Yuhushiella deserti gen. nov., sp. nov., a new member of the suborder *Pseudonocardineae*

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A thermotolerant, Gram-stain-positive, aerobic, sporangium-forming actinomycete, strain RA45^T, was isolated from a desert region in Xinjiang Uigur Autonomous Region, north-western China. Comparative analysis of the 16S rRNA gene sequence and phenotypic characterization revealed that strain RA45^T belonged phylogenetically to the family *Pseudonocardiaceae* of the suborder *Pseudonocardineae*. Strain RA45^T showed more than 5% 16S rRNA gene sequence divergence from recognized species of genera in the family *Pseudonocardiaceae*, forming a distinct lineage within the evolutionary radiation occupied by the genera *Amycolatopsis*, *Prauserella*, *Thermocrispum*, *Saccharomonospora*, *Saccharopolyspora* and *Sciscionella*, but distinct from each of them. The affiliation to the family was supported by the presence of suborder- and family-specific 16S rRNA signature nucleotides, a DNA G + C content of 69.9 mol%, the presence of meso-diaminopimelic acid, ribose, arabinose, glucose and galactose, which are characteristic components of cell-wall chemotype IV of actinomycetes, the presence of menaquinone MK-9(H₄) as the major respiratory lipoquinone, a lack of mycolic acids and the presence of an *N*-acetylated type of muramic acid. However, strain RA45^T differed from known genera of the family in its polar lipid composition: the major phospholipids were phosphatidylethanolamine, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, diphosphatidylglycerol, phospholipids of unknown structure and phospholipids of unknown structure containing glucosamine (phospholipid type IV). Based on its morphological, chemotaxonomic and phylogenetic characteristics, strain RA45^T is considered to represent a novel species of a new genus in the family *Pseudonocardiaceae*, for which the name *Yuhushiella deserti* gen. nov., sp. nov. is proposed. The type strain of *Yuhushiella deserti* is RA45^T (=CGMCC 4.5579^T =JCM 16584^T).

INTRODUCTION

The family *Pseudonocardiaceae* was described by Embley *et al.* (1988), and the description was emended by Stackebrandt *et al.* (1997) on the basis of 16S rRNA gene sequence analysis. As recognized by Stackebrandt *et al.* (1997), the genera *Actinosynnema*, *Saccharothrix* and *Streptoalloteichus*

were included in the family. Later studies by Labeda (1998) and Labeda & Kroppenstedt (2000) suggested that the genera *Actinokineospora*, *Actiosynnema*, *Lentzea* and *Saccharothrix* should be assigned to a putative new taxon, the family *Actinosynnemataceae*. The genera *Kutzneria* and *Streptoalloteichus* might also belong to this group, according to the 16S rRNA gene sequence data given by Kim & Goodfellow (1999). At the beginning of 2009, the family *Pseudonocardiaceae* included 15 genera with validly published names: *Actinoalloteichus* (Tamura *et al.*, 2000), *Actinomycetospora* (Jiang *et al.*, 2008), *Allokutzneria* (Labeda & Kroppenstedt, 2008), *Amycolatopsis* (Lechevalier *et al.*, 1986), *Crossiella* (Labeda, 2001), *Goodfellowiella*

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RA45^T is FJ526746.

(Labeda *et al.*, 2008) (previous illegitimate name *Goodfellowia* Labeda and Kroppenstedt 2006), *Kibdelosporangium* (Shearer *et al.*, 1986), *Kutzneria* (Stackebrandt *et al.*, 1994), *Prauserella* (Kim & Goodfellow, 1999), *Pseudonocardia* (Henssen, 1957), *Saccharomonospora* (Nonomura & Ohara, 1971), *Saccharopolyspora* (Lacey & Goodfellow, 1975), *Sciscionella* (Tian *et al.*, 2009), *Streptoalloteichus* (Tomita *et al.*, 1978) and *Thermocrispum* (Korn-Wendisch *et al.*, 1995). Recently, Zhi *et al.* (2009) proposed a new suborder *Actinopolysporineae* and family *Actinopolysporaceae* to accommodate the genus *Actinopolyspora* and assigned the genus *Thermobispora* to the suborder *Streptosporangineae*, although the family-level affiliation of the latter genus remains uncertain. However, the family *Pseudonocardiaceae* remains more diverse and loosely grouped than its sister family *Actinosynnemataceae*, with its characteristics including the absence of mycolic acids, the presence of an *N*-acetylated type of muramic acid, cell-wall type IV or III, phospholipids of type II or III (type IV for *Crossiella* only; Labeda, 2001) and MK-9(H₄) or MK-8(H₄) as the major menaquinone.

To allow rapid exploration of the biodiversity of the family *Pseudonocardiaceae*, genus-specific 16S rRNA gene primers have been designed for one-step identification of members of the genera *Amycolatopsis* (Tan *et al.*, 2006; McVeigh *et al.*, 1994), *Saccharomonospora* (Salazar *et al.*, 2000), *Saccharopolyspora* and *Pseudonocardia* (Morón *et al.*, 1999). The utility of genus-specific primers is defined by both their specificity and their coverage. These properties are influenced directly by the quality and quantity of sequences used to design the primers (Stach *et al.*, 2003). So, newly sequenced 16S rRNA genes of related taxa serve as a check to evaluate primers, as well as to evaluate the latest taxonomic system.

Strain RA45^T was isolated during an investigation of actinomycete diversity in soil from a desert region in Xinjiang Uigur Autonomous Region, north-western China. In this paper, we report the characterization and classification of strain RA45^T and propose a novel genus and species of the suborder *Pseudonocardineae* to accommodate it.

METHODS

Organisms and culture conditions. Strain RA45^T was isolated during the course of a study on the microbial diversity in soil samples from different biotopes from a barren desert region. Sampling and isolation were carried out in 2007, and samples were stored at 4 °C. Strain RA45^T was isolated through a serial dilution plate technique after 2 weeks of incubation at 28 °C on Sauton's agar (10 g tryptone, 2 g yeast extract, 10 g glucose, 6 g NaCl, 2 g acid-hydrolysed casein, 1000 ml distilled water, pH 7.0). To inhibit fungal growth, cycloheximide and nystatin (50 µg ml⁻¹ each) were added to all media.

Morphology. Cultural characteristics of the strain were determined after growth at 28, 37 and 45 °C for 2 weeks by methods used in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966) as well as by using Gause's asparagine agar (Gause *et al.*, 1983) and Bennett's agar (Jones, 1949). Colour determination was performed with colour chips from the ISCC-NBS Color Charts Standard Samples no. 2106 (Kelly, 1964). Morphological observations of spore chains

and mycelium were made by light microscopy (Olympus BH-2) and scanning electron microscopy (Quanta 200 FEG) after 7 days of incubation at 45 °C. Gram staining (Hucker, 1921) and Ziehl-Neelsen preparations (Gordon, 1967) were evaluated by light microscopy.

PCR amplification using genus-specific primers. 16S rRNA gene primers AMY2 (Tan *et al.*, 2006) and ATOP (McVeigh *et al.*, 1994) specific for the genus *Amycolatopsis* were employed in this study to screen soil samples and to test the genus-specificity of these primers. Genomic DNA extraction, PCR amplification and electrophoresis were performed as described by Tan *et al.* (2006).

PCR amplification of the 16S rRNA gene and sequence analysis. Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene of strain RA45^T were carried out according to the procedures described by Xu *et al.* (2003).

Phylogenetic analysis. The 16S rRNA gene sequence of strain RA45^T (1493 nt) was determined and compared with sequences downloaded from the DDBJ, EMBL and GenBank databases. For initial taxonomic classification, a combination of BLAST search (Altschul *et al.*, 1997; <http://www.ncbi.nlm.nih.gov/blast>) and the classifier program of the Ribosomal Database Project II (<http://rdp.cme.msu.edu/index.jsp>) was used. For phylogenetic analysis, sequences of the type strain of the type species of the type genus of each recognized suborder belonging to the phylum *Actinobacteria* (and some other sequences commonly used by taxonomists) were selected as the outgroup and, in a second step, sequences from the type species of all recognized genera (including *Actinopolyspora* and *Thermobispora*) in the family *Pseudonocardiaceae* and representatives of all six recognized genera in the sister family *Actinosynnemataceae* were used. Sequences shorter than 1300 bp or which contained ambiguous nucleotides were excluded. A similarity matrix of all 16S rRNA gene sequences was generated after multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). Representative sequences were chosen to make a small and robust phylogenetic dendrogram with high bootstrap values at each node. Three tree construction methods were employed in this study. Neighbour-joining (NJ) trees (Saitou & Nei, 1987) were calculated by using distances corrected according to Kimura's two-parameter model (Kimura, 1980, 1983), with the software package TREECONW version 1.3b (Van de Peer & De Wachter, 1994, 1997). For construction of the maximum-parsimony (MP) tree, the software package MEGA version 4.0 (Tamura *et al.*, 2007) was used. For construction of the maximum-likelihood (ML) tree, the online version of PhyML (Guindon *et al.*, 2005) was used. The topologies of the trees were evaluated by performing a bootstrap analysis (Felsenstein, 1985) of 1000 resamplings for the NJ and MP trees and 500 resamplings for the ML tree. The root position of the tree based on the NJ method was estimated by using 28 outgroup organisms, *Escherichia coli* (unknown strain; GenBank accession no. J01695), *Bacillus subtilis* W168 (K00637), *Acidimicrobium ferrooxidans* ICP^T (AFU75647), *Coriobacterium glomerans* PW2^T (X79048), *Rubrobacter radiotolerans* DSM 5868^T (X98372), *Bifidobacterium bifidum* KCTC 3202^T (BBU25951), *Actinomyces bovis* NCTC 11535^T (X81061), *Actinopolyspora halophila* ATCC 27976^T (X54287), *Catenulispora acidiphila* DSM 44928^T (AJ865857), *Corynebacterium diphtheriae* NCTC 11397^T (X84248), *Frankia alni* ACN14a (NC_008278), *Glycomyces harbinensis* IFO 14487^T (D85483), *Kineosporia aurantiaca* NRRL B-16913^T (AF095336), *Micrococcus luteus* DSM 20030^T (AJ536198), *Propionibacterium freudenreichii* DSM 20271^T (X53217), *Streptosporangium roseum* DSM 43021^T (X89947), *Micromonospora chalybeata* DSM 43026^T (X92594), *Streptomyces albus* DSM 40313^T (AJ621602), *Streptomyces ambifaciens* ATCC 23877^T (M27245), *Streptomyces griseus* NBRC 15744^T (AB184699), *Atopobium minutum* NCFB 2751^T (X67148), *Arthrobacter globiformis* DSM 20124^T (M23411), *Microbacterium lacticum* IFO 14135^T

(AB007415), *Sporichthya polymorpha* IFO 12702^T (AB025317), *Nocardioideus albus* DSM 43109^T (X53211), *Mycobacterium tuberculosis* H37 (AJ536031), *Sciscionella marina* SCSIO 00231^T (EU503139) and *Thermobispora bispora* ATCC 19993^T (MBU58523), as described by Labeda & Kroppenstedt (2000) and Zhi *et al.* (2009).

Signature nucleotide analysis. Two sets of selected 16S rRNA gene signature nucleotides defined for the suborder *Pseudonocardineae* (Stackebrandt *et al.*, 1997; Zhi *et al.*, 2009) were checked in this study using methods described by Zhi *et al.* (2009).

Isolation of DNA and determination of the G+C content. Cell material for genomic DNA extraction was obtained after cultivation at 28 °C for 4 days in ISP 2 broth (Shirling & Gottlieb, 1966) as a shaking culture. Chromosomal DNA for genomic DNA G+C content analysis was extracted as described by Marmur (1961). The DNA G+C content was determined by thermal denaturation (Mandel & Marmur, 1968).

Determination of physiological characteristics. Growth was tested at 4, 10, 28, 37, 45 and 50 °C on modified Bennett's agar (Jones, 1949). The ability of strain RA45^T to grow was determined at pH 4.0–12.0 on ISP 2 medium, adjusted to the appropriate pH using 0.1 M solutions of potassium dihydrogen phosphate and dipotassium hydrogen phosphate (Sambrook & Russell, 2001). After incubation for up to 21 days, growth was scored as a positive result. Tolerance of NaCl was examined at 0–10 % (w/v) on ISP 2 agar. Physiological tests including degradation of adenine, hypoxanthine, xanthine, L-tyrosine, xylan, uric acid, allantoin and urea were conducted as described previously (Kumar & Goodfellow, 2008).

Analysis of chemotaxonomic characteristics. Cell material for chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in ISP 2 broth (Shirling & Gottlieb, 1966) as a shaking culture. Extraction and analysis of mycolic acids followed the method described by Hamid *et al.* (1993). Determination of the N-acyl type of muramic acid residues followed the procedure described by Uchida *et al.* (1999). Procedures for analysis of diagnostic cell-wall amino acids and sugars followed those described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Menaquinones were extracted according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). The cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.), and the results were compared with the database of fatty acids in the Microbial Identification System.

RESULTS AND DISCUSSION

A database search based on the 16S rRNA gene sequence demonstrated that strain RA45^T belongs to the family *Pseudonocardaceae* (Stackebrandt *et al.*, 1997; Zhi *et al.*, 2009). A phylogenetic study was performed with 16S rRNA gene sequences of type strains of the type species of all genera with validly published names in the family *Pseudonocardaceae* and the sister family *Actinosynnemataceae* (Labeda & Kroppenstedt, 2000). According to the similarity matrix based on the alignment of all cited representative 16S rRNA gene sequences, the closest relatives of strain RA45^T were *Prauserella rugosa* DSM 43194^T, with 94.67 % sequence identity, *Prauserella halophila* YIM 90001^T (94.45 %), *Amycolatopsis palatopharyngis* 1BDZ^T (94.38 %), *Amycolatopsis sacchari* K24^T (94.35 %), *Saccharomonospora*

saliphila YIM 90502^T (94.34 %) and *Thermocrisium municipale* DSM 44069^T (94.32 %). The sequence identity to type strains belonging to other genera of the suborder *Pseudonocardineae* was below 94.30 %. The 16S rRNA gene sequence similarity between members of the genus *Actinopolyspora* and representatives of the emended suborder *Pseudonocardineae* (Zhi *et al.*, 2009) ranged from 85.89 to 90.52 % and the similarity between members of the genus *Thermobispora* and representatives of the emended suborder *Pseudonocardineae* (Zhi *et al.*, 2009) ranged from 85.91 to 88.43 %, supporting the decision of Zhi *et al.* (2009) to exclude these two genera from the suborder *Pseudonocardineae*.

Although the use of genus-specific primers designed for *Amycolatopsis* (AMY2 and ATOP) (Tan *et al.*, 2006; McVeigh *et al.*, 1994) showed that strain RA45^T yielded the same ~440 bp 16S rRNA gene fragment as members of the genus *Amycolatopsis*, as shown by the consensus phylogenetic tree (Fig. 1), strain RA45^T always formed a subclade distinct from the phylogenetic radiation formed by the genera *Prauserella*, *Amycolatopsis*, *Thermocrisium*, *Saccharomonospora* and *Saccharopolyspora*, adjacent to the *Thermocrisium* clade. However, the separate branching of strain RA45^T was supported by bootstrap values below 50 %. Furthermore, the whole-suborder phylogenetic tree was consistently divided into two large, approximately symmetrical clusters with high bootstrap values (>70 %), and the family *Actinosynnemataceae* formed a subclade that fell into one of the large clusters, while RA45^T fell into the other, as discussed above. Interestingly, the *Sciscionella*, *Pseudonocardia* and *Actinomycetospora* sequences formed three long, deep branches into the two large radiations.

Analysis of the patterns of signature nucleotides also supported our conclusions given above. Signature nucleotides of the 16S rRNA gene sequence of strain RA45^T were shared in all positions (according to the *Escherichia coli* numbering scheme) with the pattern of signature nucleotides reported for the suborder *Pseudonocardineae* described by Zhi *et al.* (2009) (Table 1). However, for the 16S rRNA signatures of the families *Pseudonocardaceae* and *Actinosynnemataceae* at positions 211, 480 and 142:221, our observations differed from those of Zhi *et al.* (2009). There were no differences between the two families at these positions, with members of the two families sharing A at position 211, U at position 480 and C–C at positions 142:221, with only two exceptions, *Sciscionella marina* SCSIO 00231^T and our isolate RA45^T, which both carried U at position 221.

Strain RA45^T could also be distinguished from all other representatives of the suborder *Pseudonocardineae* (Zhi *et al.*, 2009) by a combination of morphological and chemical characteristics (Table 2). Growth of strain RA45^T was good on most media tested, especially on yeast extract-malt extract (ISP 2) agar, and the optimal growth temperature was 37–45 °C. Soluble brown pigments were produced at 37

Fig. 1. NJ tree derived from 16S rRNA gene sequences showing the relationship between strain RA45^T and representatives of the suborder *Pseudonocardineae*. Asterisks indicate branches recovered with all three methods; m and p indicate branches that were also recovered by using the ML (Felsenstein, 1981) and MP (Fitch, 1971) treeing algorithms, respectively. Numbers at nodes indicate levels of bootstrap support based on an NJ (Saitou & Nei, 1987) analysis of 1000 resampled datasets; only values above 50% are given. The arrow indicates the estimated position of the ten remaining root organisms (see Methods for details). Bar, 0.02 substitutions per nucleotide position.

and 45 °C on Bennett's agar, but were absent at other temperatures. No aerial mycelium was observed under any of the culture conditions used in this study. The hyphae (0.2 µm in diameter) which covered the surfaces of colonies were straight to flexuous, smooth and branched (Fig. 2) and were pale yellow to light yellow. The vegetative hyphae frequently formed aggregates and globose or irregular-formed sporangium-like structures (5–10 µm) with irregular surfaces (Fig. 2), which differed from those formed by *Kibdelosporangium* strains and the 'pseudosporangia' of *Thermocrisum* and *Crossiella* strains. Sessile global swellings (0.2–0.3 µm) were found consistently, especially at the ends of the aerial mycelium. Fragmentation seldom occurred in the substrate mycelium. Motile spores were not detected despite extensive observation.

Strain RA45^T lacked mycolic acids and contained muramic acid in the *N*-acetylated form, which is consistent with the properties of other genera of the family *Pseudono-*

cardiaceae. The cell wall of strain RA45^T contained *meso*-diaminopimelic acid as the diagnostic peptidoglycan diamino acid. Whole-cell hydrolysates contained ribose, arabinose, glucose and galactose as diagnostic sugars (cell-wall chemotype IV; Lechevalier & Lechevalier, 1970). Analysis of phospholipids revealed that phosphatidylethanolamine, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, diphosphatidylglycerol, phospholipids of unknown structure and glucosamine-containing phospholipids of unknown structure were the major phospholipids, indicating phospholipid type IV (Lechevalier *et al.*, 1977). The predominant menaquinone was MK-9(H₄) (66.12 %), and minor amounts of MK-9 (12.69 %), MK-9(H₂) (9.08 %), MK-10(H₂) (3.52 %), MK-8(H₂) (2.10 %) and MK-8 (0.59 %) were detected. The fatty acid profile consisted of the predominant component iso-branched hexadecanoic acid (iso-C_{16:0}; 24.41 %) and smaller amounts of C_{14:0} (2.04 %), iso-C_{15:1} (1.61 %), iso-C_{16:1} (2.66 %), C_{16:1}ω7c/iso-C_{15:0} 2-OH (9.96 %),

Table 1. Signature nucleotides of the 16S rRNA sequences of members of the family *Pseudonocardiaceae*

Data were analysed in this study unless indicated, from the type strain of the type species of each genus. For the family *Pseudonocardiaceae*, 'other genera' represents *Amycolatopsis*, *Prauserella*, *Saccharomonospora*, *Saccharopolyspora*, *Actinomycetospora*, *Pseudonocardia*, *Crossiella*, *Actinoalloteichus*, *Goodfellowiella*, *Allokutzneria*, *Kutzneria*, *Kibdelosporangium* and *Streptoalloteichus*. For the family *Actinosynnemataceae*, the genera included were *Actinokineospora*, *Umezawaea*, *Saccharothrix*, *Lentzea*, *Lechevalieria* and *Actinosynnema*. Positions are given relative to the *Escherichia coli* numbering scheme. Y, Pyrimidine. Signatures specific for the suborder *Pseudonocardineae* and for the families *Pseudonocardiaceae* and *Actinosynnemataceae* are listed. Signatures that differ from those given by Zhi *et al.* (2009) are highlighted in bold.

Position(s)	<i>Pseudonocardiaceae</i>					<i>Actinosynnemataceae</i>	
	Zhi <i>et al.</i> (2009)		Our study			Zhi <i>et al.</i> (2009)	Our study
		Strain RA45 ^T	<i>Thermocrispum</i>	<i>Sciscionella</i>	Other genera		
Suborder							
<i>Pseudonocardineae</i>							
127:234	G–C	G–C	A–U/G–C	G–C	G–C	G–C	G–C
564	U	U	U	U	U	U	U
672:734	U–G	U–G	U–G	U–G	U–G	U–G	U–G
831:855	U–G	U–G	U–G	U–G	U–G	U–G	U–G
832:854	G–Y	G–U	G–U	G–U	G–U	G–U	G–U
833:853	U–G	U–G	U–G	U–G	U–G	U–G	U–G
952:1229	U–A	U–A	U–A	U–A	U–A	U–A	U–A
986:1219	U–A	U–A	U–U	U–A	U–A	U–A	U–A
<i>Pseudonocardiaceae</i> and <i>Actinosynnemataceae</i>							
211	G	A	A	A	A	A	A
480	U	U	U	U	U	G	U
142:221	C–G	C–U	C–C	C–U	C–C	C–C	C–C

C_{16:0} (7.55 %), 10-methyl C_{16:0} (2.12 %), iso-C_{17:0} (3.54 %), anteiso-C_{17:0} (6.04 %), C_{17:1ω8c} (3.50 %), C_{17:1ω6c} (11.37 %), C_{17:0} (1.25 %), 10-methyl C_{17:0} (1.53 %), iso-C_{18:0} (2.60 %), C_{18:1ω9c} (4.24 %), C_{18:1ω7c} (1.06 %) and C_{18:0} (8.48 %). The G + C content of the genomic DNA of the strain was 69.9 mol%.

On the basis of a combination of phylogenetic distinctness and differences in morphological and chemotaxonomic features, we consider that strain RA45^T represents a novel genus and species, for which the name *Yuhushiella deserti* gen. nov., sp. nov. is proposed.

Description of *Yuhushiella* gen. nov.

Yuhushiella (Yu.hu.shi.el'la. N.L. dim. ending -ella; N.L. fem. n. *Yuhushiella* named after Professor Yuhu Shi, a Chinese microbiologist, in recognition of his leadership and contributions to the exploration of the microbial resources of Xinjiang Uigur Autonomous Region, China).

Aerobic, Gram-stain-positive, non-acid-fast, non-motile actinomycetes. Vegetative mycelium is straight to flexuous, smooth and branched. Swelling and aggregation of the hyphae occur, but fragmentation is seldom observed. The cell wall contains meso-diaminopimelic acid. Whole-cell hydrolysates contain ribose, arabinose, glucose and galac-

tose (cell-wall chemotype IV). Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol mannosides, phospholipids of unknown structure and glucosamine-containing phospholipids of unknown structure are the diagnostic phospholipids. Mycolic acids are absent and *N*-acetylated-type muramic acid is present. The predominant menaquinone is MK-9(H₄). The genus is a member of the family *Pseudonocardiaceae* in the suborder *Pseudonocardineae*. The type species is *Yuhushiella deserti*.

Description of *Yuhushiella deserti* sp. nov.

Yuhushiella deserti (de.ser'ti. L. gen. n. *deserti* of a desert).

In addition to the characteristics given in the genus description, the species has the following properties. Vegetative mycelium is pale yellow to light yellow. Two types of sporangia are observed: swellings (0.5–2 µm) are formed at the ends or branch joints of the mycelium and 'post-pseudosporangia' (5–10 µm) are formed from mycelial aggregation and merging (Fig. 2). The 'post-pseudosporangia', similar to the pseudosporangia of *Thermocrispum* and *Crossiella* strains, are not surrounded by a well-defined wall and contain hyphae that fragment into rod-like structures, but the hyphae are not totally septate. A brown

Table 2. Diagnostic characteristics of strain RA45^T and phylogenetically closely related genera in the suborder *Pseudonocardineae*

Data for reference genera were taken from Lechevalier *et al.* (1986), Kim & Goodfellow (1999), Li *et al.* (2003a, b), Bian *et al.* (2009), Chun *et al.* (1999), Goodfellow *et al.* (2001), Huang *et al.* (2004), Kim *et al.* (2002), Tseng *et al.* (2006), Al-Zarban *et al.* (2002), Dolashka *et al.* (1998), Jin *et al.* (1998), Syed *et al.* (2008), Yoon *et al.* (2000), Lu *et al.* (2001), Pimentel-Elardo *et al.* (2008), Qin *et al.* (2008), Zhang *et al.*, (2008) and Korn-Wendisch *et al.* (1989, 1995). None of the genera show acropetal budding (primordial reproduction on hyphal tips). Only *Amycolatopsis taiwanensis* shows formation of bud-like structures on substrate hyphae (primordial hyphal differentiation). +, Present or positive; –, absent or negative; ND, not detected (may be no sporal differentiation); NA, not applicable (no aerial hyphae).

Characteristic	Strain RA45 ^T	<i>Thermocristum</i>	<i>Amycolatopsis</i>	<i>Prauserella</i>	<i>Saccharomonospora</i>	<i>Saccharopolyspora</i>	<i>Scissionella</i>
Aerial hyphae (hyphal differentiation)	–	+	+	+	+	+	Sparse
Substrate hyphae							
Fragmentation	Seldom	+	+	+	–	+	+
Spores†	–	–	Primordial spore chains from hyphal fragmentation*	–	Petiolate spores singly or in longitudinal pairs	From hyphal fragmentation	–
Sporangium-like structures	+	Pseudosporangia (hyphal aggregates)	–	–	–	–	–
Aerial hyphae							
Fragmentation	NA	+	+	–	–	+	–
Spores†	NA	ND	Primordial spore chains from hyphal fragmentation*‡	Well-developed, long, branched spore chains	Petiolate spores, singly or in longitudinal pairs	In chains (from hyphal segregation)	–
Sporangium-like structures	NA	Pseudo-sporangia (hyphal aggregates)	–	–	–	–	–
Chemical properties							
Phospholipid type§	IV	II	II	II	II	III	III
Sugar pattern	Ara, Gal, Glc, Rib	Ara, Man, Glc, (Gal)	Ara, Gal	Ara, Gal	Ara, Gal	Ara, Gal	Ara, Gal, Glc
Predominant menaquinone(s)¶	9(H ₄)	9(H ₄)	9(H ₂ , H ₄ , H ₆)	9(H ₂ , H ₄)	8(H ₄), 9(H ₄)	9(H ₄), 10(H ₄)	9(H ₄)
DNA G + C content (mol%)	69.9	69–73	66–73	65.8–69.9	66–74	70–77	69

*Except *Amycolatopsis taiwanensis*.

†Refers to the presence of sporal differentiation, motile spores, conidiospores, etc.

‡*A. taiwanensis* forms bud-like structures separated from the substrate hyphae.

§II, Phosphatidylethanolamine; III, phosphatidylcholine (with phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphatidylglycerol variable; no phospholipids containing glucosamine); IV, phospholipids containing glucosamine, phosphatidylethanolamine variable, phosphatidylmethylethanolamine variable, no phosphatidylglycerol, no phosphatidylcholine (Lechevalier *et al.*, 1977).

||Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rib, ribose. Compounds in parentheses are present in trace amounts.

¶Abbreviations are exemplified by 9(H₄), menaquinones with four of the nine isoprene units hydrogenated.

soluble pigment is produced during cultivation at 37–45 °C. Grows well on all tested media; optimal culture conditions are 37–45 °C, pH 9.0 and 3.5 % (w/v) NaCl. Casein, starch, L-tyrosine, uric acid and urea are hydrolysed or decomposed. Adenine, allantoin, xylan, hypoxanthine and xanthine are not hydrolysed or decomposed. The major cellular fatty acids are iso-C_{16:0}, C_{16:1}ω7c/iso-C_{15:0} 2-OH,

C_{16:0}, anteiso-C_{17:0}, C_{17:1}ω6c and C_{18:0}. The DNA G + C content of the type strain is 69.9 mol%.

The type strain, RA45^T (=CGMCC 4.5579^T =JCM 16584^T), was isolated from soil collected from a desert region in Xinjiang Uigur Autonomous Region, north-western China.

Fig. 2. Scanning electron micrographs of 'post-pseudosporangia' of a 7-day-old coverslip culture of strain RA45^T grown on Gause's asparagine agar at 45 °C. Bars, 5.0 µm (a, b, d) and 10.0 µm (c).

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