Proposal to combine the genera *Actinobispora* and *Pseudonocardia* in an emended genus *Pseudonocardia*, and description of *Pseudonocardia zijingensis* sp. nov.

Ying Huang,1 Liming Wang,1 Zhitang Lu,1 Long Hong,2 Zhiheng Liu,1 Geok Yuan Annie Tan3 and Michael Goodfellow3

1 Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People’s Republic of China
2 Department of Biochemistry and Molecular Biology, College of Life Sciences, Peking University, Beijing 100871, People’s Republic of China
3 Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

The 16S rDNA sequences of four strains, i.e. three type strains of *Actinobispora* and strain 6330T, were determined and compared with those of representatives of the family *Pseudonocardiaceae* by using two tree-making algorithms. All the validly described species of the genera *Actinobispora* and *Pseudonocardia* were consistently recovered as a mixed group in phylogenetic trees, and were distinct from the other genera of the family *Pseudonocardiaceae*. Strain 6330T formed a distinct phyletic line in the 16S rDNA tree and was most closely associated with the type strain of *Actinobispora aurantiaca*. The use of specific PCR primers designed for differentiating the genus *Pseudonocardia* from other genera of the family *Pseudonocardiaceae* showed that all the *Actinobispora* species and strain 6330T have the same amplified 640 bp 16S rDNA fragment as members of the genus *Pseudonocardia*. The DNA–DNA relatedness, chemotaxonomic and phenotypic data also supported classification of these taxa in the genus *Pseudonocardia*, and distinguished each from the others. On the basis of these observations, it is proposed that the genera *Actinobispora* and *Pseudonocardia* be combined in an emended genus *Pseudonocardia* and that strain 6330T be classified in the same genus as *Pseudonocardia zijingensis* sp. nov. The type strain is 6330T (= AS 4.1545T = JCM 11117T).

Keywords: *Actinobispora*, *Pseudonocardia*, *Pseudonocardia zijingensis* sp. nov., 16S rDNA sequencing, polyphasic taxonomy

INTRODUCTION

The genus *Pseudonocardia* was originally proposed by Henssen (1957) for mycolateless nocardioform actinomycetes that had a type IV cell wall, and its description was emended ceaselessly with species transferred from other genera and newly found species (Warwick et al., 1994; McVeigh et al., 1994; Reichert et al., 1998). This genus currently encompasses 11 validly described species and phylogenetically forms a coherent group within the evolutionary radiation occupied by the family *Pseudonocardiaceae* (Henssen, 1957; Henssen et al., 1983; Warwick et al., 1994; McVeigh et al., 1994; Reichert et al., 1998). Members of the genus are non-motile and biochemically versatile organisms which form chains of spores by acropetal budding or septation from the substrate or aerial mycelium, and the mycelium exhibits cell division in different directions. The organisms contain MK-8(H4) as the major menaquinone and iso-branched hexadecanoic acid as the predominant fatty acid. The phospholipid pattern is type PII or PIH, and the G+C content of the DNA is 68–79 mol%.

The genus *Actinobispora* was described by Jiang et al. (1991) for mycolateless actinomycetes that form branched, but not fragmented, mycelia and produce spores in longitudinal pairs on both the substrate and aerial mycelium. There are currently four validly described species: *Actinobispora yunnanensis*, *Actinobispora alaminiphila*, *Actinobispora aurantiaca*...
and *Actinobispora xinjiangensis* (Xu et al., 1999). The genus has a type IV cell wall, a type PIV phospholipid pattern (phosphatidylcholine, phosphatidyethanolamine, unknown glucosamine-containing phospholipids and phosphatidylglycerol) and major menaquinones MK-9(H₃) and MK-7(H₂); it was placed in the family *Pseudonocardiaceae*. Recently, Lee et al. (2000) sequenced the 16S rDNA of *A. yunnanensis*, and suggested the union of the genera *Pseudonocardia* and *Actinobispora*. In this investigation, we determined the 16S rDNA sequences of the other three species of the genus *Actinobispora* and a new isolate, strain 6330ᵀ, to clarify the relationships between these taxa and the family *Pseudonocardiaceae* and the genus *Pseudonocardia*. The genotypic and phenotypic data show that the genus *Actinobispora* forms a junior taxon of the genus *Pseudonocardia*, and strain 6330ᵀ represents a new species of this genus.

**METHODS**

**Strains and culture conditions.** Strain 6330ᵀ was isolated on a yeast extract/starch agar (Emerson, 1958) plate, which had been seeded with a soil suspension and incubated at 28 °C for 3 weeks. The soil sample was collected from Zijing Mountain, Yunnan Province, China. The isolate and the type strains of the genera *Actinobispora* and *Pseudonocardia* used in this study were maintained on trypticase soy broth (TSB; BBL) slants at 4 °C and as a glycerol suspension (20%, v/v) at −20 °C. Biomasses for the chemical and molecular systematic studies were prepared by growing the strains in shake flasks of TSB at 28 °C for 7–14 days. Cells were harvested by centrifugation and washed with distilled water; those used for the chemical studies were freeze-dried.

**Morphology and physiology.** The morphological characteristics of strain 6330ᵀ were observed by light microscopy and scanning electron microscopy of 14-day-old cultures grown on TSB agar. Physiological tests were carried out by the procedures of Gordon et al. (1974) and Reichert et al. (1998).

**Chemotaxonomy.** The isomers of dianimonipelic acid and whole-organism sugars were analysed by following the procedures developed by Hasegawa et al. (1983) and Lechevalier & Lechevalier (1980). Mycolic acids were checked for by using the acid methanalysis method as described previously (Minnikin et al., 1980). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985); the purified preparations were analysed using an HPLC procedure (Wu et al., 1989).

**PCR amplification and 16S rDNA sequencing.** Genomic DNA preparation and PCR amplification of 16S rDNA were performed as described by Chun & Goodfellow (1995). The almost complete amplified 16S rDNAs were directly sequenced as described previously (Huang et al., 2001). For identification of members of the genus *Pseudonocardia*, a pair of oligonucleotides, AMP3 (5’-GCGGACAGAG-ACCGTGGAAT-3’) and AMP2 (5’-GTGGGAAAGTTTT-TTCCGGCTGGGG-3’), were used as specific primers, according to the procedures of Morón et al. (1999).

**Phylogenetic analysis.** The 16S rDNA sequences obtained in this study were aligned manually using the clustal_X program (version 1.64b; Thompson et al., 1997) with corresponding sequences retrieved from the GenBank (Benson et al., 1997) database. Evolutionary trees were inferred by using the neighbour-joining (Saitou & Nei, 1987) and least-squares (Fitch & Margoliash, 1967) tree-making algorithms. Evolutionary distance matrices were generated as described by Jukes & Cantor (1969), and phylogenetic trees were constructed using the PHYLIP package (Felsenstein, 1993). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings using the SEQBOOT and CONSENSE programs in the PHYLIP package.

**RESULTS AND DISCUSSION**

**Morphological and physiological characteristics**

Strain 6330ᵀ showed morphology typical of the genus *Pseudonocardia*, in that chains of spores formed by acropetal budding from the branched substrate mycelium, and both substrate and aerial mycelium fragment into rod-shaped elements. The diameter of the hyphae was 0.3–0.5 μm (Fig. 1). The strain did not produce any pigment. The physiological properties of strain 6330ᵀ and the four *Actinobispora* type strains are shown in Table 1.

![Fig. 1. Scanning electron micrograph of *P. zijingensis* 6330ᵀ grown on TSB agar for 14 days at 28 °C. Bar, 3.8 μm.](image-url)
Table 1. Physiological properties of strain 6330T and the Actinobispora species

<table>
<thead>
<tr>
<th>Test</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D (+)Glucose</td>
<td>+</td>
<td>w</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D (+)Xylose</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D (+)Fructose</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>D (+)Trehalose</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Cellobiose</td>
<td>w</td>
<td>+</td>
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<tr>
<td>D (−)Mannitol</td>
<td>w</td>
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<tr>
<td>D (−)Sorbitol</td>
<td>+</td>
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<tr>
<td>Maltose</td>
<td>w</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>w</td>
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<tr>
<td>D (±)Lactose</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>L (±)Arabinose</td>
<td>+</td>
<td>-</td>
<td>w</td>
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<tr>
<td>Adonitol</td>
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<td>Salicin</td>
<td>-</td>
<td>-</td>
<td>w</td>
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<td>Decomposition of:</td>
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<tr>
<td>L-Tyrosine</td>
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<tr>
<td>Xanthine</td>
<td>+</td>
<td>-</td>
<td>w</td>
<td>-</td>
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<tr>
<td>Growth on:</td>
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<tr>
<td>3% NaCl</td>
<td>+</td>
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<tr>
<td>4% NaCl</td>
<td>+</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>5% NaCl</td>
<td>w</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Urease production</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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</tbody>
</table>

Chemotaxonomic properties

Strain 6330T contained meso-diaminopimelic acid as the wall diamino acid, and contained arabinose and galactose as major wall sugars (cell wall type IV, according to Lechevalier & Lechevalier, 1970 or Lechevalier & Lechevalier, 1980). It lacked mycolic acids but contained phosphatidylcholine, phosphatidylglycerol, phosphatidylmethylthanolamine, phosphatidylinositol mannosides and diphosphatidylglycerol (phospholipid type PIII, according to Lechevalier et al., 1977 or Lechevalier & Lechevalier, 1980). No glucosamine-containing phospholipids were detected. The principal menaquinone was tetra-hydrogenated with eight isoprene units [MK-8(H4)], consistent with that of the genus Pseudonocardia.

The genus Actinobispora was distinguished from the genus Pseudonocardia in the compositions of menaquinones and phospholipids. Jiang et al. (1991) and Xu et al. (1999) reported that the major menaquinones of the genus Actinobispora were MK-9(H4) and MK-7(H4). However, our chemotaxonomic data showed that all the Actinobispora species had MK-8(H4) as the major menaquinone, which is the same as the genus Pseudonocardia. This result is supported by a previous study of Lee et al. (2000). Thus, the genera Actinobispora and Pseudonocardia are different only in phospholipid pattern, and the difference of a single chemical characteristic is not sufficient to justify forming a new genus.

PCR identification of members of the genus Pseudonocardia

The results of PCR assays using Pseudonocardia-specific primer pair AMP3/AMP2 (Morón et al., 1999) also support the classification of species of Actinobispora and strain 6330T into the genus Pseudonocardia. As shown in Fig. 2, the specific amplification product (640 bp) was obtained from the four Actinobispora type strains, strain 6330T and members of Pseudonocardia as the positive control. No amplification products were observed with the other reference strains as negative controls.

Phylogenetic analysis

The genus Actinobispora was established only by chemotaxonomic and phenotypic characteristics (Jiang et al., 1991). In a previous study (Xu et al.,
Y. Huang and others

Fig. 3. Neighbour-joining tree (Saitou & Nei, 1987) showing the positions of the Actinobispora species and P. zijingensis 6330T within the family Pseudonocardiaceae. Asterisks indicate the branches that were also recovered using the least-squares methods. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values over 50% are given. Scale bar, 0.01 substitutions per nucleotide position.

The 16S rRNA sequences of the four species of Actinobispora were reported but contained lots of ‘n’ (not determined) bases. Comparison of the sequences with only those of the type species of the genus Pseudonocardia, Pseudonocardia thermophila and representatives of the genus Saccharopolyspora resulted in the undoubtable conclusion that the four species of Actinobispora clustered into a distinct group in the family Pseudonocardiaceae. (1997) and Lee et al. (2000) had sequenced the 16S rDNA of A. yunnanensis and had obtained very similar results, so, in this study, again we sequenced only the 16S rDNAs of the other three Actinobispora species, A. alaniniphila AS 4.1536T (= CCTCC AA97001T), A. aurantiaca AS 4.1537T (= CCTCC AA97002T) and A. xinjiangensis AS 4.1538T (= CCTCC AA97020T), and strain 6330T (= AS 4.1545T). Comparison of these sequences with those of all validly described species of the genus Pseudonocardia and representatives of the other nine genera in the family Pseudonocardiaceae showed that the four species of Actinobispora, Pseudonocardia petroleophila, Pseudonocardia saturnea, P. thermophila and Pseudonocardia asaccharolytica, always formed an intermixed group (83% bootstrap value) within the monophyletic clade of the genus Pseudonocardia (Fig. 3). The levels of 16S rDNA sequence similarity between the members of the genera Actinobispora and Pseudonocardia were 98.0–95.2%, which fell into the range found between the Pseudonocardia species (99.6–93.6%) and was higher than those found between members of the other genera of the family Pseudonocardiaceae and either Pseudonocardia species (94.9–86.1%) or Actinobispora species (94.2–86.8%). The phylogenetic analysis also showed that strain 6330T belongs to the Actinobispora–Pseudonocardia group and formed a distinct phyletic line. The 16S rDNA sequence similarity values between strain 6330T and its nearest neighbour, A. aurantiaca, was 98.5%. In addition, A. yunnanensis and A. alaniniphila were always closely related to each other (100% bootstrap value) and share a high 16S rDNA similarity value (99.7%). Accordingly, there is strong evidence from our phylogenetic analysis that all the members of the genera Actinobispora and Pseudonocardia and strain 6330T should be classified within the same genus.
Pseudonocardia

relatedness between the four

and

aurantiaca

Some species are facultative autotrophs and some


vegetative hyphae and in longitudinal pairs or singly

ding or septation from the substrate or aerial my-

normally smooth and form chains by acropetal bud-

strains form swollen hyphal segments. Aerial mycelium

may or may not be present. The mycelium may or may

with the additions and modifications made in this

publication.

Description of Pseudonocardia zijingensis sp. nov.

Pseudonocardia zijingensis (zi.jing.en’sis. M.L. adj. zijingensis pertaining to Zijing, the source of the soil

from which the organism was isolated).

Forms a branching yellow substrate mycelium and

white aerial mycelium on TSB agar. The mycelium

fragments into rod-shaped elements. Smooth spores

are borne in chains by acropetal budding from the

substrate mycelium. No pigment is produced. Not

tolerant of lysozyme (0-005, w/v). Growth occurs at

15–45 °C. Additional physiological properties are

listed in Table 1. The cell wall chemotype is IV. The

main menaquinone is MK-8(H4). It contains phospha-

Table 2. Percentage DNA relatedness among test strains

<table>
<thead>
<tr>
<th>Species</th>
<th>P. saturnea IMSNU 20052T</th>
<th>P. petroleophila IMSNU 22140T</th>
<th>P. zijingensis 6330T</th>
<th>A. yunnanensis AS 4.1542T</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. alaniniphila AS 4.1536T</td>
<td>31</td>
<td>41</td>
<td>42</td>
<td>48</td>
</tr>
<tr>
<td>A. aurantiaca AS 4.1537T</td>
<td>34</td>
<td>47</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>A. xinjiangensis AS 4.1538T</td>
<td>52</td>
<td>31</td>
<td>33</td>
<td>ND</td>
</tr>
<tr>
<td>A. yunnanensis AS 4.1542T</td>
<td>37</td>
<td>29</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>P. thermophila IMSNU 20112T</td>
<td>36</td>
<td>27</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P. petroleophila IMSNU 22140T</td>
<td>43</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, Not determined.

DNA–DNA hybridization

The results of DNA–DNA hybridization are shown in Table 2. The levels of DNA relatedness between strain 6330T and A. aurantiaca (40%), and between A. yunnanensis and A. alaniniphila (48%), clearly show that they are different genospecies. The levels of DNA relatedness between the four Actinobispora species and the two Pseudonocardia species (P. petroleophila and P. saturna) ranged from 29% to 52%. Similar or lower relatedness values were observed between P. thermophila (the type species), P. petroleophila and P. saturna (27–43%). These results strongly support the view that all the species mentioned above should be classified within the same genus.

DNA base composition

The G + C contents of strain 6330T, A. alaniniphila, A. aurantiaca, A. xinjiangensis and A. yunnanensis were 70-9, 69-3, 71-5, 72-1 and 73-4 mol % (Tm), respectively.

On the basis of the results of this investigation, we propose that the genus Actinobispora should be recognized as a junior taxon of the genus Pseudonocardia, that the description of this genus should be emended again, and that strain 6330T should be recognized as a new Pseudonocardia species.

Emended description of the genus Pseudonocardia (Henissen 1957)

The vegetative mycelium varies in thickness (0.3–2.0 μm) and in the degree of branching. Some strains form swollen hyphal segments. Aerial mycelium may or may not be present. The mycelium may or may not be fragmented; if fragmented, both types of mycelium exhibit cell division in different directions. In some species, the mycelium is covered by an electron-dense outer layer. Spore sizes vary; the spores are normally smooth and form chains by acropetal budding or septation from the substrate or aerial mycelium, or else are formed in longitudinal pairs on vegetative hyphae and in longitudinal pairs or singly on aerial hyphae. Non-motile. Biochemically versatile. Some species are facultative autotrophs and some strains can oxidize hydrocarbons. Some species can oxidize methyl sulfides to sulfate. P. thermophila can degrade cellulose. The major menaquinone is MK-8(H4). Iso-branched fatty acids are predominant. The main compound is iso-branched hexadecanoic acid. Phosphatidymethylethanolamine is present in most species. The occurrence of phosphatidylethanolamine, phosphatidylcholine and glucosamine-containing phospholipids is variable between the species. Cell wall type IV. Mycolates are absent. The G + C content of the DNA is 68–79 mol %. Members of this genus form a coherent group on the basis of 16S rRNA sequence data. The genus contains the species Pseudonocardia alni, P. asascharolytica, Pseudonocardia autotrophica, Pseudonocardia compacta, Pseudonocardia halophobica, Pseudonocardia hydrocarbonsynox, P. petroleophila, P. saturnae, Pseudonocardia spinosa, Pseudonocardia sulfivoxydans, P. thermophila and Pseudonocardia zijingensis. The type species is P. thermophila.

The species of the genus Actinobispora should be transferred to the genus Pseudonocardia as Pseudonocardia alani comb. nov., Pseudonocardia aurantiaca comb. nov., Pseudonocardia xinjiangensis comb. nov. and Pseudonocardia yunnanensis comb. nov. The descriptions of these species are identical to those given by Jiang et al. (1991) and Xu et al. (1999), with the additions and modifications made in this publication.
tidylycholine, phosphatidylglycerol, phosphatidyl-
methylethanolamine, phosphatidylinositol manno-
sides and diphosphatidylglycerol, but does not contain
glucosamine-containing phospholipids or mycolic
acids. The G+C content of the DNA is 70.9 mol %
\(T_m\). Isolated from a soil sample collected from Zijing
Mountain, Yunnan Province, China. The type strain is
\(6330^T\) (AS 4.1545\(^T\) = JCM 11117\(^T\)).

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