Emended description of the genus *Actinokineospora* Hasegawa 1988 and transfer of *Amycolatopsis fastidiosa* Henssen *et al.* 1987 as *Actinokineospora fastidiosa* comb. nov.

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The species *Amycolatopsis fastidiosa* (ex Celmer *et al.* 1977) Henssen *et al.* 1987 was proposed, based on morphological and chemotaxonomic observations, for a strain originally described as '*Pseudonocardia fastidiosa*' Celmer *et al.* 1977 in a US patent. In the course of a phylogenetic study of the taxa with validly published names within the suborder *Pseudonocardineae* based on 16S rRNA gene sequences, it became apparent that this species was misplaced in the genus *Amycolatopsis*. After careful evaluation of the phylogeny, morphology, chemotaxonomy and physiology of the type strain, it was concluded that this strain represents a species of the genus *Actinokineospora* that is unable to produce motile spores. The description of the genus *Actinokineospora* is therefore emended to accommodate species that do not produce motile spores, and it is proposed that *Amycolatopsis fastidiosa* be transferred to the genus *Actinokineospora* as *Actinokineospora fastidiosa* comb. nov. The type strain is NRRL B-16697^T = ATCC 31181^T = DSM 43855^T = JCM 3276^T = NBRC 14105^T = VKM Ac-1419^T.

The name '*Pseudonocardia fastidiosa*' was proposed by Celmer *et al.* (1977) for an actinomycete strain that displayed acropetal development of spore chains and which produced a macrobicyclic peptide antibiotic, although the name was never validly published. A subsequent study by Henssen *et al.* (1987) reported that the strain deviated from the typical morphology exhibited by the genus *Pseudonocardia* in that acropetal budding was not observed. Moreover, they noted that phosphatidylethanolamine was the predominant polar lipid (type PII *sensu* Lechevalier *et al.*, 1977) rather than phosphatidylcholine (type PIII *sensu* Lechevalier *et al.*, 1977), which is characteristic of *Pseudonocardia*. They proposed that this species be

transferred to the genus *Amycolatopsis* as *Amycolatopsis fastidiosa* because these morphological and chemotaxonomic characteristics were typical of members of this genus. During the course of a recent phylogenetic analysis of all taxa within the suborder *Pseudonocardineae* on the basis of 16S rRNA gene sequences, it became obvious that this species is misplaced within the genus *Amycolatopsis*, and a study was undertaken to clarify its taxonomic position. In addition, it was noted that *Amycolatopsis fastidiosa* did not yield an amplification product using *Amycolatopsis*-specific oligonucleotide primers (Tan *et al.*, 2006) and that Everest & Meyers (2009) had determined that the *gyrB* sequence of *Amycolatopsis fastidiosa* was quite different (only 79.5–83.3 % similarity) from those of the other species of the genus.

Several 16S rRNA gene sequences for the type strain of *Amycolatopsis fastidiosa* are available in the public databases and, to confirm their accuracy, DNA was isolated from NRRL B-16697^T, the type strain held in the ARS Culture Collection, using an UltraClean microbial DNA

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NRRL B-16697^T is GQ200601.

Whole-cell carbohydrate profiles of *Actinokineospora riparia* NRRL B-16432^T and *Amycolatopsis fastidiosa* NRRL B-16697^T and details of the grouped sequences used in the construction of Fig. 1 are available as supplementary material with the online version of this paper.

isolation kit (MoBio Laboratories). The 16S rRNA gene sequence was determined by standard procedures (Labeda & Kroppenstedt, 2000). The sequence of NRRL B-16697^T was found to be identical to the other deposited sequences for this type strain (GenBank accession no. AJ400710 from IMSNU 20054^T; AY389140 from AS 4.1172^T).

Phylogenetic analyses were performed on the aligned sequences of the type strains of all taxa currently described within the suborder *Pseudonocardineae* using ARB (Ludwig *et al.*, 2004) and it was observed that *Amycolatopsis fastidiosa* was positioned consistently as the most distant member of the *Actinokineospora* genus clade (Fig. 1), which is quite far from that of the genus *Amycolatopsis*. The 16S rRNA gene sequence similarity of NRRL B-16697^T to the species within the genus *Actinokineospora* ranged from a high of 97.4% for *Actinokineospora terrae* NBRC 15668^T (GenBank accession no. AB058394) to 95.7% for *Actinokineospora enzanensis* NBRC 16517^T (AB058395), clearly suggestive of membership of this genus.

A key characteristic of all *Actinokineospora* species has been the production of motile spores. Morphological studies were performed on the media suggested by Shirling & Gottlieb (1966) as well as a modified version of the humic acid medium introduced by Hayakawa & Nonomura (1987) in which 0.1% yeast extract was substituted for the vitamin stock solution. The procedure of Tamura *et al.* (1995) was used to observe the strain for the presence of motile spores. Colonial growth from glycerol-asparagine agar or humic acid-yeast extract agar plates was suspended in 0.01 M sodium phosphate buffer (pH 7.0) containing 10% soil extract (Lochhead & Burton, 1957) and incubated for 30 min at 28 °C and aliquots were then observed by phase-contrast microscopy for the presence of motile spores. *Amycolatopsis fastidiosa* NRRL B-16697^T did not form motile spores on any of the media evaluated. Agar blocks containing colonial growth of NRRL B-16697^T were fixed with osmium tetroxide vapour, dehydrated through a graded acetone series, critical-point dried from liquid CO₂ and sputter-coated with gold prior to observation using a JEOL

Fig. 1. Phylogenetic tree for taxa of the suborder *Pseudonocardineae* calculated within ARB (Ludwig *et al.*, 2004) from almost-complete 16S rRNA gene sequences using Kimura's two-parameter evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987) illustrating the taxonomic position of *Amycolatopsis fastidiosa* NRRL B-16697^T relative to the other species of *Actinokineospora* and the other taxa within the suborder. Percentages at nodes represent levels of bootstrap support from 1000 resampled datasets; values less than 60% are not shown. Asterisks indicate that the corresponding branches were also recovered in maximum-parsimony (Felsenstein, 1993) and maximum-likelihood (Stamatakis *et al.*, 2002) trees. Bar, 0.01 nucleotide substitutions per site. Details of the grouped sequences used in construction of the tree are given in Supplementary Table S1, available in IJSEM Online.

Fig. 2. Scanning electron micrograph of growth of *Amycolatopsis fastidiosa* NRRL B-16697^T incubated for 6 weeks on glycerol-asparagine agar. Note the zigzag morphology of the aerial mycelium in (a) and the rod-shaped elements of varying length in (b) that may be spores. Bars, 1 µm.

model JSM 6400V scanning electron microscope. The observations of Henssen *et al.* (1987) regarding the zigzag morphology of the aerial mycelium (Fig. 2a) and the production of smooth-surfaced, rod-shaped spores of varying length (Fig. 2b) were confirmed.

A comparison of the chemotaxonomic characteristics of *Amycolatopsis fastidiosa*, determined by the methods of Grund & Kroppenstedt (1989), with those of phylogenetically nearest neighbouring genera (Table 1) provided strong evidence to support the placement of this taxon within the genus *Actinokineospora*, since it exhibits compositional traits virtually identical to those of species of this genus, including phosphatidylethanolamine and hydroxy fatty acid-containing phosphatidylethanolamine as the major polar lipids and MK-9(H₄) as the predominant menaquinone. It was felt that the whole-cell sugar profile might be crucial; hence a quantitative determination of sugar composition was performed by hydrolysing 50 mg freeze-dried biomass in 1.5 ml 0.5 M HCl by autoclaving for 20 min. The hydrolysate was neutralized after cooling by addition of 300 µl 17 M NH₄OH and then peracetylated aldonitrile derivatives of the sugars present were prepared and analysed by GC/MS by the method of Price (2004). The whole-cell sugar content of *Amycolatopsis fastidiosa* NRRL B-16697^T was compared directly with that of *Actinokineospora riparia* NRRL B-16432^T, the type strain of the type species of *Actinokineospora*, and was found to be identical (Supplementary Fig. S1) in containing galactose with smaller quantities of arabinose, mannose and rhamnose, further supporting the placement of this species in the genus *Actinokineospora*.

Table 1. Comparison of the chemotaxonomic profile of *Amycolatopsis fastidiosa* NRRL B-16697^T with the phylogenetically nearest taxa

Taxa: 1, *Amycolatopsis fastidiosa* NRRL B-16697^T; 2, *Actinokineospora*; 3, *Actinoalloteichus*; 4, *Actinosynnema*; 5, *Allokutzneria*; 6, *Crossiella*; 7, *Goodfellowiella*; 8, *Kibdelosporangium*; 9, *Kutzneria*; 10, *Streptoalloteichus*.

Character	1	2	3	4	5	6	7	8	9	10
Motile spores	—	+	—	+	—	—	—	—	—	+
Whole-cell sugars*	Gal, Ara, Rha, Man	Gal, Ara, Rha, Man	Glc, Gal, Man, Rib	Gal, Man	Ara, Gal, Man	Gal, Man, Rha, Rib	Gal, Rib	Ara, Gal, Glc, Rha	Gal, Rha	Gal, Man, Rha, Rib
Phospholipids†	PE, OH-PE, PI, DPG	PE, OH-PE	PIM, PI, PG, DPG, PME	PE, OH-PE, PG	PE, PME, OH-PE, PI, <i>lyso</i> -PME, DPG, PG, <i>lyso</i> -PE	PE, DPG, PI, PIM, PME	PE, DPG, OH-PE, PME	PE, PME, PG, PI	PE, DPG, PI, PG, PME	PE, DPG, PI, PIM, DPG, PME
Predominant menaquinone(s)	9(H ₄), 9(H ₂), 8(H ₄)	9(H ₄), 7(H ₄), 8(H ₄)	9(H ₄)	9(H ₄), 9(H ₆)	9(H ₄)	9(H ₄)	9(H ₄), 10(H ₄)	9(H ₄)	9(H ₄)	9(H ₆), 10(H ₆)
DNA G + C content (mol%)	73	69.1–72.0	72–72.5	71	71.6	71.4	69.2	66	70.3–70.7	71.6

*Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose.

†DPG, Diphosphatidylglycerol; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; *lyso*-PME, phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine.

The physiological characteristics of *Amycolatopsis fastidiosa* NRRL B-16697^T were evaluated by the methods of Celmer *et al.* (1977), Gordon *et al.* (1974), Shirling & Gottlieb (1966), de Boer *et al.* (1990) and Goodfellow *et al.* (1997, 2001). The differential morphological and physiological properties that distinguish *Amycolatopsis fastidiosa* from the described species of *Actinokineospora* are shown in Table 2.

The phylogenetic position of strain NRRL B-16697^T, along with its chemotaxonomic profile, clearly place it within the genus *Actinokineospora*. Production of motile spores, however, is a major trait in the formal description of the genus *Actinokineospora* (Hasegawa, 1988a); its absence from strain NRRL B-16697^T requires that the description of the genus be emended to accommodate species that have not been observed to produce them.

Emended description of the genus *Actinokineospora* Hasegawa 1988b

Actinokineospora (Ac.ti.no.ki'ne.o.spo'ra. Gr. n. *aktis* -inos a ray; Gr. v. *kineo* to set in motion; Gr. fem. n. *spora* a seed

and, in biology, a spore; N.L. fem. n. *Actinokineospora* actinomycete bearing zoospores).

Form hyphae (approx. 0.5 µm in diameter) that differentiate into a vegetative mycelium that penetrates the agar medium and forms colonies on the surface; aerial mycelium arises from the vegetative hyphae. Aerial hyphae generally bear chains of conidia capable of forming flagella in an aqueous environment, although non-motile spores may be produced by some species. Gram-positive. Catalase-positive. Aerobic. The cell wall contains *meso*-diaminopimelic acid as the diamino acid along with glycine, D-glutamic acid and L-alanine, properties characteristic of a type A1γ peptidoglycan. The characteristic whole-cell sugars are arabinose, galactose, mannose and rhamnose, but very little arabinose is found in purified cell walls. The phospholipid pattern consists of significant amounts of phosphatidylethanolamine, including phosphatidylethanolamine containing hydroxylated fatty acids. The principal menaquinone is MK-9(H₄). The G+C content of the DNA is 69.1–73.0 mol% (*T_m*). Placed phylogenetically within the suborder *Pseudonocardineae*

Table 2. Differential physiological properties of the type strains of *Actinokineospora* species

Strains: 1, *Actinokineospora fastidiosa* comb. nov. NRRL B-16697^T; 2, *Actinokineospora auranticolor* NBRC 16518^T (data from Otoguro *et al.*, 2001); 3, *Actinokineospora diospyrosa* NBRC 15665^T (Tamura *et al.*, 1995); 4, *Actinokineospora enzanensis* NBRC 16517^T (Otoguro *et al.*, 2001); 5, *Actinokineospora globicatena* NBRC 15664^T (Tamura *et al.*, 1995); 6, *Actinokineospora inagensis* NBRC 15663^T (Tamura *et al.*, 1995); 7, *Actinokineospora riparia* NBRC 14541^T (Hasegawa, 1988a); 8, *Actinokineospora terrae* NBRC 15668^T (Tamura *et al.*, 1995). +, Positive reaction or growth; –, negative reaction or no growth; v, variable reaction; w, weak reaction; ND, no data available.

Property	1	2	3	4	5	6	7	8
Colony reverse colour*	PY/Y	O	Y/B	G	Y/B	Y/B	Y/B	Y/B
Colour of aerial mycelium*	WH/PP	WH/G	WH/G	WH	WH/G	WH/G	WH	WH/G
Motile spores	–	+	+	+	+	+	+	+
Hydrolysis of starch	–	ND	+	ND	+	–	–	+
Production of:								
Hydrogen sulfide	–	+	+	+	+	+	–	+
Nitrate reductase	+	–	–	+	–	+	+	–
Growth on sole carbon sources (1.0 % w/v):								
L-Arabinose	+	–	–	–	–	–	–	w
D-Fructose	+	–	+	–	+	–	w	+
myo-Inositol	–	–	–	–	–	–	–	–
D-Mannitol	–	–	–	–	–	–	–	–
Raffinose	+	–	–	–	–	–	–	–
L-Rhamnose	–	–	w	–	+	w	–	w
D-Xylose	+	–	–	–	–	–	–	–
Growth in the presence of:								
2.0 % (w/v) NaCl	+	+	+	+	+	–	+	+
3.0 % (w/v) NaCl	+	v	+	–	+	–	–	+
Growth at:								
10 °C	+	+	+	–	+	–	–	+
37 °C	+	+	v	–	–	–	+	v
45 °C	+	ND	–	–	–	–	ND	–

*B, Brown; G, grey; O, orange; PP, pale pink; PY, pale yellow; WH, white; Y, yellow.

based on 16S rRNA gene sequences. The type species is *Actinokineospora riparia* Hasegawa 1988.

Description of *Actinokineospora fastidiosa* (Henssen *et al.* 1987) comb. nov.

Actinokineospora fastidiosa (fas.ti.di.o'sa. L. fem. adj. *fastidiosa* fastidious).

Basonym: *Amycolatopsis fastidiosa* Henssen *et al.* 1987.

Other synonym: '*Pseudonocardia fastidiosa*' Celmer *et al.* 1977.

The description is based on data from Celmer *et al.* (1977), Henssen *et al.* (1987) and the present study. Substrate mycelium is pale yellow to pale salmon, hyphae are irregularly branched, up to 1 µm wide and frequently zigzag shaped, occasionally with intercalary swellings. Aerial mycelium is sparse and white to pale pink and spores are rod-shaped, smooth (1.2–4.5 µm) and non-motile. Good growth on complex agar media, but aerial mycelium is only observed on a few media, such as Czapek sucrose agar and inorganic salts-starch agar. Yellowish to brown soluble pigments are produced on several media. Casein, aesculin and tyrosine are hydrolysed, but not allantoin, gelatin, hypoxanthine, starch or xanthine. Nitrate reductase and urease are produced, but hydrogen sulfide is not. Acid is produced from cellobiose and produced weakly from D-fructose but not from adonitol, L-arabinose, dextrin, dulcitol, *meso*-erythritol, D-galactose, D-glucose, glycerol, glycogen, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, methyl α-D-glucoside, D-ribose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose, turanose, D-xylose or xylitol. No utilization of carbohydrates on ISP 9 medium but, when ISP 4 medium without starch is used as the basal medium, L-arabinose, D-fructose, D-glucose, raffinose and D-xylose are utilized, while *myo*-inositol, D-mannitol and L-rhamnose are not. Sodium citrate, DL-lactic acid and sodium propionate are decarboxylated, but sodium benzoate, sodium mucate, sodium oxalate and sodium L-tartrate are not. Grows in the presence of 5% NaCl and at pH 10; no growth in lysozyme broth or at pH 5. Temperature range for growth is 10–45 °C, with an optimum of 28 °C. The G+C content of the DNA of the type strain is 73 mol% (*T_m*).

The type strain, NRRL B-16697^T = DSM 43855^T = ATCC 31181^T = NBRC 14105^T = JCM 3276^T = VKM Ac-1419^T, was isolated from a soil sample from Egypt.

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