Lechevalieria fradiae sp. nov., a novel actinomycete isolated from soil in China

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The taxonomic position of a soil isolate, strain Z6^T, was established using a polyphasic approach. The organism showed a range of chemical and morphological properties consistent with its classification in the genus *Lechevalieria*. An almost complete 16S rRNA gene sequence determined for the strain was aligned with corresponding sequences of representatives of the genus *Lechevalieria* and related taxa using three tree-making algorithms. The organism formed a distinct phyletic line within the evolutionary radiation occupied by the genus *Lechevalieria* and was more closely related to the type strain of *Lechevalieria aerocolonigenes* than to that of *Lechevalieria flava*. Strain Z6^T could be distinguished from both these strains using DNA–DNA relatedness data and by a combination of phenotypic properties. The combined genotypic and phenotypic data show that strain Z6^T should be assigned to the genus *Lechevalieria* as a representative of a novel species. The name proposed for this new taxon is *Lechevalieria fradiae* sp. nov. The type strain is Z6^T (=CGMCC 4.3506^T=JCM 14205^T).

The genus *Lechevalieria* was proposed by Labeda *et al.* (2001) to accommodate aerobic, Gram-positive actinomycetes that form an extensively branched substrate mycelium and scant aerial hyphae, and contain *meso*-diaminopimelic acid in whole-organism hydrolysates (wall chemotype III *sensu* Lechevalier & Lechevalier, 1970), galactose, mannose and rhamnose as diagnostic sugars, tetrahydrogenated menaquinones as the predominant isoprenologue, phosphatidylethanolamine as the key polar lipid (phospholipid type II *sensu* Lechevalier *et al.*, 1977) and fatty acids rich in saturated and monounsaturated iso- and anteiso-components. The genus encompasses two species, *Lechevalieria aerocolonigenes*, the type species, and *Lechevalieria flava*; these taxa form a distinct branch within the evolutionary radiation occupied by the family *Actinosynnemataceae* Labeda and Kroppenstedt 2000. This family also contains the genera *Actinosynnema* Hasegawa *et al.* 1978, *Lentzea* Labeda *et al.* 2001 and *Saccharothrix* Labeda *et al.* 1984.

The present investigation was designed to establish the taxonomic position of a soil organism, isolate Z6^T, which had been considered by Xunchu Yan in 1978 (unpublished) to be a strain of '*Streptomyces fradiae*' on the basis of a few biochemical, morphological and physiological properties.

Here, a polyphasic taxonomic approach was used to study this isolate; the data showed that it should be recognized as a representative of a novel species of *Lechevalieria*.

Strain Z6^T was isolated on a glucose-yeast extract-malt extract agar plate, which had been seeded with a soil suspension and incubated at 28 °C for 2 weeks. The soil sample was collected from Wutaishan Mountain in Shanxi Province, China. It was maintained on modified Sauton's agar (Mordarska *et al.*, 1972) at 4 °C and as suspensions of mycelial fragments in 20 % (v/v) glycerol at –20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared as described by Zhang *et al.* (2002).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product from isolate Z6^T were carried out after Rainey *et al.* (1996) and the PCR product was sequenced directly following the procedure described by Lu *et al.* (2001). The resultant 16S rRNA gene sequence was aligned manually with corresponding sequences of representatives of genera classified in the suborder *Pseudonocardineae* retrieved from the DDBJ/EMBL/GenBank databases using CLUSTAL_X 1.8 software (Thompson *et al.*, 1997). Phylogenetic trees were generated using the least-squares, maximum-likelihood and neighbour-joining algorithms from the PHYLIP suite of programs (Felsenstein, 1993); evolutionary distance matrices for the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Z6^T is AY114175.

least-squares and neighbour-joining methods were produced after Kimura (1980). Topologies of the resultant unrooted trees were evaluated by bootstrap analyses of the neighbour-joining dataset based on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP package. In a corresponding second analysis, the sequence of the tested strain was compared with those of members of genera classified in the family Actinosynnemataceae.

It is apparent from Fig. 1 that strain Z6^T forms a distinct phyletic line that lies towards the periphery of the *Lechevalieria* 16S rRNA clade. It shared 16S rRNA gene similarities of 98.4 and 98.0% with the type strains of

L. aerocolonigenes and *L. flava*, respectively, values that correspond to 22 and 28 nt differences at 1407 positions. It is also significant that the 16S rRNA gene sequence of the isolate includes signature nucleotides that are characteristic of members of the genus *Lechevalieria* (Labeda *et al.*, 2001), the family Actinosynnemataceae (Labeda & Kroppenstedt, 2000) and the suborder Pseudonocardineae (Stackebrandt *et al.*, 1997).

The isolate was examined for a range of chemotaxonomic markers to determine whether its chemical profile was typical of members of the genus *Lechevalieria* (Labeda *et al.*, 2001). Standard chromatographic procedures were used for

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain Z6^T and representatives of known species classified in genera assigned to the family Actinosynnemataceae. Asterisks indicate branches of the tree that were also recovered using the least-squares and maximum-likelihood tree-making algorithms; 'f' denotes branches of the tree that were recovered using the least-squares method. Numbers at nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given. Bar, 0.02 substitutions per nucleotide position.

the extraction and analysis of the diagnostic isomers of diaminopimelic acid (A₂pm; Hasegawa *et al.*, 1983), menaquinones (Collins *et al.*, 1987; Wu *et al.*, 1989), mycolic acids (Minnikin *et al.*, 1975), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Lechevalier & Lechevalier, 1980), using appropriate controls. In turn, fatty acids were extracted, purified, methylated and quantified by GC using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The base composition of the genomic DNA of the isolate was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as the control. The organism contained: *meso*-A₂pm as the wall diamino acid; galactose, mannose and rhamnose as diagnostic sugars; tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue and dihydrogenated menaquinones with nine isoprene units as the minor isoprenologue (peak area MK-9(H₄): MK-9(H₂) ratio of 27:10); phosphatidylethanolamine and diphosphatidylglycerol as the key polar lipids; and fatty acids rich in saturated and mono-unsaturated iso- and anteiso-components. However, strain Z6^T lacked mycolic acids. The G + C content of the DNA of the isolate was 68.0 mol%. All of these properties are consistent with the classification of the isolate in the genus *Lechevalieria*.

Colonial and micromorphological properties were examined on glucose-yeast extract-malt extract agar, modified Sauton's agar (Mordarska *et al.*, 1972) and on standard media used in the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966). Micromorphological features were observed using the coverslip technique of Kawato & Shinobu (1959) following the procedure described by Zhou *et al.* (1998). Peptone-yeast extract-iron agar and tyrosine agar plates (Shirling & Gottlieb, 1966) were examined for the production of melanin pigments following incubation at 28 °C for 14 days. Additional phenotypic properties were determined using standard media and methods (Gordon *et al.*, 1974; Zhang *et al.*, 2003). DNA–DNA relatedness studies were carried out between the isolate and the type strains of *L. aerocolonigenes* and *L. flava* using a thermal denaturation procedure (De Ley *et al.*, 1970; Huß *et al.*, 1983) with a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermobath and standard software (Jahnke, 1992); the results were expressed as the mean of two determinations.

Strain Z6^T exhibited phenotypic properties typical of members of the genus *Lechevalieria* (Labeda *et al.*, 2001). It was an aerobic, Gram-positive, non-motile, catalase-positive actinomycete, which formed an extensively branched substrate mycelium that fragmented into coccoid- and rod-shaped elements. The mean DNA–DNA hybridization values found between the isolate and the type strains of *L. aerocolonigenes* and *L. flava* were 45 and 37 %, respectively; values well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for the delineation of genomic species. The isolate could also be distinguished

Table 1. Differential phenotypic properties of strain Z6^T and the type strains of the two *Lechevalieria* species

Strains: 1, strain Z6^T; 2, *L. aerocolonigenes* NRRL B-3298^T; 3, *L. flava* NRRL B-16131^T. Data were taken from this and previous studies (Labeda, 1986; Labeda *et al.*, 2001; Grund & Kroppenstedt, 1989). All strains grow at 37 °C. +, Positive; –, negative; W, weak; ND, not determined.

Property	1	2	3
Nitrate reduction	+	+	–
Decomposition of:			
Casein	+	+	–
Hypoxanthine	–	+	+
Production of acid from:			
Adonitol	–	+	W
Galactose	–	+	ND
Inositol	–	+	ND
Lactose	–	+	ND
Salicin	–	+	–
Assimilation of:			
Citrate	–	+	–
Lactate	+	+	–
Malate	+	+	–
Malonate	+	+	–
Oxalate	–	+	ND
Tartrate	–	+	ND
Growth on sole carbon sources:			
Sorbitol	+	–	–
Xylose	+	+	–
Growth in the presence of:			
5 % NaCl	+	+	–
6 % NaCl	W	–	–
Growth at 45 °C	+	–	+

from the two type strains using a combination of phenotypic properties (Table 1).

It is evident from the genotypic and phenotypic data that strain Z6^T can be distinguished from the two known species of *Lechevalieria*. It is therefore proposed that the strain be classified in the genus *Lechevalieria* as *Lechevalieria fradiae* sp. nov.

Description of *Lechevalieria fradiae* sp. nov.

Lechevalieria fradiae (fra' di. ae. N.L. gen. n. *fradiae* of Fradia, a patronymic).

Aerobic, Gram-positive, catalase-positive, non-motile actinomycete. Forms an extensively branched substrate mycelium that fragments *in situ* into coccoid- and rod-shaped elements. A yellow to orange mycelium is formed on ISP media 2, 3 and 4, glucose-yeast extract-malt extract agar and modified Sauton's agar. Soluble pigments are not produced nor are melanin pigments formed on glucose-yeast extract-iron or tyrosine agars. Colony elevation is convex to irregular and colony margins are filamentous.

Grows at 15–45 °C and pH 5–10. Aesculin, arbutin, gelatin and urea are hydrolysed and nitrate is reduced. H₂S is not produced. Degrades casein, starch and tyrosine, but not adenine, guanine, hypoxanthine or xanthine. Acid is formed from (+)-L-arabinose, meso-erythritol, methyl α -D-glucoside, (+)-D-raffinose, (+)-D-trehalose and (+)-D-xylose, but not from (+)-D-cellobiose, dextrin, (+)-D-fructose, (+)-D-glucose, glycerol, (+)-D-maltose, (+)-D-mannitol, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, α -L-rhamnose, (+)-D-sorbitol or (+)-D-sucrose. Sodium acetate, sodium lactate, sodium malate, sodium malonate and sodium succinate are used as sole carbon and energy sources, but not sodium benzoate, sodium citrate, sodium oxalate or sodium tartrate. Growth occurs in the presence of crystal violet (0.001 %, w/v) and phenol at 0.05 % (w/v), but not in the presence of phenol at 0.1 % (w/v). Resistant to (in $\mu\text{g ml}^{-1}$ unless otherwise stated): lysozyme (0.005 %, w/v); amoxycillin plus clavulanic acid (10); ampicillin (10); aztreonam (30); clindamycin hydrochloride (2); mezlocillin (75); and penicillin G (10 international units). Susceptible to (in $\mu\text{g ml}^{-1}$): amikacin (30); cefotaxime (30); chloramphenicol (30); ciprofloxacin (5); erythromycin (15); gentamicin sulfate (10); kanamycin sulfate (30); ofloxacin (5); rifampicin (5); streptomycin sulfate (10); tetracycline hydrochloride (30); and tobramycin sulfate (10). Additional phenotypic properties are shown in Table 1. Chemotaxonomic properties are typical of the genus *Lechevalieria*. The fatty acid profile is composed mainly of iso-C_{16:0} (56.8 %), iso-C_{14:0} (13.8 %), H-iso-C_{16:1} (12.3 %), C_{17:1} ω 6c (3.5 %), iso-C_{15:0} (3.3 %) and C_{15:1} ω 6c (3.0 %).

The type strain is Z6^T (=CGMCC 4.3506^T = JCM 14205^T), isolated from a soil sample collected from Wutaishan Mountain, Shanxi Province, China. The DNA G + C content of strain Z6^T is 68.0 mol%. The species description is based on a single strain and hence serves as a description of the species.

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