Lechevalieria xinjiangensis sp. nov., a novel actinomycete isolated from radiation-polluted soil in China

Wei Wang,¹ Zhidong Zhang,¹ Qiyong Tang,¹ Jun Mao,¹ Dong Wei,¹ Ying Huang,² Zhiheng Liu,² Yuhu Shi¹ and Michael Goodfellow³

Correspondence

Ying Huang

huangy@im.ac.cn

¹Institute of Microbiology, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, Xinjiang Uygur Autonomous Region, People's Republic of China

²State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

³Division of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

A novel actinomycete was isolated from radiation-polluted soil collected from Xinjiang in north-western China. The isolate, strain R24^T, was found to have chemical and morphological properties associated with members of the genus *Lechevalieria*. An almost-complete 16S rRNA gene sequence of the isolate was aligned with corresponding sequences from representatives of the genus *Lechevalieria* and related taxa, using three tree-making algorithms, and was shown to form a distinct phyletic line in the *Lechevalieria* phylogenetic tree. DNA–DNA relatedness and phenotypic data served to distinguish strain R24^T from members of the three *Lechevalieria* species with validly published names. The genotypic and phenotypic data show that the isolate should be classified as a novel species within the genus *Lechevalieria*. The name proposed for this taxon is *Lechevalieria xinjiangensis* sp. nov. The type strain is R24^T (=CGMCC 4.3525^T=DSM 45081^T).

The genus *Lechevalieria* Labeda *et al.* (2001) belongs to the family *Actinosynnemataceae* (Labeda & Kroppenstedt, 2000) which also includes the genera *Actinosynnema*, *Actinokineospora*, *Lentzea* (Labeda *et al.*, 2001) and *Saccharothrix*. The genus currently encompasses three species, *Lechevalieria aerocolonigenes* (Labeda *et al.*, 2001), *Lechevalieria flava* (Labeda *et al.*, 2001) and *Lechevalieria fradiae* (Zhang *et al.*, 2007), which form a distinct branch in the *Actinosynnemataceae* phylogenetic tree based on 16S rRNA gene sequences (Zhang *et al.*, 2007); representatives of these species can be distinguished using a combination of phenotypic properties. During an investigation of microbial sources in Xinjiang, north-western China, an actinomycete, strain R24^T, was isolated from a radiation-contaminated soil sample and was found to have morphological properties characteristic of the genus *Lechevalieria*. The aim of the present study was to determine the taxonomic status of the isolate by using a polyphasic approach. It was evident from the resultant data that the organism represents a novel species of the genus *Lechevalieria*.

Strain R24^T was isolated from a plate containing modified Bennett's agar (Jones, 1949) that had been inoculated with

a soil suspension and incubated at 28 °C for 14 days. The soil sample was collected from radiation-polluted soil in the Xinjiang Uygur Autonomous Region of China. The organism was maintained on modified Sauton's agar slopes (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 analyses was prepared as described previously (Xie *et al.*, 2002); the cells for the chemical studies were freeze-dried.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA and purification of the PCR product from isolate R24^T were carried out as described by Chun & Goodfellow (1995); the PCR product was sequenced directly using a *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer. The resultant 16S rRNA gene sequence was aligned manually with the corresponding sequences of representatives of genera of the family *Actinosynnemataceae*, as retrieved from the GenBank/EMBL/DDBJ databases using the CLUSTAL_X 1.8 software (Thompson *et al.*, 1997). Evolutionary distances for the neighbour-joining algorithm were calculated using the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were generated using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms from MEGA, version 3.1 (Kumar *et al.*,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R24^T is DQ898283.

2004), and the maximum-likelihood (Felsenstein, 1981) method from PHYLIP, version 3.5c (Felsenstein, 1993). The topologies of the resultant unrooted trees were evaluated in an analysis of the neighbour-joining dataset based on 1000 replicates. It is apparent from Fig. 1 that strain R24^T falls within the evolutionary radiation occupied by the genus *Lechevalieria*.

Isolate R24^T was examined for a range of diagnostic chemotaxonomic markers to determine whether or not it belongs to the genus *Lechevalieria*. Standard chromatographical procedures were used for the extraction and analysis of the isomers of diaminopimelic acid (Hasegawa *et al.*, 1983; Lechevalier & Lechevalier, 1980), the menaquinones (Collins *et al.*, 1987), the mycolic acids (Minnikin *et al.*, 1975), the polar lipids (Minnikin *et al.*, 1984) and the whole-cell sugars (Hasegawa *et al.*, 1983; Lechevalier & Lechevalier, 1980), using appropriate controls. Fatty acids were extracted, purified, methylated and quantified by gas chromatography using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G+C content of the genomic DNA of the strain was determined using the thermal denaturation method (Marmur & Doty, 1962), with *Escherichia coli* K-12 as the control.

Strain R24^T contained *meso*-diaminopimelic acid as the wall diamino acid, galactose, mannose, rhamnose, glucose and ribose as the diagnostic whole-cell sugars (cell-wall chemotype III *sensu* Lechevalier & Lechevalier, 1970), phosphatidylethanolamine and phosphatidylmethyl-ethanolamine as major polar lipids (phospholipid type II

sensu Lechevalier *et al.*, 1977), tetrahydrogenated menaquinones with nine isoprene units [MK-9(H₄)] as the predominant isoprenologues, fatty acids rich in saturated iso and anteiso components and lacked mycolic acids. These properties correspond with classification of the isolate as belonging to the genus *Lechevalieria* (Labeda, 1986; Grund & Kroppenstedt, 1989; Zhang *et al.*, 2007).

It is evident from Fig. 1 that isolate R24^T is most closely related to the type strain of *L. flava*. This relationship is supported by a relatively high bootstrap percentage and by all three tree-making algorithms. The isolate shares 16S rRNA gene sequence similarities with the type strains of *L. flava*, *L. aerocolonigenes* and *L. fradiae* of 98.8, 98.3 and 97.6 %, respectively. The 16S rRNA gene sequence of the isolate contains 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 levels of DNA–DNA relatedness between strain R24^T and the type strains of the three *Lechevalieria* species were determined using the renaturation method (De Ley *et al.*, 1970) with a Perkin Elmer Lambda 35UV/VIS spectrophotometer fitted with the Peltier temperature controller, PTP-1. Genomic DNA was sheared by ultrasonication to give a mean fragment size of 300–700 bp and the sheared preparations were adjusted to an OD₂₆₀ of 2.0 in 2 × SSC buffer (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate). Denaturation was achieved at 99 °C after 10 min; the hybridization was carried out at 82 °C. The values for DNA–DNA relatedness between strain R24^T and *L. aerocolonigenes* NRRL B-3298^T, *L. flava* NRRL B-16131^T and *L. fradiae* Z6^T were 44, 28 and 34 %, respectively; these values are well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987).

The morphological properties of cells of strain R24^T were examined by light microscopy (Axioskop 20; Zeiss) and scanning electron microscopy (FEI Quanta) after incubation for 14 days at 28 °C. These incubation conditions were also used to determine colonial and pigmentation properties on inorganic salts-starch agar (ISP medium 4), yeast extract-malt extract agar (ISP medium 2), as described by Shirling & Gottlieb (1966) and on modified Bennett's agar (Jones, 1949), Gauze's synthetic medium no. 1 (DSMZ medium no. 1048) and yeast-starch agar (DSMZ medium no. 1027). The production of melanin pigments was examined on peptone-yeast extract-iron agar and tyrosine agar plates (Shirling & Gottlieb, 1966). The physiological characteristics of strain R24^T and type strains of the recognized *Lechevalieria* species were tested together using standard media and standard methods (Gordon *et al.*, 1974; Yassin *et al.*, 1995). Strain R24^T was shown to have phenotypic properties that are characteristic of the members of the genus *Lechevalieria* (Labeda *et al.*, 2001; Zhang *et al.*, 2007). The strain is an aerobic, Gram-positive, catalase-positive actinomycete that forms an extensively

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain R24^T and representatives of the family *Actinosynnemataceae*. Asterisks indicate branches that were also recovered using maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. Numbers at the nodes indicate bootstrap percentages (based on 1000 resampled datasets). Bar, 0.005 substitutions per nucleotide position.

branched substrate mycelium that carries moderate amounts of aerial hyphae; the mycelium fragments into rod-like elements (Fig. 2). A range of phenotypic properties (Table 1) serve to distinguish this organism from representatives of the three *Lechevalieria* species with validly published names.

On the basis of the combined genotypic and phenotypic data, strain R24^T warrants classification as a novel species of the genus *Lechevalieria*, for which the name *Lechevalieria xinjiangensis* sp. nov. is proposed.

Description of *Lechevalieria xinjiangensis* sp. nov.

Lechevalieria xinjiangensis (xin.jiang.en'sis. N.L. fem. adj. *xinjiangensis* referring to Xinjiang, north-western China, the source of the isolate).

Aerobic, Gram-positive, catalase-positive, non-motile actinomycete that forms an extensively branched substrate mycelium that fragments *in situ* into rod-shaped elements. Moderate amounts of white to yellow aerial hyphae are formed on modified Bennett's agar, Gauze's no. 1 agar, inorganic salts-starch agar, yeast extract-malt extract agar and yeast extract-starch agar. Soluble pigments are not produced and melanin pigments are not produced on peptone-yeast extract-iron agar or tyrosine agar. Grows at temperatures between 10 and 45 °C and at pH 5.0–12.0. Does not degrade elastin or Tween 80. L-Arabinose, L-cellobiose, L-fucose, *myo*-inositol, D-lactose, lactulose, D-mannitol, L-rhamnose and sucrose are used as sole carbon sources for energy and growth (all at 1 %, w/v). Similarly, D-alanine, L-cysteine, D-glutamic acid, hydroxy-L-proline, DL-isoleucine, L-leucinamide, L-leucine, sodium acetate, sodium propionate and sodium suberate are used as sole carbon sources at 0.1 % (w/v). L-Cysteine, L-phenylalanine

Fig. 2. Scanning electron micrograph showing rod-shaped fragments of substrate mycelium of strain R24^T grown on modified Bennett's agar for 14 days at 28 °C. Bar, 5.0 µm.

Table 1. Differential phenotypic characteristics of strain R24^T and the type strains of *Lechevalieria* species

Strains: 1, R24^T; 2, *L. aerocolonigenes* NRRL B-3298^T; 3, *L. flava* NRRL B-16131^T; 4, *L. fradiae* Z6^T. Data were taken from this study. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Nitrate reduction	+	+	–	+
Decomposition of:				
Casein	+	+	–	+
Aesculin	–	+	+	–
Hypoxanthine	–	+	+	–
Tyrosine	–	–	–	+
Urea	–	+	+	+
Production of acid from:				
Dextrin	–	–	+	+
D-Glucose	+	+	+	–
D-Mannose	–	+	+	–
D-Melezitose	–	+	+	+
L-Rhamnose	–	+	–	+
D-Sorbitol	–	–	+	–
Sucrose	–	–	+	+
Trehalose	–	+	+	–
D-Fructose	–	+	–	–
D-Galactose	+	+	+	–
D-Mannitol	+	+	+	–
Inositol	+	+	+	–
Lactose	+	+	+	–
Salicin	–	+	–	–
Sodium succinate	+	–	–	–
Assimilation of:				
Citrate	+	+	–	–
Malonate	+	+	–	+
Oxalate	+	+	–	–
Growth on sole carbon sources:				
Sorbitol	+	–	–	+
Xylose	+	+	–	+
Growth in the presence of:				
4 % NaCl	+	+	–	+
5 % NaCl	w	+	–	w
0.1 % NaN ₃	–	+	+	+
0.1 % Phenol	–	+	+	+
0.001 % Methyl violet	+	–	+	+
Growth at 45 °C	+	–	+	+

and potassium nitrate are used as sole sources of nitrogen. Acid is produced from L-arabinose, D-cellobiose, glycerol, maltose and D-xylose, but not from D-raffinose, sodium acetate, sodium lactate, sodium malonate, sodium benzoate, sodium citrate, sodium oxalate or sodium tartrate. 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_{15:0} (10.7 %), anteiso-C_{15:0} (9.8 %), iso-C_{16:0} (19.9 %), anteiso-C_{17:0} (13 %), iso-C_{17:0} (4.0 %), C_{16:1} (7.4 %) and C_{18:0} (4.5 %). The DNA G+C content is 68.6 mol%.

The type strain, R24^T (=CGMCC 4.3525^T=DSM 45081^T), was isolated from a radiation-polluted soil sample collected in the Xinjiang Uygur Autonomous Region of China. The species description is based on a single strain and hence serves as a description of the species.

Acknowledgements

The work was supported by the Hi-Tech Research and Development Program of China (grant no. 2004AA227110) and through the Royal Society–Chinese Academy of Sciences Exchange Scheme (grant no. Q814). The authors are grateful to Dr Yuhua Xin (CGMCC, China General Microbiological Culture Collection, Chinese Academy of Sciences) for providing the type strains of the *Lechevalieria* species.

References

- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Collins, M. D., Howarth, O. W., Grund, E. & Kroppenstedt, R. M. (1987). Isolation and structural determination of new members of the vitamin K₂ series in *Nocardia brasiliensis*. *FEMS Microbiol Lett* **41**, 35–39.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurements of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Grund, E. & Kroppenstedt, R. M. (1989). Transfer of five *Nocardiopsis* species to the genus *Saccharothrix* Labeda et al. 1984. *Syst Appl Microbiol* **12**, 267–274.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Labeda, D. P. (1986). Transfer of “*Nocardia aerocolonigenes*” (Shinobu and Kawato 1960) Pridham 1970 into the genus *Saccharothrix* Labeda, Testa, Lechevalier, and Lechevalier 1984 as *Saccharothrix aerocolonigenes* sp. nov. *Int J Syst Bacteriol* **36**, 109–110.
- Labeda, D. P. & Kroppenstedt, R. M. (2000). Phylogenetic analysis of *Saccharothrix* and related taxa: proposal for *Actinosynnemataceae* fam. nov. *Int J Syst Evol Microbiol* **50**, 331–336.
- Labeda, D. P., Hatano, K., Kroppenstedt, R. M. & Tamura, T. (2001). Revival of the genus *Lentzea* and proposal for *Lechevalieria* gen. nov. *Int J Syst Evol Microbiol* **51**, 1045–1050.
- Lechevalier, H. A. & Lechevalier, M. P. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, Special Publication 6, pp. 227–291. Arlington, VA: Society for Industrial Microbiology.
- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Marmur, J. & Doty, P. (1962). Determination of base composition of deoxyribonucleic acid from its denaturation temperature. *J Mol Biol* **5**, 109–118.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolytates. *J Gen Microbiol* **88**, 200–204.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Mordarska, H., Mordarski, M. & Goodfellow, M. (1972). Chemotaxonomic characters and classification of some nocardioform bacteria. *J Gen Microbiol* **71**, 77–86.
- Saitou, N. & Nei, M. (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Xie, Q., Wang, Y., Huang, Y., Wu, Y., Ba, F. & Liu, Z. (2002). Description of *Lentzea flaviverrucosa* sp. nov. and transfer of *Saccharothrix aerocolonigenes* subsp. *stauroporea* to *Lentzea albidia*. *Int J Syst Evol Microbiol* **52**, 1815–1820.
- Yassin, A. F., Rainey, F. A., Brzezinka, H., Jahnke, K.-D., Weissbrodt, H., Budzikiewicz, H., Stackebrandt, E. & Schaal, K. P. (1995). *Lentzea* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **45**, 357–363.
- Zhang, J., Xie, Q., Liu, Z. & Goodfellow, M. (2007). *Lechevalieria fradiae* sp. nov., a novel actinomycete isolated from soil in China. *Int J Syst Evol Microbiol* **57**, 832–836.