioides albus</i> IMET 7801 (= DSM 43364)	R	0.08 (2.1)	0.97 (25.1)	2.71 (70.0)	0.06 (1.6)	Tr	0.05 (1.3)	Tr
<i>Nocardioides albus</i> IMET 7815 (= DSM 43365)	R	0.14 (1.9)	3.48 (48.1)	3.45 (47.7)	0.12 (1.7)	–	0.02 (0.3)	0.03 (0.4)
<i>Nocardioides albus</i> IMET 7815 (= DSM 43365)	MM	–	0.21 (20.6)	0.78 (76.5)	0.03 (2.9)	–	–	–
<i>Nocardioides albus</i> IMET 7822 (= DSM 43873)	R	0.04 (0.5)	3.11 (39.4)	4.59 (58.1)	0.12 (1.5)	Tr	0.04 (0.5)	–
<i>Nocardioides albus</i> IMET 7835 (= DSM 43874)	R	0.23 (14.9)	0.47 (30.5)	0.82 (53.2)	0.02 (1.3)	–	Tr	Tr
<i>Nocardioides simplex</i> IMET 10368 ^T (= NCIB 8929 ^T , DSM 20130 ^T)	R	0.20 (4.2)	0.72 (15.0)	2.71 (56.6)	0.89 (18.6)	Tr	0.27 (5.6)	–
<i>Nocardioides simplex</i> IMET 10283	R	0.16 (15.4)	–	0.40 (38.5)	0.05 (4.8)	–	0.28 (26.9)	0.15 (14.4)
<i>Nocardioides plantarum</i> NCIMB 12834 ^T (= DSM 11054 ^T)	R	0.04 (6.8)	–	0.20 (33.9)	0.03 (5.1)	–	0.25 (42.4)	0.07 (11.9)
<i>Nocardioides jensenii</i> IMET 10678 ^T (= NCIB 9770 ^T , DSM 20641 ^T)	R	0.07 (2.5)	0.61 (21.9)	1.51 (54.1)	0.34 (12.2)	–	0.26 (9.3)	–
<i>Nocardioides luteus</i> IMET 7830 ^T (= DSM 43366 ^T)	R	0.15 (2.6)	1.63 (28.2)	3.10 (53.7)	0.65 (11.3)	–	0.14 (2.4)	0.10 (1.7)
<i>Aeromicrobium erythreum</i> NRRL B-3381 ^T (= DSM 8599 ^T)	R	0.18 (3.9)	0.16 (3.5)	2.22 (48.6)	1.70 (37.2)	–	0.31 (6.8)	–
<i>Aeromicrobium fastidiosum</i> IMET 7849 ^T (= NCIB 12713 ^T , DSM 10552 ^T)	R	0.03 (3.0)	0.11 (10.9)	0.25 (24.8)	0.03 (3.0)	–	0.59 (58.4)	Tr
<i>Sporichthya polymorpha</i> IMET 7840 ^T (= DSM 46113 ^T)	R	0.07 (2.2)	0.48 (15.4)	0.30 (9.6)	2.08 (66.7)	0.04 (1.3)	0.06 (1.9)	0.09 (2.9)
<i>Friedmanniella antarctica</i> AA-1042 ^T (= DSM 11053 ^T)	R	0.15 (2.0)	0.26 (3.5)	0.16 (2.1)	2.93 (38.9)	0.03 (0.3)	3.84 (51.0)	0.16 (2.1)
<i>Propioniferax innocua</i> NCTC 11082 ^T (= DSM 8251 ^T)	R	Tr	0.21 (13.0)	0.14 (8.7)	0.77 (47.8)	Tr	0.49 (30.4)	–
<i>Microtholus phosphovorus</i> JCM 9379 ^T (= DSM 10555 ^T)	R	0.91 (13.3)	0.05 (0.7)	0.96 (14.0)	2.64 (38.5)	0.02 (0.3)	2.19 (32.0)	0.08 (1.2)
<i>Luteococcus japonicus</i> IFO 12422 ^T (= DSM 10546 ^T)	R	0.05 (3.2)	0.08 (5.1)	0.11 (7.1)	0.80 (51.3)	–	0.52 (33.3)	–

* DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IFO, Institute for Fermentation, Osaka, Japan; IMET, Nationale Kultursammlung für Mikroorganismen, Zentralinstitut für Mikrobiologie und Experimentelle Therapie, Jena, Germany (now included in the DSMZ, Braunschweig, Germany); JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Saitama, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen, UK; NCFB, National Collection of Food Bacteria, AFRC Institute of Food Research, Reading, UK; NCIMB, National Collection of Industrial and Marine Bacteria, Torry Research Station, Aberdeen, UK; NCPPB, National Collection of Plant-pathogenic Bacteria, Central Science Laboratory, York, UK; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, UK; NRRL, ARS Culture Collection, Northern Regional Research Laboratory, US Department of Agriculture, Peoria, IL, USA.

† Growth conditions as described in the text.

‡ DAP, 1,3-diaminopropane; PUT, putrescine; CAD, cadaverine; SPD, spermidine; HSPD, *sym*-homospermidine; SPM, spermine; TYR, tyramine; Tr, trace [less than 0.01 μ mol (g dry weight)⁻¹]; –, none detected. Values in boldface type are those for major polyamines (which accounted for more than 20% of the total polyamines). Values in parentheses are percentages of the total polyamine content.

Table 2. Differential chemotaxonomic characteristics of coryneform and nocardioform actinomycetes with LL-A₂pm as diagnostic diamino acid of their peptidoglycan

Taxon	Polyamine pattern*	Peptidoglycan interpeptide bridge	Diamino acid and amino acid at position 1 of peptide subunit†	Polar lipids‡	Major menaquinone(s)§	References
Propionibacteriaceae						
<i>Friedmanniella</i>	SPD, SPM	Gly	LL-A ₂ pm, Gly	PI, PG, DPG, PL	MK-9(H ₄)	Schumann <i>et al.</i> (1997)
<i>Microtholunatus</i>	SPD, SPM	Gly	LL-A ₂ pm, Gly	PI, PG, DPG, PL	MK-9(H ₄)	Nakamura <i>et al.</i> (1995); Schumann <i>et al.</i> (1997)
<i>Luteococcus</i>	SPD, SPM	Gly	LL-A ₂ pm, L-Ala	PI, PG, DPG, GL	MK-9(H ₄)	Schumann <i>et al.</i> (1997); Tamura <i>et al.</i> (1994)
<i>Propioniferax</i>	SPD, SPM	Gly	LL-A ₂ pm, L-Ala	PE, PG, PL, GL	MK-9(H ₄)	Schumann <i>et al.</i> (1997); Yokota <i>et al.</i> (1994)
Nocardioidaceae						
<i>Aeromicrobium</i>	CAD (SPD)	Gly	LL-A ₂ pm, L-Ala	PE, PG	MK-9(H ₄)	Miller <i>et al.</i> (1991); Tamura & Yokota (1994)
<i>Nocardioides</i>	CAD (PUT)	Gly	LL-A ₂ pm, L-Ala	PG, DPG, PL, PG-OH	MK-8(H ₄)	Collins <i>et al.</i> (1989); O'Donnell <i>et al.</i> (1982); Prauser (1986)
Intrasporangiaceae						
<i>Terrabacter</i>	PUT	Gly ₃	LL-A ₂ pm, L-Ala	PE, PI, DPG, PL	MK-8(H ₄)	Collins <i>et al.</i> (1989)
<i>Terracoccus</i>	PUT, SPD, SPM	Gly ₃	LL-A ₂ pm, L-Ala	PE, PI, DPG, PL	MK-8(H ₄)	Prauser <i>et al.</i> (1997)
<i>Intrasporangium</i>	PUT, SPD	Gly ₃	LL-A ₂ pm, L-Ala	PI, PIM, PG, DPG	MK-8	Kalakoutsii (1989); Schumann <i>et al.</i> (1997)
<i>Janibacter</i>	PUT, CAD, (SPD)	None	meso-A ₂ pm, none	DPG, PG, PI	MK-8(H ₄)	Martin <i>et al.</i> (1997)
<i>Sanguibacter</i>	SPD	Ser-D-Glu	L-Lys, L-Ala	ND	MK-9(H ₄)	Fernández-Garayzábal <i>et al.</i> (1995)
Sporichthyaceae						
<i>Sporichthya</i>	SPD	Gly	LL-A ₂ pm, L-Ala	PI, PG, DPG, PL	MK-9(H ₆), MK-9(H ₈)	Rainey <i>et al.</i> (1993); Schumann <i>et al.</i> (1997)

*CAD, cadaverine; PUT, putrescine; SPD, spermidine; SPM, spermine. Polyamines given in parentheses were not detected in all strains of the respective taxon.

†LL-A₂pm, LL-diaminopimelic acid; *m*-A₂pm, *m*-diaminopimelic acid.

‡PI, phosphatidylinositol; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PL, unknown phospholipid(s); GL, unknown glycolipid(s); PE, phosphatidylethanolamine; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids. ND, Not determined.

§Abbreviations for menaquinones are exemplified by MK-9(H₄), menaquinone with two of nine isoprene units hydrogenated.

tumescens IMET 10431^T have been grouped together in the family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997) and *Terracoccus luteus* IMET 7848^T, which has been described recently (Prauser *et al.*, 1997), can be considered as another member of this family. The polyamine patterns of these three phylogenetic relatives were similar in respect to the presence of putrescine as a predominant polyamine. In addition, in strain IMET 7816^T a high concentration of spermidine was detected and in IMET 7848^T high amounts of

spermidine and spermine were found. Thus the polyamine pattern of these three organisms does not only display their phylogenetic relatedness but also appears to be useful for their differentiation at the genus level. However, the genus specificity of the polyamine patterns has to be confirmed by inclusion of more strains of the three genera, which are not yet available. In order to substantiate the predominant diamine putrescine as a family characteristic feature in the polyamine pattern of the *Intrasporangiaceae*,

members of two more genera belonging to the family, *Janibacter* (Martin *et al.*, 1997) and *Sanguibacter* (Fernández-Garayzábal *et al.*, 1995) were included in our study. *Janibacter limosus* is known to possess meso-A₂pm whereas the two species *Sanguibacter keddiei* and *Sanguibacter suarezii* contain L-lysine in their peptidoglycans. The two strains of *J. limosus*, DSM 11140^T and DSM 11141, were found to have a polyamine pattern which contained the predominant compounds putrescine and cadaverine. In addition, strain DSM 11141 contained significant amounts of spermidine. The presence of putrescine in the polyamine pattern is in agreement with the affiliation of these two strains with the family *Intrasporangiaceae* but the polyamine pattern was clearly distinct from the other members of the family and also appears to be genus-specific. A common characteristic of the polyamine patterns of the two species *S. keddiei* DSM 10542^T and *S. suarezii* DSM 10543^T was the relatively low polyamine content when compared to the other members of the family. In addition, these two species contained spermine as the predominant compound whereas significant amounts of putrescine were lacking. This observation demonstrates that putrescine as a predominant compound is not present in all members of the family and thus cannot be considered as a family-specific characteristic. However, the two *Sanguibacter* species are the deepest phylogenetic branching within the family (Prauser *et al.*, 1997). They differ from the other members of the family in respect to their peptidoglycan diamino acid (L-lysine), their menaquinone MK-9(H₄) and their fatty acid profile (Prauser *et al.*, 1997). These data, as well as the polyamine patterns, confirm the phylogenetic analyses indicating that the genus *Sanguibacter* represents a separate branch within the *Intrasporangiaceae* (Prauser *et al.*, 1997), which might be considered as a subfamily. For pragmatic reasons it would be appropriate to exclude the genus *Sanguibacter* from the family *Intrasporangiaceae* and to propose the new family *Sanguibacteriaceae*. This transfer would be justified by the significant differences in the chemotaxonomic characteristics (Table 2). The remainder of the family *Intrasporangiaceae* would be restricted to the genera *Intrasporangium*, *Terrabacter*, *Terracoccus* and *Janibacter*.

The only representative of a separate line of descent, *Sporichthya polymorpha* IMET 7840^T, could be distinguished from the other LL-A₂pm-containing taxa by its distinct polyamine pattern. It was characterized by spermidine as the predominant compound and smaller amounts of putrescine and cadaverine. As long as no more strains of this line of descent are available, the significance of this polyamine pattern cannot be evaluated. In respect to the major menaquinone and peptidoglycan characteristics, *Sporichthya polymorpha* cannot be distinguished from the genus *Streptomyces* (Schumann *et al.*, 1997). So far, only eight species of the genus *Streptomyces* have been analysed for their polyamine content (Hamana & Matsuzaki, 1987)

including *Streptomyces griseus*, *Streptomyces chrysomallus*, *Streptomyces coelicolor*, *Streptomyces caeruleus*, '*Streptomyces thermoolivaceus*', *Streptomyces thermoviolaceus*, *Streptomyces thermodiastaticus* and *Streptomyces thermovulgaris*. All these species contain putrescine as a major polyamine and, in addition, most of the species also contain cadaverine and spermidine as major compounds. Due to the limited data on the polyamine content of *Streptomyces* species it cannot be decided whether the polyamine approach is applicable for distinction between *Sporichthya* and *Streptomyces*. However, the detection of putrescine as a predominant polyamine in *Streptomyces* species might be a distinguishing characteristic.

The polyamine patterns detected in the heterogeneous group of coryne- and nocardioform actinobacteria with LL-A₂pm in the peptidoglycan demonstrated that this approach is also useful for distinction of the major lines of descent within this group, usually at the family level, and supports a reliable identification at the genus level. Classification and identification of these organisms at the genus level necessitates the additional investigation of other chemotaxonomic characteristics such as peptidoglycan type, fatty acid and polar lipid profile, and quinone system (Schumann *et al.*, 1997). The examination of supplementary characteristics is necessary, since the polyamine patterns detected for LL-A₂pm-containing members of the class *Actinobacteria* are not unique within this class. Similar patterns are found in other groups of the class *Actinobacteria*, for example the polyamine pattern characteristic for the *Nocardioideae* branch and *J. limosus* is similar to that found in *Brevibacterium* species and the polyamine pattern of the family *Propionibacteriaceae* resembles that found in the genera *Clavibacter*, *Rathayibacter* and *Curtobacterium* (Altenburger *et al.*, 1997; Hamana, 1995). On the other hand, the distinguishing polyamine patterns appear to facilitate differentiation between LL-A₂pm-containing taxa which have other characteristics in common, such as the genera *Terrabacter* and *Nocardioideae*, both containing MK-8(H₄), or the genus *Aeromicrobium* and taxa of the family *Propionibacteriaceae*, which both contain MK-9(H₄) (Table 2). Thus, the polyamine approach is considered as an important method for classification of coryne- and nocardioform LL-A₂pm-containing actinobacteria when applied in combination with other, well-established chemotaxonomic investigations.

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