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Pseudonocardia cypriaca sp. nov., *Pseudonocardia salamisensis* sp. nov., *Pseudonocardia hierapolitana* sp. nov. and *Pseudonocardia kujensis* sp. nov., isolated from soil

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The taxonomic positions of four novel actinomycetes isolated from soil samples, designated KT2142^T, PM2084^T, K236^T and A4038^T, were established by using a polyphasic approach. The organisms had chemical and morphological features that were consistent with their classification in the genus *Pseudonocardia*. Whole-cell hydrolysates of the four strains contained *meso*-diaminopimelic acid and arabinose and galactose as the diagnostic sugars (cell-wall type IV). Their predominant menaquinone was found to be MK-8(H_4). The major fatty acid was iso-$C_{16:0}$. 16S rRNA gene sequence data supported the classification of the isolates in the genus *Pseudonocardia* and showed that they formed four distinct branches within the genus. DNA–DNA relatedness studies between the isolates and their phylogenetic neighbours showed that they belonged to distinct genomic species. The four isolates were readily distinguished from one another and from the type strains of species classified in the genus *Pseudonocardia* based on a combination of phenotypic and genotypic properties. In conclusion, it is proposed that the four isolates be classified in four novel species of the genus *Pseudonocardia*, for which the names *Pseudonocardia cypriaca* sp. nov. (type strain KT2142^T=KCTC 29067^T=DSM 45511^T=NRRL B-24882^T), *Pseudonocardia hierapolitana* sp. nov. (type strain PM2084^T=KCTC 29068^T=DSM 45671^T=NRRL B-24879^T), *Pseudonocardia salamisensis* sp. nov. (type strain K236^T=KCTC 29100^T=DSM 45717^T) and *Pseudonocardia kujensis* sp. nov. (type strain A4038^T=KCTC 29062^T=DSM 45670^T=NRRL B-24890^T) are proposed.

The genus *Pseudonocardia* was originally proposed by Henssen (1957) for mycolateless nocardioform actinomycetes that possessed type IV cell walls with *meso*-diaminopimelic acid and arabinose and galactose as characteristic sugars (Lechevalier & Lechevalier, 1980). The description of the genus has been emended based on chemotaxonomic and morphological variations observed for subsequently described species of the genus (Warwick *et al.*, 1994; Reichert *et al.*, 1998; Huang *et al.*, 2002; Park *et al.*, 2008). The predominant menaquinone is MK-8(H_4), iso-branched hexadecanoic acid is the major fatty acid, the phospholipid pattern is type PII or PIII, with either phosphatidylethanolamine or phosphatidylcholine as diagnostic polar lipids (Lechevalier *et al.*, 1981), and strains exhibit a high DNA G+C content. The members of the genus constitute a distinct, albeit heterogeneous, clade in the 16S rRNA gene tree of the family *Pseudonocardiaceae*,

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Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethanolamine.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KT2142^T, K236^T, PM2084^T and A4038^T are respectively HQ157191, JQ864427, JN989288 and JN989287.

Eleven supplementary figures and three supplementary tables are available with the online version of this paper.

and can be distinguished from other genera classified in this family by using a combination of chemotaxonomic and morphological properties (Labeda *et al.*, 2011; Huang & Goodfellow, 2012).

At the time of writing, the genus encompassed 48 recognized species (http://www.bacterio.net/pseudonocardia.html), most of which have been described in the last decade. Novel species of the genus *Pseudonocardia* have been isolated from diverse environments such as active sludge soils, including those polluted by chemical compounds (Lee *et al.*, 2004; Kämpfer & Kroppenstedt, 2004; Mahendra & Alvarez-Cohen, 2005; Liu *et al.*, 2006; Kämpfer *et al.*, 2006; Park *et al.*, 2008), wastewater activated sludge (Cuesta *et al.*, 2013), plant samples (Evtushenko *et al.*, 1989; Reichert *et al.*, 1998; Gu *et al.*, 2006; Chen *et al.*, 2009; Sakiyama *et al.*, 2010; Kaewkla & Franco, 2010, 2011; Qin *et al.*, 2010; Zhao *et al.*, 2011a, b, c), soil (Lee *et al.*, 2002; Park *et al.*, 2008; Qin *et al.*, 2008; Li *et al.*, 2010; Ara *et al.*, 2011) and deep-sea sediments (Tian *et al.*, 2013).

In the present study, four *Pseudonocardia*-like strains, designated KT2142^T, K236^T, PM2084^T and A4038^T, were isolated and presumptively assigned to the genus *Pseudonocardia*. The aim of the present study was to establish the taxonomic position of these organisms, and the results indicate that the strains represent four novel species.

Strains KT2142^T and PM2084^T were isolated in Stevenson's medium no. 2 (Tan *et al.*, 2006) supplemented with filter-sterilized cycloheximide (50 μg ml^{-1}), nystatin (50 μg ml^{-1}), neomycin sulphate (4 μg ml^{-1}) and (+)-melezitose (1 %, w/v) from soil samples collected from Karpaz, Magusa, Northern Cyprus, and Pamukkale, Denizli, Turkey, respectively. Strain A4038^T was isolated from a soil sample collected from Kuje, Abuja, Nigeria, by using SM3 medium (Tan *et al.*, 2006) supplemented with filter-sterilized rifampicin (5 μg ml^{-1}), nalidixic acid (10 μg ml^{-1}) and novobiocin (10 μg ml^{-1}). Strain K236^T was isolated from a soil sample collected from Karpaz, Magusa, Northern Cyprus, by using humic acid-vitamin agar (Hayakawa & Nonomura, 1987) supplemented with filter-sterilized cycloheximide (50 μg ml^{-1}) and nalidixic acid (10 μg ml^{-1}). Isolation plates were incubated at 28 °C for 21 days. The strains were maintained on yeast extract-malt extract (ISP 2 medium; Shirling & Gottlieb, 1966) agar slopes at room temperature and as glycerol suspensions (20 %, v/v) at −20 °C.

Genomic DNA extraction, PCR-mediated amplification and sequencing of the 16S rRNA gene were performed as described by Chun & Goodfellow (1995), using an ABI PRISM 3730 XL automatic sequencer. The resultant 16S rRNA gene sequences were aligned with corresponding sequences of representatives of the genus *Pseudonocardia* (retrieved from the EzTaxon-e server; Kim *et al.*, 2012) by using CLUSTAL W in MEGA version 5 (Tamura *et al.*, 2011). Phylogenetic analysis was carried out by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. The software MEGA version 5.0 (Tamura *et al.*, 2011) was used for phylogenetic tree reconstruction with the neighbour-joining, maximum-likelihood and maximum-parsimony tree-making algorithms. Evolutionary distances were calculated using the model of Jukes & Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Almost-complete 16S rRNA gene sequences of strains KT2142^T (1469 bp), PM2084^T (1458 bp), A4038^T (1465 bp) and K236^T (1465 bp) were determined. These sequences were analysed by preliminary comparison with sequences in the GenBank database and the results indicated that the four isolates were most closely related to members of the genus *Pseudonocardia*. Strains KT2142^T and PM2084^T formed a subclade supported by all of the tree-making algorithms and a bootstrap value of 71 % (Fig. 1 and Figs S1–S3, available in the online Supplementary Material). These two novel strains share 99.18 % 16S rRNA gene sequence similarity and share relatively low sequence similarities with strains K236^T (96.22–96.98 %) and A4038^T (95.26–95.19 %). 16S rRNA gene sequence similarity calculations indicated that the closest relatives of strain KT2142^T were *Pseudonocardia zijingensis* 6330^T (99.15 %; 12 nt differences at 1417 sites), *P. adelaidensis* EUM 221^T (99.01 %; 14/1410), *P. kunmingensis* YIM 63158^T (98.20 %; 25/1387) and *P. sichuanensis* KLBMP 1115^T (98.02 %; 29/1468). Strain PM2084^T showed the highest 16S rRNA gene sequence similarities to *P. zijingensis* 6330^T (99.01 %; 14/1419), *P. adelaidensis* EUM 221^T (98.73 %; 18/1412), *P. aurantiaca* DSM 44773^T (98.48 %; 22/1450), *P. kunmingensis* YIM 63158^T (98.20 %; 25/1388) and *P. sichuanensis* KLBMP 1115^T (98.08 %; 28/1456). It is evident from Fig. 1 that strains K236^T and A4038^T formed two distinct clades in the 16S rRNA gene tree of the genus *Pseudonocardia*, with the most closely related type strains being *Pseudonocardia alaniniphila* YIM 16303^T (98.36 %; 24/1464) and *P. yunnanensis* IFO 15681^T (98.22 %; 26/1457), and *P. halophobica* DSM 43089^T (98.54 %; 21/1435) and *P. yuanmonensis* YIM 75926^T (98.16 %; 27/1465), respectively. Lower sequence similarities were found to other species of the genus *Pseudonocardia* with validly published names.

DNA–DNA relatedness was determined between strains KT2142^T and PM2084^T and the closest related type strains *P. adelaidensis* DSM 45352^T and *P. zijingensis* DSM 44774^T; between strain K236^T and the closest related type strains *P. alaniniphila* DSM 44660^T and *P. yunnanensis* DSM 44253^T; and between strain A4038^T and the closest related type strains *P. halophobica* DSM 43089^T and *P. yuanmonensis* DSM 45676^T. Genomic DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huss *et al.* (1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

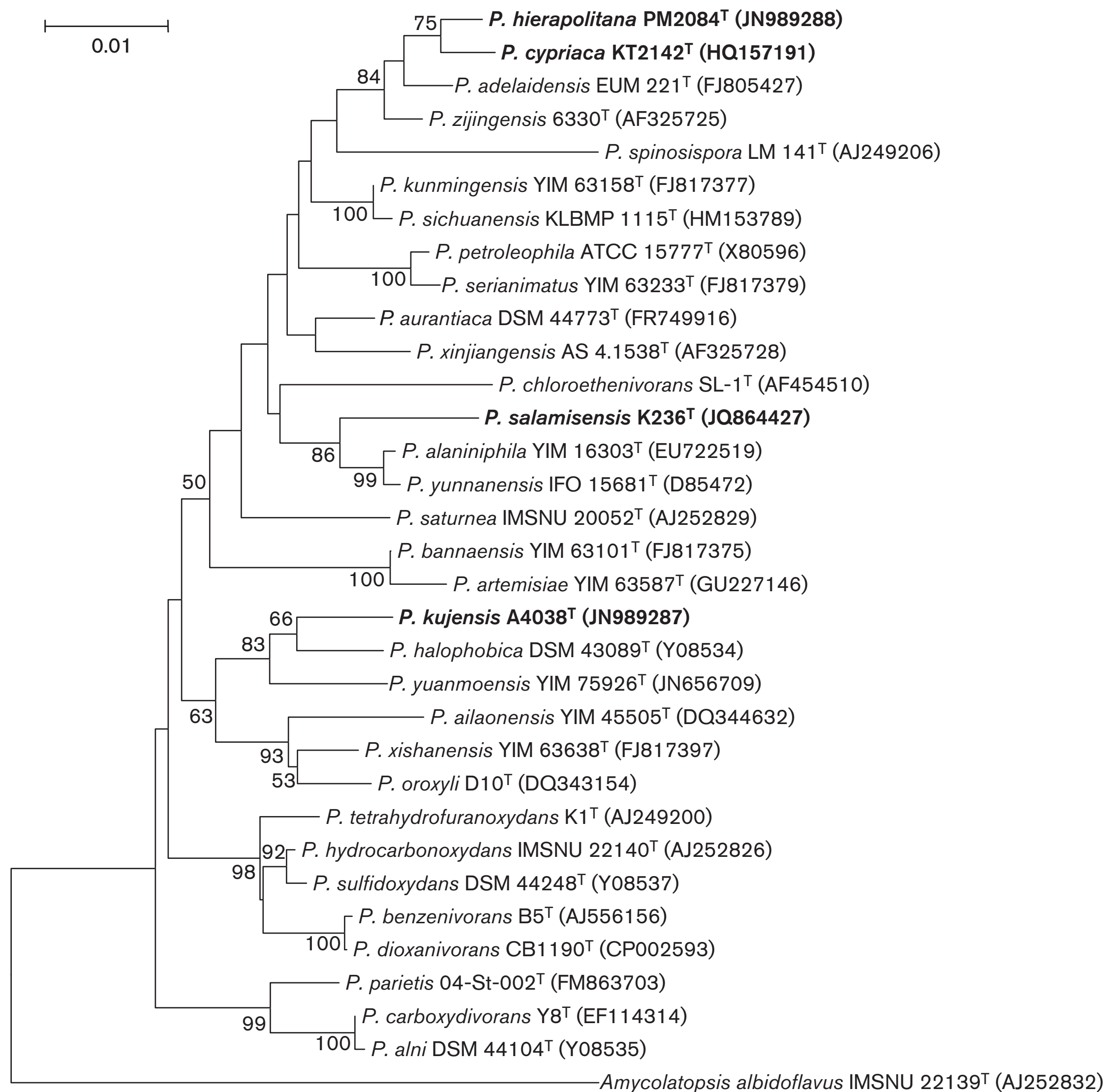

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the positions of strains PM2084^T, KT2142^T, K236^T and A4038^T amongst their phylogenetic neighbours. *Amycolatopsis albidoflavus* IMSNU 22139^T was used as an outgroup. Numbers at nodes indicate levels of bootstrap support (%); only values ≥50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per site.

The taxonomic integrity of the test strains were supported by DNA–DNA relatedness data. Strain KT2142^T showed DNA–DNA relatedness of 18.8 ± 1.0 % to *P. adelaidensis* DSM 45352^T, 11.2 ± 4.2 % to *P. zijingensis* DSM 44774^T and 19.2 ± 4.2 % to PM2084^T, while strain PM2084^T showed DNA–DNA relatedness of 36.2 ± 6.1 % to *P. adelaidensis* DSM 45352^T and 28.8 ± 7.2 % to *P. zijingensis* DSM 44774^T. Strain K236^T showed DNA–DNA relatedness of 43.6 ± 7.3 and 39.4 ± 4.1 % to *P. yunnanensis* DSM 44253^T and *P. alaniniphila* DSM 44660^T, respectively, and strain A4038^T showed DNA–DNA relatedness of 37.4 ± 8.4 and 40.0 ± 6.2 % to *P. halophobica* DSM 43089^T and *P. yuanmonensis* DSM 45676^T, respectively. These values are well below the 70 % cut-off point recommended for recognition of genomic species (Wayne *et al.*, 1987), indicating that strains KT2142^T, K236^T, PM2084^T and A4036^T should be considered to represent different genomic species of the genus *Pseudonocardia*.

Biomass for chemotaxonomic studies was prepared by growing strains K236^T, KT2142^T, PM2084^T and A4038^T in ISP 2 broth at 160 r.p.m. for 18 days at 28 °C; cells were harvested by centrifugation, washed in distilled water, recentrifuged and freeze-dried. Whole-cell amino acids and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed by TLC (Staneck & Roberts, 1974). Polar lipids were extracted and analysed by the method of Minnikin *et al.* (1984) as modified by Kroppenstedt &

Goodfellow (2006). Isoprenoid quinones were extracted and purified using the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1982). Mycolic acids were extracted and analysed by the method of Minnikin *et al.* (1980). For extraction and analysis of cellular fatty acids, the physiological age of each strain was standardized by consistently choosing the last quadrant streaked on ISP 2 agar plates incubated at 28 °C for 4 days. Analysis was conducted using the Microbial Identification System (MIDI) Sherlock software version 4.5 (method TSBA40, TSBA6 database) as described by Sasser (1990). Fatty acid methyl ester peaks were analysed using software version TSBA 5.0. The DNA G+C contents of strains K236^T, KT2142^T, PM2084^T and A4038^T were determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

The cell walls of the four novel strains contained *meso*-diaminopimelic acid. Whole-cell hydrolysates of all four isolates contained arabinose and galactose as diagnostic sugars (type IV; Lechevalier & Lechevalier, 1970), and also glucose and ribose. MK-8(H_4) was the major respiratory quinone (>80.0 %) for all four isolates, and minor amounts of MK-9(H_4), MK-9(H_2), MK-8(H_2), MK-8(H_0), MK-7(H_4) and an unidentified component were also detected [the ratio of MK-8(H_4), MK-9(H_4), MK-9(H_2), MK-8(H_2), MK-8(H_0), MK-7(H_4) and the unidentified component was 85.4 : 0.3 : 1.2 : 2.5 : 2.8 : 1.7 : 5.6 for strain KT2142^T, 83.8 : 0.1 : 1.4 : 2.5 : 1.4 : 1.7 : 8.2 for strain A4038^T, 87.7 : 0.5 : 1.1 : 1.1 : 0.3 : 1.0 : 6.4 for strain K236^T and 80.3 : 0 : 0.5 : 3.3 : 0 : 1.7 : 13.7 for strain PM2084^T]. The polar lipid patterns of the strains were different. Strain KT2142^T contained diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylmethanolamine (PME) and an unknown phospholipid, while phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylinositol mannoside (PIM) were absent. Strain PM2084^T contained PI, PC, traces of PME and two unknown phospholipids; while DPG, PE, PG and PIM were absent. Strain K236^T contained DPG, PI, PC, PME, two unknown phospholipids and one unknown glycolipid, while PE, PG and PIM were absent. Strain A4038^T contained PE, PI and PME and traces of DPG and PC, one unknown phospholipid and one unknown glycolipid, while PG and PIM were absent (Figs S4–S7). Mycolic acids were not detected. The cellular fatty acid compositions of the strains were similar, although not identical: strains KT2142^T and A4038^T contained 10-methyl $C_{16:0}$ at 7.10 and 10.15 %, respectively, while strains K236^T and PM2084^T contained 9-methyl $C_{16:0}$ at 10.76 and 10.07 %, respectively. The major cellular fatty acid for all four strains was iso-$C_{16:0}$. The fatty acid compositions of isolates KT2142^T, PM2084^T, K236^T and A4038^T and related type strains are shown in Table S1. The G+C content of the genomic DNA was 74.6, 72.8, 73.2 and 71.9 mol% for strains KT2142^T, PM2084^T, K236^T and A4038^T, respectively.

Cultural characteristics were investigated on media from the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966), modified Bennett's agar (MBA; Jones, 1949), Czapek's agar and tryptic soy agar (TSA; Difco). The degree of growth, aerial mycelium and pigmentation were recorded after 21 days of incubation at 28 °C. The National Bureau of Standards colour name charts (Kelly, 1964) were used to determine colour designations and names. Colony morphology and micromorphological properties of strains KT2142^T, PM20824^T, K236^T and A4038^T were determined by examining gold-coated dehydrated specimens of 4–6 week cultures from ISP 3 medium using a JEOL JSM 6060 instrument. Growth at 4, 10, 20, 28, 37, 45 and 50 °C, at pH 4.0–12.0 (at intervals of 1.0 pH unit) and in the presence of 0–10 % (w/v) NaCl (at 1.0 % intervals) was determined on ISP 2. Established methods were used to determine whether the strains degraded Tweens 40 and 80 (Nash & Krent, 1991); the remaining degradation tests were carried out employing methods described by Williams *et al.* (1983). Carbon-source utilization was tested using carbon source utilization (ISP 9) medium (Shirling & Gottlieb, 1966) supplemented with final concentrations of 1 % (w/v) of the tested carbon sources. Nitrogen-source utilization was examined using the basal medium recommended by Williams *et al.* (1983) supplemented with final concentrations of 0.1 % (w/v) of the tested nitrogen sources. Activity of strains KT2142^T, PM2084^T, K236^T and A4038^T to inhibit the growth of 21 micro-organisms, including Gram-positive and Gram-negative bacteria and fungi, was observed using an overlay technique described by Williams *et al.* (1983). Spot-inoculated colonies on MBA plates were inverted over 2 ml chloroform for 40 min. Killed colonies were overlaid with 5–7 ml sloppy modified Bennett's broth inoculated with the test organisms. Zones of inhibition were scored as positive after 24 h at 37 °C. Except for antimicrobial activity tests, the type strains *P. adelaidensis* DSM 45352^T, *P. zijingensis* DSM 44774^T, *P. aurantiaca* DSM 44773^T, *P. yunnanensis* DSM 44253^T, *P. alaniniphila* DSM 44660^T, *P. yuanmonensis* DSM 45676^T and *P. halophobica* DSM 43092^T were included for comparison.

Strains KT2142^T, PM2084^T, A4038^T and K236^T had morphological properties consistent with their classification in the genus *Pseudonocardia*. The organisms formed an extensively branched substrate mycelium and aerial mycelium that fragmented into smooth or spiny, rod-shaped spores (Figs S8–S11). No diffusible pigment was detected on any tested medium. Melanoid pigments were not produced on ISP 6 or ISP 7 medium. Physiological and biochemical properties are given in Tables 1 and S1–S3 and the species descriptions.

The genotypic and phenotypic data presented here show that the four isolates can be distinguished from one another and from the type strains of species previously classified in the genus *Pseudonocardia*. Therefore, it is concluded that strains KT2142^T, PM2084^T, K236^T and A4038^T represent four novel species within the genus *Pseudonocardia*, for which the names *Pseudonocardia cypriaca* sp. nov., *Pseudonocardia salamisensis* sp. nov.,

Pseudonocardia hierapolitana sp. nov. and *Pseudonocardia kujensis* sp. nov. are proposed.

Description of *Pseudonocardia cypriaca* sp. nov.

Pseudonocardia cypriaca (cy.pri.a′ca. L. fem. adj. *cypriaca* from Cyprus, source of the type strain).

Aerobic, Gram-reaction-positive, non-motile, non-acid–alcohol-fast actinomycete that forms extensively branched substrate mycelium and aerial mycelium that differentiates into spiny-surfaced coccoid or rod-like elements. Grows well on ISP 2 and moderately well on ISP 4, ISP 5, nutrient agar, MBA and Czapek's agar, but poorly on ISP 3, ISP 6, ISP 7 and TSA. Aerial mycelium is formed rarely on ISP 4, but does not appear on the remaining tested media. Growth occurs at pH 4.0–10 and at 28–45 °C, but not at pH 11 or 12 or at 4, 10, 20 or 50 °C. Optimum growth occurs on ISP 2 at 28 °C and pH 7.2. Arbutin, allantoin and urea are hydrolysed. Nitrate reduction is positive. Tweens 40 and 80 are degraded but adenine, casein, elastin, guanine, hypoxanthine, xanthine and xylan are not. Utilizes starch and succinic acid as sole carbon sources, but not D- or L-arabinose, cellobiose, D-fructose, D-galactose, D-mannose, D-mannitol, melezitose, D-ribose, L-rhamnose, adonitol, dextrin, inulin, lactose, maltose, sucrose, xylose, *myo*-inositol, D-sorbitol, xylitol, L-glutamic acid or L-sorbose. Utilizes DL-phenylalanine, glycine, L-methionine, L-threonine and L-serine as sole nitrogen sources, but not α-isoleucine, L-alanine, L-arginine, L-cysteine, L-histidine, L-hydroxyproline, L-phenylalanine, L-proline, L-tyrosine or L-valine. The cell-wall chemotype is type IV. The predominant menaquinone of the type strain is MK-8(H_4). The polar lipid profile contains DPG, PI, PC, PME and one unknown phospholipid. The major cellular fatty acids are iso-$C_{16:0}$, $C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and 10-methyl $C_{16:0}$.

The type strain, KT2142^T (=KCTC 29067^T=DSM 45511^T= NRRL B-24882^T), was isolated from soil collected from Karpaz, Magusa, Northern Cyprus. The G+C content of the genomic DNA of the type strain is 74.6 mol%.

Description of *Pseudonocardia hierapolitana* sp. nov.

Pseudonocardia hierapolitana (hi.e.ra.po.li.ta′na. L. fem. n. *hierapolitana* from the ancient city of Hierapolis, Pamukkale, Denizli, Turkey, from where the type strain was isolated).

Aerobic, Gram-stain-positive, non-motile, non-acid–alcohol-fast actinomycete that forms extensively branched substrate mycelium and aerial mycelium that differentiates into spiny-surfaced coccoid or rod-like elements. Good growth occurs on ISP 2, ISP 6, MBA, nutrient agar and TSA at 28 °C, and grows moderately well on ISP 3, 4 and 7; no growth is observed on ISP 5 or Czapek's agar. Forms pale orange–yellow to light yellowish-brown aerial mycelium on ISP 2, ISP 3 and ISP 4 agar and orange–yellow substrate mycelium on most tested media. Growth occurs at pH 4.0–10 and at 28–45 °C, but not at pH 11–12 or at 4, 10, 20 or 50 °C. Optimum growth occurs on ISP 2 agar at 28 °C and pH 7.2. Grows well in the presence of 0–3.0 % (w/v) NaCl. Arbutin and allantoin are hydrolysed but not urea. Nitrate reduction is positive. Hypoxanthine and Tweens 40 and 80 are degraded but adenine, casein, elastin, guanine, xanthine and xylan are not. Utilizes dextrin, cellobiose, D-fructose, D-galactose, D-mannose, D-ribose, D-mannitol, D-sorbitol, L-rhamnose, sucrose, xylose, xylitol, *myo*-inositol, starch and succinic acid as sole carbon sources, but not D- or L-arabinose, melezitose, lactose, maltose, adonitol, inulin, L-sorbose or L-glutamic acid. Most amino acids are utilized as sole nitrogen sources, but L-hydroxyproline is not. The cell-wall chemotype is type IV. The predominant menaquinone of the type strain is MK-8(H_4). The polar lipid profile contains PI, PC, PME, two unknown phospholipids and one unknown glycolipid. The major cellular fatty acids are iso-$C_{16:0}$, 9-methyl $C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and $C_{17:1}$ *cis*9.

The type strain, PM2084^T (=KCTC 29068^T=DSM 45671^T= NRRL B-24879^T), was isolated from soil collected from the ancient city of Hierapolis, Pamukkale, Denizli, Turkey. The G+C content of the genomic DNA of the type strain is 72.8 mol%.

Description of *Pseudonocardia salamisensis* sp. nov.

Pseudonocardia salamisensis (sa.la.mi.sen′sis. L. fem. adj. *salamisensis* from the ancient city of Salamis, Dipkarpaz, Magusa, Northern Cyprus, from where the type strain was isolated).

Aerobic, Gram-reaction-positive, non-motile, non-acid–alcohol-fast actinomycete that forms extensively branched substrate mycelium and aerial mycelium that differentiates into smooth-surfaced coccoid or rod-like elements. Grows moderately well on ISP 2, ISP 4, ISP 5 and ISP 7, MBA, nutrient agar and Czapek's agar; no growth detected on ISP 3, ISP 6 or TSA. White aerial mycelium is formed sparsely on ISP 4, but does not appear on the remaining tested media. Growth occurs at pH 7.0–8.0 and at 28 °C, but not at pH 4.0–6.0 or 9.0–12.0 or at 4, 10, 20, 37, 45 or 50 °C. Optimum growth occurs on ISP 2 at 28 °C and pH 7.2. Arbutin is hydrolysed and nitrate reduction is positive, but allantoin and urea are not hydrolysed. Tween 40 is degraded, but adenine, casein, elastin, guanine, hypoxanthine, Tween 80, xanthine and xylan are not. Most sugars are not utilized as sole carbon sources for growth, but starch is. Utilizes only L-arginine as sole nitrogen source, and not α-isoleucine, DL-phenylalanine, glycine, L-alanine, L-cysteine, L-histidine, L-hydroxyproline, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine or L-valine. The cell-wall chemotype is type IV. The predominant menaquinone of the type strain is MK-8(H_4). The polar lipid profile contains DPG, PI, PC, PME, two unknown phospholipids and one unknown glycolipid. The

Table 1. Phenotypic properties of strains PM2084^T, KT2142^T, K236^T and A4038^T and closely related type strains

Strains: 1, PM2084^T; 2, KT2142^T; 3, *P. adelaidensis* DSM 45352^T; 4, *P. zijingensis* DSM 44774^T; 5, *P. aurantiaca* DSM 44773^T; 6, K236^T; 7, *P. yunnanensis* DSM 44253^T; 8, *P. alaniniphila* DSM 44660^T; 9, A4038^T; 10, *P. halophobica* DSM 43092^T; 11, *P. yuanmonensis* DSM 45676^T. All strains grew at pH 8 and 28 °C and in the absence of NaCl. All strains were negative for degradation of adenine (0.5 %), casein (1 %), elastin (0.3 %), guanine (0.5 %) and xylan (0.4 %), the ability to grow with dextran as a sole carbon source (1.0 %) and growth at 4 °C. Glucose was used as a positive control. All data were obtained in this study. Carbon sources were tested at 1.0 % (w/v) unless indicated; nitrogen sources were tested at 0.1 % (w/v). +, Positive; W, weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Hydrolysis of:											
Arbutin	+	+	−	+	+	+	+	−	+	−	−
Allantoin	+	+	−	+	−	−	−	−	−	−	−
Urea	−	+	−	−	+	−	−	−	−	−	+
Nitrate reduction	+	+	+	+	−	+	+	+	+	+	+
Growth at/in:											
pH 4.0	+	+	−	−	−	−	+	+	+	+	+
pH 5.0, 6.0 and 10.0	+	+	+	+	−	−	+	+	+	+	+
pH 11.0	−	−	−	−	−	−	+	−	−	+	−
pH 12.0	−	−	−	−	−	−	+	−	−	−	−
10 °C	−	−	−	−	−	−	−	−	−	−	w
20 °C	−	−	+	+	−	−	+	−	+	+	+
37 and 45 °C	+	+	+	+	−	−	+	−	+	+	+
50 °C	−	−	−	−	−	−	+	−	−	−	−
1.0–3.0 % NaCl	+	−	+	+	−	−	+	−	+	+	+
4.0–5.0 % NaCl	−	−	+	+	−	−	+	−	+	+	+
6.0–7.0 % NaCl	−	−	−	−	−	−	+	−	−	+	−
8.0–10.0 % NaCl	−	−	−	−	−	−	−	−	−	+	−
Degradation of:											
Hypoxanthine (0.4 %)	+	−	+	+	+	−	+	+	+	+	+
Tween 40 (1 %)	+	+	+	+	−	+	+	+	+	+	+
Tween 80 (1 %)	+	+	+	+	−	−	+	+	−	+	+
Xanthine (0.4 %)	−	−	−	+	w	−	−	−	−	+	−
Use as a sole carbon source of:											
Adonitol	−	−	−	+	−	−	+	−	−	−	+
myo-Inositol	+	−	+	+	−	−	−	+	−	−	+
D-Arabinose	−	−	−	+	w	−	−	+	−	+	+
L-Arabinose	−	−	−	+	−	−	−	−	−	−	+
Cellobiose	+	−	−	+	+	−	+	+	+	+	−
D-Fructose	+	−	−	−	+	−	+	+	+	+	+
D-Sorbitol	+	−	+	+	−	−	−	−	+	+	+
D-Galactose	+	−	+	+	−	−	+	+	+	+	−
D-Mannose	+	−	+	+	+	−	w	+	+	+	−
Melezitose	−	−	−	+	−	−	−	−	−	−	+
D-Ribose	+	−	+	+	+	−	+	−	+	+	+
D-Mannitol	+	−	+	+	−	−	+	+	+	+	−
Dextrin	+	−	−	+	+	−	−	+	+	−	+
Inulin	−	−	−	+	−	−	w	−	−	−	−
L-Sorbose	−	−	−	+	−	−	−	−	+	+	+
L-Rhamnose	+	−	+	+	+	−	+	+	−	+	−
Lactose	−	−	−	+	−	−	−	−	−	−	+
L-Glutamic acid	−	−	−	−	−	−	+	−	+	+	−
Maltose	−	−	−	+	+	−	+	−	−	−	+
Starch	+	+	−	+	+	+	−	+	+	−	+
Sucrose	+	−	+	+	−	−	−	+	+	−	+
Xylitol	+	−	−	+	−	−	−	−	+	+	−
Xylose	+	−	+	+	−	−	+	+	+	+	+
Succinic acid (0.1 %)	+	+	−	+	−	−	+	+	+	+	−

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Use as a sole nitrogen source of:											
α-Isoleucine	+	−	−	−	−	−	−	+	+	+	+
DL-Phenylalanine	+	+	−	+	+	−	−	+	+	+	+
Glycine	+	+	−	+	+	−	+	+	+	+	+
L-Alanine	+	−	−	−	+	−	+	+	+	+	+
L-Arginine	+	−	−	−	+	+	+	+	+	+	+
L-Cysteine	+	−	−	−	−	−	+	+	+	+	+
L-Histidine	+	−	−	−	+	−	−	+	+	+	+
L-Hydroxyproline	−	−	−	−	−	−	−	+	+	+	−
L-Methionine	+	+	−	−	+	−	+	+	+	+	+
L-Phenylalanine	+	−	−	−	−	−	−	−	+	+	+
L-Proline	+	−	−	−	+	−	+	+	+	+	+
L-Serine	+	+	−	+	+	−	+	+	+	+	+
L-Threonine	+	+	−	−	+	−	+	+	+	+	+
L-Valine	+	−	−	−	+	−	−	−	+	+	+
L-Tyrosine	+	−	−	−	−	−	−	+	+	+	+

major cellular fatty acids are iso-$C_{16:0}$, iso-$C_{16:1}$ H, iso-$C_{15:0}$ and 9-methyl $C_{16:0}$.

The type strain, K236[T] (=KCTC 29100[T]=DSM 45717[T]), was isolated from soil collected from the ancient city of Salamis, Karpaz, Magusa, Northern Cyprus. The G+C content of the genomic DNA of the type strain is 73.2 mol%.

Description of *Pseudonocardia kujensis* sp. nov.

Pseudonocardia kujensis (ku.jen′sis. N.L. fem. adj. *kujensis* of or belonging to Kuje, Abuja, Nigeria, the source of the type strain).

Aerobic, Gram-reaction-positive, non-motile, non-acid–alcohol-fast actinomycete that forms extensively branched substrate mycelium and aerial mycelium that differentiates into smooth-surfaced coccoid or rod-like elements. Good growth occurs on ISP 2, ISP 6, ISP 7 and nutrient agar, moderate growth on MBA, ISP 3, ISP 4, ISP 5 and Czapek's agar at 28 °C. Aerial mycelium on agar is white, substrate mycelium is pale yellow to deep orange on tested media. No diffusible pigment is produced. Growth occurs at pH 4.0–10.0, at 20–45 °C and at 0–5.0 % (w/v) NaCl, but not at pH 11.0 or at 4, 10 or 50 °C. Optimum growth occurs on ISP 2 at 28 °C and pH 7.2. Arbutin is hydrolysed and nitrate reduction is positive, but allantoin and urea are not hydrolysed. Hypoxanthine and Tween 40 are degraded, but adenine, casein, elastin, guanine, Tween 80, xanthine and xylan are not. Utilizes cellobiose, D-fructose, D-sorbitol, D-galactose, D-mannose, D-mannitol, D-ribose, dextrin, L-glutamic acid, L-sorbose, starch, succinic acid, sucrose, xylitol and xylose as sole carbon sources, but not adonitol, D-arabinose, dextran, inulin, lactose, L-arabinose, maltose or *myo*-inositol. Utilizes all tested nitrogen sources. The cell-wall chemotype is type IV. The predominant menaquinone of the type strain is MK-8(H_4). The polar lipid profile contains PE, PI and PME and traces of DPG, PC, one unknown phospholipid and one unknown glycolipid. The major cellular fatty acids are iso-$C_{16:0}$, 10-methyl $C_{16:0}$, iso-$C_{15:0}$, $C_{16:1}$ *cis*9 and $C_{16:0}$.

The type strain, A4038[T] (=KCTC 29062[T]=DSM 45670[T]=NRRL B-24890[T]), was isolated from soil collected from Kuje, Abuja, Nigeria. The G+C content of the genomic DNA of the type strain is 71.9 mol%.

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