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ORIGINAL ARTICLE

Simple and rapid characterization of mycolic acids from *Dietzia* strains by using MALDI spiral-TOFMS with ultra high mass-resolving power

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Mycolic acids have been used as important chemotaxonomic markers. In this study, a newly developed matrix-assisted laser desorption/ionization time-of-flight mass spectrometer with a spiral ion trajectory (MALDI spiral-TOFMS) was applied to the characterization of mycolic acids of three type strains of validated species belonging to the genus *Dietzia* (*D. papillomatosis* 105045^T, *D. kunjamensis* NBRC 105042^T and *D. timorensis* NBRC 104184^T), by analysis of total fatty acid methyl ester fractions. In addition, owing to the high mass-resolving power of MALDI spiral-TOFMS, adjacent peaks (0.036 Da mass differences) were successfully separated, and weak peaks corresponding to oxygenated mycolic acids were detected. For all samples, the distributions of carbon-chain lengths were mainly in the range of C_{30}–C_{42} and the average number of carbon-chain lengths was about 37, which agreed reasonably well with reported results for the genus *Dietzia*. The number of double bonds and/or cyclopropane rings was 0–2. Relative peak intensities of each mycolic acid methyl ester were used to compare the mycolic acids of the three strains. The mycolic acids of *D. papillomatosis* and *D. kunjamensis* were characterized by a high content of mycolic acids with 0–1 double bond or cyclopropane ring and an almost equal content of mycolic acids with odd- and even-numbered carbon-chain lengths. In contrast, mycolic acids of *D. timorensis* were characterized by a high content of mycolic acids with 1–2 double bonds and/or cyclopropane rings with an even-numbered carbon-chain length. By using MALDI spiral-TOFMS, mycolic acids from three type strains of the genus *Dietzia* were characterized easily and rapidly.
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Keywords: *Dietzia*; high mass-resolving power; MALDI spiral-TOFMS; mycolic acid; rapid characterization

INTRODUCTION

Mycolic acids are 2-alkyl 3-hydroxy fatty acids specifically contained in the cell envelopes of actinobacteria of the suborder Corynebacterineae, including the genera *Mycobacterium*, *Nocardia*, *Gordonia*, *Rhodococcus*, *Dietzia* and *Corynebacterium*. They are natural organic substances with large carbon-chain length (C_{20}–C_{90}).[1–5] Mycolic acids in mycobacterium and related species possess functional groups such as a cyclopropane ring, methoxy-, keto-, epoxy- or ester-group, and they are classified into sub-classes according to their functional groups.[6–8] The presence or absence of mycolic acids, the distribution of carbon-chain lengths, the types of functional groups and the number of double bonds differ depending on the genus.[9] Therefore, mycolic acids have been used as important chemotaxonomic markers.

In general, TLC, HPLC, and GC coupled with MS have been used to analyze mycolic acids.[6] Although TLC has been traditionally used as a simple method of separating the different types of mycolic acids, it requires reference specimens and cannot provide an accurate determination of carbon-chain length. HPLC can provide characteristic HPLC peak patterns of mycolic acids, and this could enable us to identify *Mycobacterium* species.[10] HPLC, however, frequently requires cumbersome purification of the samples and regular maintenance to keep the retention time constant for good reproducibility, and these are not easy tasks for many clinical laboratories.[10] GC/MS is a far more advanced method than the previous two, but additional derivatization procedures such as trimethylsilylation are essential before measurement. Furthermore, proficiency and special knowledge are required to interpret the complicated fragmentation patterns in the mass spectra observed by GC/MS.

Matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOFMS), which has been widely used to analyze various lipids and proteins,[11] is also a powerful tool for the characterization of mycolic acids.[12–16] The high sensitivity and very simple measurement

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procedures of MALDI-TOFMS have a great advantage over traditional methods for analyzing the trace amounts of mycolic acids available from slow growers such as *Mycobacterium tuberculosis*.[12] One approach is the combination of TLC and MALDI-TOFMS, whereby mycolic acids are separated into each sub-class by TLC, which is followed by the identification of molecular species and carbon-chain lengths by MALDI-TOFMS.[17,18] Laval *et al.*[12] have analyzed total fatty acid methyl ester fractions including mycolic acid methyl esters without prior TLC separation; this is a simple and rapid method[12] that enables the successful assignment of the major peaks corresponding to mycolic acids. In the direct measurement of mycolic acids by MALDI-TOFMS; however, consideration should be given to the resolving power of TOFMS instruments, because it is possible that several sub-classes of mycolic acids with 0.036 Da mass differences will be observed. These mass differences caused by the differences in elemental compositions between CO and C_2H_4 would be quite difficult to separate by using conventional TOFMS instruments.

This can be overcome using a new type of high-resolution TOFMS instrument with a spiral ion trajectory (spiral-TOFMS) in which the long flight path of the ions along the spiral ion trajectory of approximately 17 m (2.1 m × 8 turns) gives a high mass-resolving power of 80 000 at full width at half maximum for *m/z* 2564 of a peptide sample.[19] MALDI spiral-TOFMS, which has easily succeeded in separating adjacent peaks (Δ 0.036 Da) of copolymers up to *m/z* 3000[20] will be useful for structure assignment of individual mycolic acids.

In this study, high-resolution MS by means of MALDI spiral-TOFMS was applied to characterize mycolic acids from three type strains of validated species belonging to the genus *Dietzia* (*D. papillomatosis* 105045^T, *D. kunjamensis* NBRC 105042^T and *D. timorensis* NBRC 104184^T). Properties of mycolic acids have been described in terms of the proposal of new species, but only their presence[21,22] or at best the carbon-chain length[23] has been reported. Detailed structural characteristics of mycolic acids based on carbon-chain length, degree of unsaturation and/or cyclopropanation, and relative peak intensity observed by MALDI spiral-TOFMS, therefore, will aid in the chemotaxonomy of actinobacteria.

RESULTS AND DISCUSSION

Figure 1 shows the MALDI mass spectra of total fatty acid methyl ester fractions for *D. papillomatosis* (a), *D. kunjamensis* (b) and *D. timorensis* (c) in the range of *m/z* 500–670. Peak assignments were made by comparing the masses of mycolic acid methyl esters (Table 1). A peak resolution of 40 000–70 000 (full width at half maximum) was achieved, enabling the peak separation of components with mass differences of only 0.036 Da. The top panel of Figure 1a is an example of high-resolution peak separation: the oxygenated mycolic acid B(3) with C_{37} at *m/z* 615.533 was found to precede the major peak at *m/z* 615.569 for the α-type mycolic acid A(2) with C_{38}. Similarly, the peak observed at *m/z* 617.549 corresponding to the oxygenated mycolic acid B(2) with C_{37} could be clearly separated front the peak at *m/z* 617.585 for the α-type saturated mycolic acid A(1) with C_{38}. In this way, most of the observed peaks, including minor components, could be assigned as the respective types of mycolic acid methyl esters. Thanks to high-resolution MALDI spiral-TOFMS, the α-type mycolic acids and the oxygenated mycolic acids with very close mass values (ca. 0.036 Da), that is, [A(1) with C_{n+1}]/[B(2) with C_n] and [A(2) with C_{n+1}]]/[B(3) with C_n] could be discriminated without the need for pre-separation by, for example, TLC and HPLC.

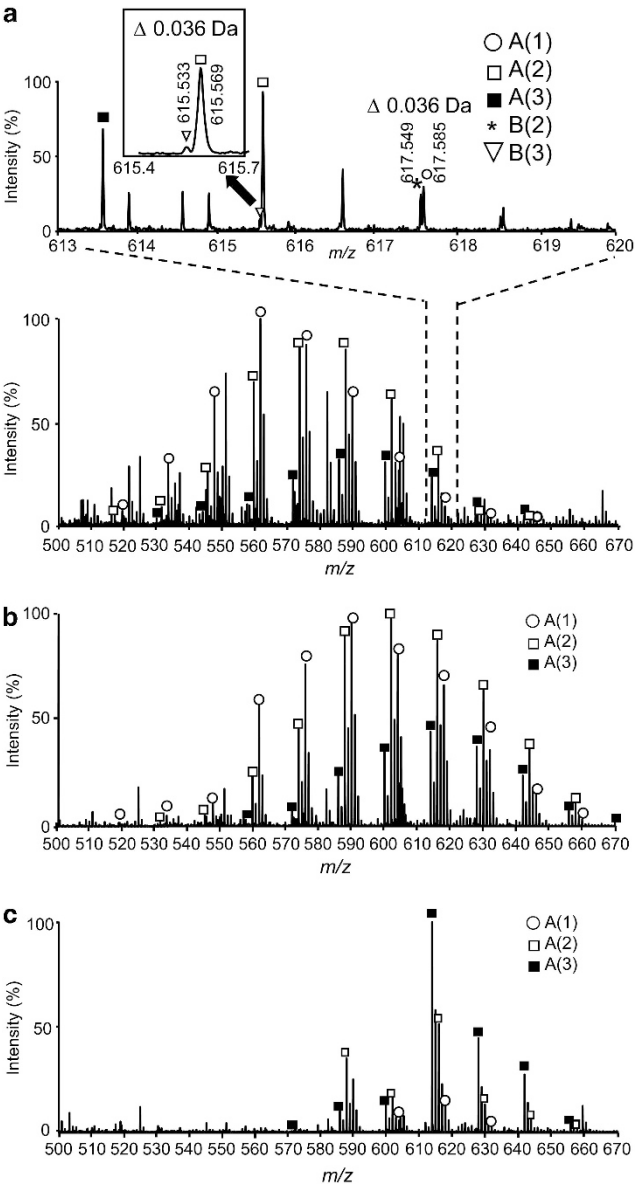

Figure 1 MALDI mass spectra of mycolic acid methyl esters of *D. papillomatosis* (**a**), *D. kunjamensis* (**b**) and *D. timorensis* (**c**). ○: α-Type saturated mycolic acid methyl esters [A(1)]. □: α-Type mycolic acid methyl esters whose IHDs were two [A(2)]. ■: α-Type mycolic acid methyl esters whose IHDs were three [A(3)]. *: Oxygenated mycolic acid, which corresponds to methoxy mycolic acid methyl ester [B(2)]. ▼: Oxygenated mycolic acid, which corresponds to keto, epoxy and/or ω-1 methoxy mycolic acid methyl ester [B(3)].

The components and the distributions of carbon-chain lengths of mycolic acid methyl esters differed for each species. The major types observed for both *D. papillomatosis* and *D. kunjamensis* were the α-type mycolic acids A(1) and A(2). However, the distribution of carbon-chain lengths observed for *D. kunjamensis* (Figure 1b) was slightly higher than that for *D. papillomatosis* (Figure 1a). The MALDI mass spectrum of *D. timorensis* (Figure 1c) was somewhat different from the previous two. The notable components were the α-type mycolic acids A(3), whose index of hydrogen deficiency (IHD)

Table 1 Representative structures of mycolic acid methyl esters

Category	*Structure*[a,b] *(methyl ester form)*	*Sub-class name*[c]
α-type mycolic acids		
A(1)	$CH_3\text{-}(CH_2)_m\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_n\text{-}CH_3$	saturated mycolic acids
A(2)	$CH_3\text{-}(CH_2)_m\text{-}CH{=}CH\text{-}(CH_2)_n\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_o\text{-}CH_3$	α-mycolic acids
A(3)	$CH_3\text{-}(CH_2)_m\text{-}A\text{-}(CH_2)_n\text{-}A\text{-}(CH_2)_o\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_p\text{-}CH_3$	α-mycolic acids with two cyclopropane rings and/or double bonds
Oxygenated mycolic acids		
B(2)	$CH_3\text{-}(CH_2)_m\text{-}CH(CH_3OCH_3)\text{-}CH\text{-}(CH_2)_n\text{-}A\text{-}(CH_2)_o\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_p\text{-}CH_3$	methoxy mycolic acids
B(3)	$CH_3\text{-}(CH_2)_m\text{-}CH(CH_3C{=}O)\text{-}CH\text{-}(CH_2)_n\text{-}A\text{-}(CH_2)_o\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_p\text{-}CH_3$	keto mycolic acids
	$CH_3\text{-}(CH_2)_m\text{-}CH(CH_3)\text{-}CH(O)\text{-}CH\text{-}(CH_2)_n\text{-}A\text{-}(CH_2)_o\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_p\text{-}CH_3$	epoxy mycolic acids
	$OH\text{-}C(=O)\text{-}(CH_2)_m\text{-}A\text{-}(CH_2)_n\text{-}CH(OH)\text{-}CH(COOCH_3)\text{-}(CH_2)_o\text{-}CH_3$	ω-1 methoxy mycolic acids

[a]A: C_2H_2 or C_3H_4.
[b]In the structures, m, n, o, and p are arbitrary integer values.
[c]Sub-class names are referred to in Barry *et al*.[6]

was three; saturated mycolic acids A(1) appeared to be minor components.

The relative peak intensities of each mycolic acid methyl ester as a function of the carbon-chain length could be derived from Figure 1. Figure 2 compares the relative peak intensities for *D. papillomatosis* (a), *D. kunjamensis* (b) and *D. timorensis* (c). For *D. papillomatosis* and *D. kunjamensis*, the major components were A(1) and A(2), and the peak intensities with even-numbered carbon-chain lengths did not differ from those with odd numbers. On the other hand, for *D. timorensis*, peak intensities with even-numbered carbon-chain lengths were markedly stronger than those with odd numbers. Although the pathways of odd-numbered mycolic acid biosynthesis are unknown, *D. maris* is characterized by even- and odd-numbered carbon-chain mycolic acid contents that are almost the same.[4] In this study, the contents of even- and odd-numbered carbon-chain mycolic acids of *D. papillomatosis* and *D. kunjamensis* were almost equal, suggesting that these sample strains have odd-numbered mycolic acid biosynthesis pathways that are the same as those of *D. maris*. From the dendrogram derived from 16S rRNA gene sequences, it is apparent that *D. timorensis*, which has a mycolic acid profile distinct from those of other *Dietzia* strains, forms the outermost phylogenetic lineage.[22] In the genus *Dietzia*, it seems that there is variation in the contents of even- and odd-numbered carbon-chain mycolic acids.

The distributions of carbon-chain lengths, the average numbers of carbon-chain lengths, and the distributions of HIDs are summarized in Table 2. The observed carbon-chain lengths for all samples were distributed mainly in the range of C_{30}–C_{42}, with the maxima at C_{35} for *D. papillomatosis*, C_{36} for *D. kunjamensis* and C_{38} for *D. timorensis*. The mycolic-acid-containing genera can be distinguished by the carbon-chain lengths of their mycolic acids,[10,24] which are, for *Corynebacterium*, C_{20}–C_{38}; *Dietzia*, C_{34}–C_{38}; *Rhodococcus*, C_{34}–C_{52}; *Nocardia*, C_{40}–C_{60}; *Gordonia*, C_{48}–C_{66}; *Tsukamurella*, C_{64}–C_{78}; and *Mycobacterium*, C_{60}–C_{90}. The carbon-chain lengths of mycolic acids of *Dietzia* species have also been characterized, by MS, as follows: *D. maris*, C_{30}–C_{38},[4] C_{33}–C_{38}[25] and C_{34}–C_{38};[5] *D. psychralcaliphila*, C_{34}–C_{39};[25] *D. natronolimnaea*, C_{34}–C_{38};[25] and *D. kunjamensis*, C_{33}–C_{40}.[23] The average numbers of carbon-chain length for samples were about 37: that is, 35.4 for *D. papillomatosis*, 36.9 for *D. kunjamensis* and 38.0 for *D. timorensis*. The results observed for the three type strains of *Dietzia* used here were in good agreement with the distributions observed for the genus *Dietzia*.

The IHDs were 1–3 in the three sample strains. However, there were differences in the distributions of IHD, as calculated from the relative peak intensities: for IHD = 1; 15–42%, IHD = 2; 31–46% and IHD = 3; 14–54%. The distributions of IHD for *D. papillomatosis* and *D. kunjamensis* showed almost the same pattern: IHDs of the major mycolic acids were 1–2. On the other hand, *D. timorensis* had a unique distribution of IHDs: IHDs of the major mycolic acids were 2–3. The distribution of double bonds for *D. maris* has been analyzed by using GC/MS; the content of saturated mycolic acids is 98%.[4]

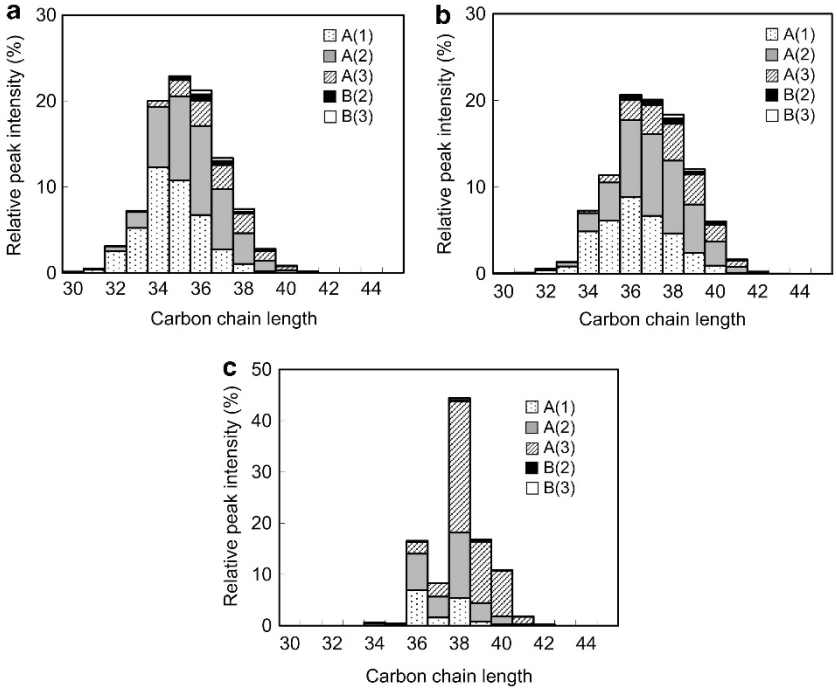

Figure 2 Relative peak intensities and distributions of carbon-chain length of mycolic acid methyl esters extracted from *D. papillomatosis* (**a**), *D. kunjamensis* (**b**) and *D. timorensis* (**c**).

Table 2 Profiles of mycolic acids of *Dietzia*

			IHD dependency of relative peak intensity		
Sample strain	CCL[a]	AvCCL[b]	1	2	3
D. papillomatosis	30–42	35.4	42	44	14
D. kunjamensis	31–42	36.9	36	46	18
D. timorensis	34–42	38.0	15	31	54

Abbreviations, AvCCL, average number of carbon-chain lengths; CCL, carbon-chain lengths; IHD, index of hydrogen deficiency; RPI, relative peak intensity.
[a]Carbon-chain length.
[b]AAvCCL obtained by summing the multiplication product of the CCL and the RPI of each molecular species: AvCCL $=\sum$(CCL $\times$ RPI/100).

For the genus *Rhodococcus*, the reported distributions of double bonds analyzed by GC/MS were in the range from 1–99%.[4] The diversity in the IHDs in mycolic acids would be revealed by further analysis of mycolic acids from another species belonging to the genus *Dietzia*.

In conclusion, mycolic acids from *D. papillomatosis*, *D. kunjamensis* and *D. timorensis* were characterized on the basis of carbon-chain length, IHDs and relative peak intensity by an analysis of total fatty acid methyl ester fractions using MALDI spiral-TOFMS. Mycolic acids from the three type strains were classified into two types on the basis of the distributions of IHDs and the content of mycolic acids with odd-numbered carbon chains. Additionally, owing to the high mass-resolving power, each type of mycolic acid could be discriminated without the need for prior separation by TLC or HPLC. Although this method cannot distinguish structural isomers with the same molecular formulas, it is overwhelmingly simple and rapid, although further analyses of mycolic acids from other bacteria are required to verify the results obtained by our proposed method. An exhaustive characterization of mycolic acids would reveal the chemotaxonomic diversity of the genus *Dietzia*.

MATERIALS AND METHODS

Cell culture and preparation of mycolic acid methyl esters

D. papillomatosis NBRC 105045^{T},[21] *D. kunjamensis* NBRC 105042^{T},[23] and *D. timorensis* NBRC 104184^{T},[22] used in this study were supplied by the National Institute of Technology and Evaluation (NITE) Biological Resource Center (NBRC, Kisarazu, Japan). Each experimental culture was grown at 30 °C until the late logarithmic stage on a shaker in Luria–Bertani medium.

Total fatty acid methyl ester fractions containing mycolic acid methyl esters were prepared by saponification with a 10% KOH aqueous solution for 2 h at 100 °C, followed by methyl esterification with methanol-toluene-sulfuric acid (25:25:1) for 3 h at 100 °C.[26]

MALDI spiral-TOFMS Sample preparation for MS followed the method of Laval *et al.*[12] Three types of solution were prepared: (1) a sample solution of each species at a concentration of about 1 mg ml^{-1} in tetrahydrofuran; (2) a 2,5-dihydroxybenzoic acid solution of 10 mg ml^{-1} in tetrahydrofuran; and (3) a sodium iodide (NaI) solution of 1 mg ml^{-1} in tetrahydrofuran. First, 1 µl of NaI solution was dropped onto a MALDI target plate and dried to form a cationization salt layer. Then, the same amount of sample solution was dropped onto the spot pre-coated with NaI and dried. Finally, as the matrix for sample ionization, 1 µl of 2,5-dihydroxybenzoic acid solution was overcoated at the same spot.

MALDI-TOF mass spectra were collected on a JMS-S3000 MALDI spiral-TOFMS[19] (JEOL, Tokyo, Japan) with a flight length of ca. 17 m and eight cycles of the spiral trajectory to achieve high mass-resolving power. Ions generated by irradiation with a 349-nm Nd:YLF laser were accelerated at 20 kV. The settings of delay time and grid voltage were optimized to stay constant at $\Delta M = 0.02$–0.03 Da over the range of *m/z* 450–1000. Mass calibration was made with a poly(methyl methacrylate) standard (peak-top MW, $M_p = 625$) purchased from Polymer Laboratories (Church Stretton, UK). Three

mass spectra for each sample were collected and the mass spectral data were processed with Polymerix software (Sierra Analytics, Modesto, CA, USA).

Notation for mycolic acid methyl esters

Typical types and chemical structures of methyl ester forms of representative mycolic acids are summarized in Table 1. In this table, 'α-type' mycolic acids,[7] the aliphatic chain of which is composed only of hydrocarbon, are indicated as A(x), where x corresponds to the IHD reflecting the numbers of double bonds and/or cyclopropane rings. Here, MS cannot discriminate between double bonds and cyclopropane rings because of their same mass. The other type are termed 'oxygenated' mycolic acids;[7] in these, the main chain contains oxygen existing as methoxy, keto and/or epoxy forms together with double bonds. The oxygenated mycolic acids can be divided into two categories: methoxy mycolic acids, denoted as B(2), and others, summarized as B(3); the latter are structural isomers that have the same molecular formula.

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1 Chun, J., Blackall, L. L., Kang, S. O., Hah, Y. C. & Goodfellow, M. A proposal to reclassify *Nocardia pinensis* Blackall et al. as *Skermania piniformis* gen. nov. comb. nov. *Int. J. Syst. Bacteriol.* **47,** 127–131 (1997).
2 De Briel, D., Couderc, F., Riegel, P., Jehl, F. & Minck, R. High-performance liquid chromatography of corynomycolic acids as a tool in identification of *Corynebacterium* species and related organisms. *J. Clin. Microbiol.* **30,** 1407–1417 (1992).
3 Nishiuchi, Y., Baba, T., Hotta, H. H. & Yano, I. Mycolic acid analysis in *Nocardia* species. The mycolic acid compositions of *Nocardia asteroides*, *N. farcinica* and *N. nova*. *J. Microbiol. Methods* **37,** 111–122 (1999).
4 Nishiuchi, Y., Baba, T. & Yano, I. Mycolic acids from *Rhodococcus*, *Gordonia* and *Dietzia*. *J. Microbiol. Methods* **40,** 1–9 (2000).
5 Rainey, F. A., Klatte, S., Kroppenstedt, R. M. & Stackebrandt, E. *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris*. *Int. J. Syst. Bacteriol.* **45,** 32–36 (1995).
6 Barry, C. E. III *et al.* Mycolic acids: structure, biosynthesis and physiological functions. *Prog. Lipid Res.* **37,** 143–179 (1998).
7 Minnikin, D. E. *et al.* Mycolic acid patterns of four vaccine strains of *Mycobacterium bovis* BCG. *J. Gen. Microbiol.* **129,** 889–891 (1983).
8 Daffé, M., Lanéelle, M., Asselineau, C., Lévy-Frébault, V. & David, H. Taxonomic value of mycobacterial fatty acids: proposal for a method of analysis. *Ann. Microbiol.* **134B,** 241–256 (1983).
9 Goodfellow, M. *et al.* Numerical and chemical classification of *Nocardia amarae*. *J. Gen. Microbiol.* **128,** 1283–1297 (1982).
10 Butler, W. R. & Guthertz, L. S. Mycolic acid analysis by high-performance liquid chromatography for identification of *Mycobacterium* species. *Clin. Microbiol. Rev.* **14,** 704–726 (2001).
11 Lay, J. O. Jr. MALDI-TOF mass spectrometry of bacteria. *Mass Spectrom. Rev.* **20,** 172–194 (2001).
12 Laval, F., Laneelle, M. A., Deon, C., Monsarrat, B. & Daffe, M. Accurate molecular mass determination of mycolic acids by MALDI-TOF mass spectrometry. *Anal. Chem.* **73,** 4537–4544 (2001).
13 Flores-Valdez, M. A., Morris, R. P., Laval, F., Daffe, M. & Schoolnik, G. K. *Mycobacterium tuberculosis* modulates its cell surface via an oligopeptide permease (Opp) transport system. *FASEB J* **23,** 4091–4104 (2009).
14 Fujita, Y., Naka, T., Doi, T. & Yano, I. Direct molecular mass determination of trehalose monomycolate from 11 species of mycobacteria by MALDI-TOF mass spectrometry. *Microbiology.* **151,** 1443–1452 (2005).
15 Uenishi, Y., Fujita, Y., Kusunose, N., Yano, I. & Sunagawa, M. Comprehensive analysis of mycolic acid subclass and molecular species composition of *Mycobacterium bovis* BCG Tokyo 172 cell wall skeleton (SMP-105). *J. Microbiol. Methods* **72,** 149–156 (2008).
16 Gao, L. Y. *et al.* Requirement for kasB in *Mycobacterium* mycolic acid biosynthesis, cell wall impermeability and intracellular survival: implications for therapy. *Mol. Microbiol.* **49,** 1547–1563 (2003).
17 Bhatt, A. *et al.* Deletion of kasB in Mycobacterium tuberculosis causes loss of acid-fastness and subclinical latent tuberculosis in immunocompetent mice. *Proc. Natl Acad. Sci. USA* **104,** 5157–5162 (2007).
18 Naka, T. *et al.* Lipid phenotype of two distinct subpopulations of *Mycobacterium bovis* Bacillus Calmette-Guerin Tokyo 172 substrain. *J. Biol. Chem.* **286,** 44153–44161 (2011).
19 Satoh, T., Sato, T. & Tamura, J. Development of a high-performance MALDI-TOF mass spectrometer utilizing a spiral ion trajectory. *J. Am. Soc. Mass Spectrom.* **18,** 1318–1323 (2007).
20 Sato, H., Ishii, Y., Momose, H., Sato, T. & Teramoto, K. Application of high-resolution MALDI-TOFMS with a spiral ion trajectory for the structural characterization of free radical polymerized methacrylate ester copolymers. *Mass Spectrom.* **2,** A0014 (2013).
21 Jones, A. L., Koerner, R. J., Natarajan, S., Perry, J. D. & Goodfellow, M. *Dietzia papillomatosis* sp. nov., a novel actinomycete isolated from the skin of an immuno-competent patient with confluent and reticulated papillomatosis. *Int. J. Syst. Evol. Microbiol.* **58,** 68–72 (2008).
22 Yamamura, H. *et al. Dietzia timorensis* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* **60,** 451–454 (2010).
23 Mayilraj, S., Suresh, K., Kroppenstedt, R. M. & Saini, H. S. *Dietzia kunjamensis* sp. nov., isolated from the Indian Himalayas. *Int. J. Syst. Evol. Microbiol.* **56,** 1667–1671 (2006).
24 Kampfer, P., Andersson, M. A., Rainey, F. A., Kroppenstedt, R. M. & Salkinoja-Salonen, M. *Williamsia muralis* gen. nov., sp. nov., isolated from the indoor environment of a children's day care centre. *Int. J. Syst. Bacteriol.* **49** (Pt 2), 681–687 (1999).
25 Yumoto, I *et al. Dietzia psychralcaliphila* sp. nov., a novel, facultatively psychrophilic alkaliphile that grows on hydrocarbons. *Int. J. Syst. Evol. Microbiol.* **52,** 85–90 (2002).
26 Yano, I., Saito, K., Furukawa, Y. & Kusunose, M. Structural analysis of molecular species of nocardomycolic acids from *Nocardia erythropolis* by the combined system of gas chromatography and mass spectrometry. *FEBS Lett.* **21,** 215–219 (1972).