Alloactinosynnema album gen. nov., sp. nov., a member of the family *Actinosynnemataceae* isolated from soil

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The taxonomic position of a Gram-stain-positive, aerobic strain, designated 03-9939^T, isolated from a soil sample collected from Xinjiang Province, China, was established using a polyphasic approach. Whole-cell hydrolysates of strain 03-9939^T contained galactose and ribose as diagnostic sugars and *meso*-diaminopimelic acid as the diamino acid. The predominant menaquinone was MK-9(H₄). The phospholipids consisted of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. The major fatty acids were iso-C_{16:0} (61.5 %) and iso-C_{16:1} H (11.6 %). The genomic DNA G + C content was 68.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 03-9939^T should be placed within the family *Actinosynnemataceae*, in which the strain formed a distinct lineage. Signature nucleotides in the 16S rRNA gene sequence showed that the strain contained a genus-specific diagnostic nucleotide signature pattern. The combination of phylogenetic analysis, phenotypic characteristics and chemotaxonomic data supported the conclusion that strain 03-9939^T represents a novel species in a new genus of the family *Actinosynnemataceae*, for which the name *Alloactinosynnema album* gen. nov., sp. nov. is proposed. Strain 03-9939^T (=DSM 45114^T =KCTC 19294^T =CCM 7461^T) is the type strain of *Alloactinosynnema album*.

The family *Actinosynnemataceae* was proposed by Labeda & Kroppenstedt (2000) to accommodate actinomycetes which display a type III cell-wall composition (*meso*-diaminopimelic acid, with galactose and rhamnose as diagnostic whole-cell sugars), type PII phospholipid pattern and MK-9(H₄) as the predominant menaquinone. The G + C content of the genomic DNA ranged from 68 to 76 mol%. At the time of writing, the family encompasses six genera with validly published names, *Actinokineospora* (Hasegawa, 1988), *Actinosynnema* (Hasegawa *et al.*, 1978), *Lentzea* (Labeda *et al.*, 2001), *Saccharothrix* (Labeda, 1986; Labeda & Lechevalier, 1989), *Lechevalieria* (Labeda *et al.*, 2001) and *Umezawaea* (Labeda & Kroppenstedt, 2007). In this paper, we report a polyphasic taxonomic study on

strain 03-9939^T, isolated from a soil sample collected from Xinjiang province, China.

Strain 03-9939^T was isolated following incubation at 28 °C for 3 weeks on Czapek's agar (Waksman, 1961). The purified strain was maintained on ISP 2 agar slants at 4 °C and as glycerol suspensions (20 %, v/v) at –20 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained by cultivation in shake flasks using tryptic soy broth (Difco) at 28 °C for 7 days. Cultural characteristics of the isolate were determined after growth for 7–28 days at 28 °C on ISP 2, ISP 3, ISP 4, ISP 5 (Shirling & Gottlieb, 1966), Czapek's agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961). The morphology of spore chains and structures resembling sporangia was examined using gold-coated dehydrated specimens of 14-day cultures from tryptone soy agar (TSA; Oxoid), ISP 2 and ISP 4 agar with a scanning electron microscope (Quanta; FEI). The coverslip technique (Zhou *et al.*, 1998) was employed to observe the characteristics of hyphae and spore chains.

Good growth was observed on tested media for strain 03-9939^T. White or buff to pink vegetative hyphae were

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 03-9939^T is EU438907.

Scanning electron micrographs of growth of strain 03-9939^T and its cellular fatty acid profile are available as supplementary material with the online version of this paper.

abundant, well-developed and fragmented into rod-shaped elements. Wrapped structures resembling sporangia were produced on the surface of the substrate mycelium on TSA (Supplementary Fig. S1a, available in IJSEM Online). White aerial hyphae were produced on tested media. Rod-shaped, smooth-surfaced spores were formed by fragmentation of the aerial hyphae (Supplementary Fig. S1b). The spore chains and aerial mycelium always aggregated into clusters (Supplementary Fig. S1c). Buff, diffusible pigment was produced on ISP 2, ISP 3, ISP 5 and Czapek's agar. These morphological characters were consistent with the typical properties of members of the family *Actinosynnemataceae* (Labeda & Kroppenstedt, 2000).

Growth was tested at 0, 4, 10, 15, 20, 28–37 (at intervals of 1.0 °C), 40, 45 and 55 °C on ISP 2. Other physiological and biochemical tests were performed at 28 °C. The pH range was examined at pH 4.0–11.0 (at intervals of 0.5 pH units). Tolerance of NaCl [0, 1, 3 and 5–10 % (at intervals of 0.5 %), w/v] and phenol (0.05, 0.1, 0.5 and 1.0 %) was examined using ISP 2 as basal medium. Carbon source utilization was tested as described by Shirling & Gottlieb (1966) and also by using API 50 CH strips according to the manufacturer's instructions (bioMérieux). Enzyme activities were examined qualitatively using the API ZYM test kit (bioMérieux). Other physiological tests and antimicrobial activities were examined following procedures described previously (Yuan *et al.*, 2008).

Good growth occurred at 28–37 °C, pH 6–8 and with 0–3 % (w/v) NaCl. Gelatin, starch and aesculin were hydrolysed, but not urea. Nitrate was reduced, while H₂S was not produced. Milk was coagulated and peptonized. The strain exhibited strong growth inhibition activity against *Staphylococcus aureus* CPCC 100051 and *Pseudomonas aeruginosa* CPCC 100109. The detailed physiological and biochemical characteristics of strain 03-9939^T are given in the species description.

The whole-cell sugar pattern and diagnostic isomers of diaminopimelic acid were determined by TLC (Lechevalier & Lechevalier 1965; Lechevalier & Lechevalier 1980). Menaquinones were extracted and analysed following the method of Collins (1985). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.* 1979). Cellular fatty acids were prepared and analysed following the standard Sherlock Microbial Identification System (MIDI, Inc.) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Whole-cell hydrolysates of strain 03-9939^T contained galactose and ribose. The diagnostic diamino acid was *meso*-diaminopimelic acid. The phospholipids comprised diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. The predominant menaquinone was MK-9(H₄) (95.1 %), with minor amounts of MK-9(H₆) (4.9 %). The detailed cellular fatty acid profile is given in Supplementary Table S1; iso-C_{16:0} and iso-C_{16:1} H were the major fatty acids.

Extraction of genomic DNA and amplification of the 16S rRNA gene were carried out as described by Li *et al.* (2007). Purified PCR products were sequenced with an ABI PRISM automatic sequencer. Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson *et al.*, 1997). Subsequently, a phylogenetic analysis was performed using the software package MEGA 3 (Kumar *et al.*, 2004). Distances were calculated using distance options according to Kimura's two-parameter model (Kimura, 1980, 1983) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985). The G+C content of genomic DNA was determined to be 68.2 mol% using the thermal denaturation (*T*_m) method (Marmur & Doty, 1962), with DNA of *Escherichia coli* AS 1.365 as a control.

A nearly complete 16S rRNA gene sequence (1442 bp) of strain 03-9939^T was obtained. BLAST search results using the 16S rRNA gene sequence of strain 03-9939^T showed that the new isolate exhibited highest similarity to members of the family *Actinosynnemataceae*. In the phylogenetic tree based on the 16S rRNA gene sequences of members of all genera in the family *Actinosynnemataceae* (Fig. 1), strain 03-9939^T formed a distinct lineage next to the genus *Actinokineospora*. Additionally, analysis of the 16S rRNA sequence of strain 03-9939^T demonstrated that 03-9939^T contained the nucleotide signatures T–A (823:975) and G–C (824:874) diagnostic for the family *Actinosynnemataceae* (Labeda & Kroppenstedt, 2000); meanwhile, strain 03-9939^T also possessed unique 16S rRNA signature nucleotides compared with other genera of the family *Actinosynnemataceae*, namely 603:635 (C–G), 617:623 (U–C) and 619 (U) (this study and Labeda & Kroppenstedt, 2007).

The chemotaxonomic characteristics of strain 03-9939^T were consistent with those of members of the family *Actinosynnemataceae*, e.g. *meso*-diaminopimelic acid as the cell-wall diamino acid, MK-9 as the principal menaquinone, straight-chain saturated and mono-unsaturated and iso- and anteiso-branched fatty acids and a relatively high DNA G+C content (68.2 mol%), within the range 68–76 mol%. The high 16S rRNA gene sequence similarities between the new isolate 03-9939^T and members of the family *Actinosynnemataceae* also support the affiliation of strain 03-9939^T with this family. However, significant differences in chemotaxonomic characteristics between strain 03-9939^T and the phylogenetically most closely related genera of the family *Actinosynnemataceae* listed in Table 1 distinguish strain 03-9939^T clearly from other genera of the family *Actinosynnemataceae*; galactose and ribose are present in the whole-cell sugar pattern without mannose or rhamnose, and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine are present as polar lipids without phosphatidylethanolamine. This distinction is supported by the position of strain 03-

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships among strain 03-9939^T and type strains of related genera within the family Actinosynnemataceae. Numbers at branch nodes are bootstrap values (percentages of 1000 replicates). Bar, 1 substitution per 100 nucleotide positions.

9939^T in the 16S rRNA gene sequence-based phylogenetic tree and by the diagnostic nucleotide signature pattern. Therefore, the studied strain should not be classified within any of the known genera of the family Actinosynnemataceae. On the basis of both phylogenetic and phenotypic distinctions, we propose that strain 03-9939^T should be classified as representing a novel species within a new genus, for which the name *Alloactinosynnema album* gen. nov., sp. nov. is proposed.

Description of *Alloactinosynnema* gen. nov.

Alloactinosynnema (Al.lo.ac.ti.no.syn.ne'ma. Gr. adj. *allos* other; N.L. neut. n. *Actinosynnema* a bacterial generic name; N.L. neut. n. *Alloactinosynnema* the other *Actinosynnema*, referring to the fact that it is morphologically similar to *Actinosynnema* but chemotaxonomically and phylogenetically distinct).

Aerobic, Gram-stain-positive. Lysozyme-resistant. Extensively branched, white or buff to pink substrate mycelium may fragment into rod-shaped elements. White aerial hyphae are produced, which differentiate into long chains of smooth-surfaced spores. The spore chains and aerial mycelium often aggregate into clusters. Structures resembling sporangia are produced on some media. Diffusible buff pigments are produced on some media. Contain *meso*-diaminopimelic acid as the diamino acid. The whole-cell sugar pattern consists of galactose and ribose. The phospholipid pattern consists mainly of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. The predominant menaquinone is MK-9(H₄). The fatty acid profile consists of straight-chain saturated and mono-unsaturated and iso- and

anteiso-branched fatty acids. The G+C content of the genomic DNA of the type strain of the type species is 68.2 mol%. The 16S rRNA gene contains a genus-specific pattern of diagnostic nucleotide signatures, namely 603:635 (C–G), 617:623 (U–C) and 619 (U). The type species is *Alloactinosynnema album*.

Description of *Alloactinosynnema album* sp. nov.

Alloactinosynnema album (al'bum. L. neut. adj. *album* white).

Morphological, chemotaxonomic and general characteristics are as described for the genus. Good growth occurs on ISP 2, ISP 3, ISP 4, ISP 5, Czapek's agar, nutrient agar and potato agar. Aesculin, casein, chitin, elastin, gelatin, hypoxanthine, starch, tyrosine and xylan are degraded, but not adenine, cellulose, xanthine or urea. Milk is coagulated and peptonized. Nitrate is reduced, but H₂S is not produced. Utilizes citrate, D-ribose, glucosamine, malonate, mannose, melampyrin (dulcitol), melibiose, phenylalanine, rhamnose, sorbitol, sucrose, tartrate, trehalose, turanose and xylose as sole carbon sources for energy and growth but does not use acetate, gluconate or methyl α-D-glucoside. Positive for acid phosphatase, alkaline phosphatase, esterase lipase C8, chymotrypsin, trypsin, α-glucosidase, β-galactosidase and β-glucosidase (API ZYM). Grows at 0–3 % (w/v) NaCl, 20–37 °C and pH 6.0–8.0. Optimal growth at 28–32 °C and pH 7.0–7.5. Sensitive to 0.1 % phenol. Exhibits antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Major fatty acids are iso-C_{16:0} and iso-C_{16:1} H.

Table 1. Chemotaxonomic characteristics of *Alloactinosynnema* gen. nov. (strain 03-9939^T) and the other genera of the family *Actinosynnemataceae*

Members of all genera have *meso*-diaminopimelic acid as the cell-wall diamino acid and are of cell-wall chemotype III. Data for strain 03-9939^T are from this study; other data are from Labeda (1986) and Labeda & Lechevalier (1989) (*Saccharothrix*), Labeda *et al.* (2001) (*Lechevalieria*) and Labeda & Kroppenstedt (2007) (remaining genera).

Characteristic	Strain 03-9939 ^T	<i>Actinosynnema</i>	<i>Lechevalieria</i>	<i>Lentzea</i>	<i>Saccharothrix</i>	<i>Umezawaea</i>	<i>Actinokineospora</i>
Whole-cell sugars*	Gal, Rib	Gal, Man	Gal, Man, Rha	Gal, Man, Rib	Gal, Rha, Man (trace)	Gal, Man, Rib, Rha (trace)	Gal, Man, Rha
Phospholipid(s)†	DPG, PG, PC	PE, OH-PE, PI, PIM, DPG	PE	PE, DPG, PG, PI	PE, OH-PE, PI, PIM, DPG, PG	PE, PI, OH-PE, lyso-PE, DPG, PIM	PE
Predominant menaquinone(s)	MK-9(H ₄)	MK-9(H ₄), MK-9(H ₆)	MK-9(H ₄)	MK-9(H ₄)	MK-10(H ₄), MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Major fatty acid(s) (>5.0%)‡	i-C _{16:0} , i-C _{16:1}	C _{15:0} , C _{16:0} , C _{17:0} , C _{18:0} , ai-C _{15:0} , i-C _{16:0} , ai-C _{17:0}	i-C _{14:0} , i-C _{15:0} , i-C _{16:0} , i-C _{16:1}	i-C _{14:0} , i-C _{15:0} , i-C _{16:0} , ai-C _{15:0} , C _{16:1} ω ₉ C	i-C _{15:0} , i-C _{16:0} , i-C _{16:1} , ai-C _{17:0}	i-C _{14:0} , i-C _{15:0} , i-C _{16:0} , i-C _{16:1}	i-C _{16:0}

*Gal, Galactose; Man, mannose; Rha, rhamnose; Rib, ribose.

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

‡ai, Anteiso-branched; i, iso-branched.

The type strain, 03-9939^T (=DSM 45114^T =KCTC 19294^T =CCM 7461^T), was isolated from a soil sample collected in Xinjiang Province, China.

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