Lentzea jiangxiensis sp. nov., isolated from acidic soil

Xiaomin Li,^{1,2} Limin Zhang,¹ Yun Ding,^{1,2} Yongsheng Gao,²
Jisheng Ruan¹ and Ying Huang¹

Correspondence
Ying Huang
huangy@im.ac.cn

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

²College of Land Resources and Environment, Jiangxi Agricultural University, Nanchang 330045, Jiangxi Province, PR China

A novel actinomycete, designated strain FXJ1.034^T, was isolated from acidic soil collected in Jiangxi Province, South-east China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain FXJ1.034^T belonged to the genus *Lentzea* and showed high sequence similarities to *Lentzea kentuckyensis* NRRL B-24416^T (98.5 %) and *Lentzea albida* NBRC 16102^T (98.3 %). Morphological and chemotaxonomic characteristics supported its assignment to the genus *Lentzea*. The results of DNA–DNA hybridization, physiological and biochemical tests allowed genotypic and phenotypic differentiation of the novel strain from closely related species. Based on the evidence presented here, strain FXJ1.034^T represents a novel species of the genus *Lentzea*, for which the name *Lentzea jiangxiensis* sp. nov. is proposed. The type strain is FXJ1.034^T (=CGMCC 4.6609^T =NBRC 106680^T).

The genus *Lentzea* was first proposed by Yassin *et al.* (1995) to accommodate aerobic, Gram-positive actinomycetes that form abundant aerial hyphae, which fragment into rod-shaped elements. Members of this group were transferred to the genus *Saccharothrix* by Lee *et al.* (2000) based on phylogenetic and chemotaxonomic data but the genus was revived the following year by Labeda *et al.* (2001) based on more extensive phylogenetic and chemotaxonomic analyses and diagnostic nucleotide signatures of the 16S rRNA genes, which were published with an emended description reflecting changes in the chemotaxonomic characteristics. Members of the genus *Lentzea* contain *meso*-diaminopimelic acid as the cell-wall diamino acid, galactose, mannose and traces of ribose as diagnostic whole-cell sugars and MK-9 (H₄) as the principal menaquinone; the phospholipid pattern type is PII and the fatty acid pattern mainly comprises *iso*/*anteiso* branched fatty acids. This genus presently belongs to the large family *Pseudonocardiaceae* (Labeda *et al.*, 2011) and comprises seven species with validly published names (<http://www.bacterio.cict.fr/l/lentzea.html>). The current study was designed to establish the taxonomic position of a soil isolate, designated FXJ1.034^T. Genotypic and

phenotypic data showed that it represents a novel species of the genus *Lentzea*.

Strain FXJ1.034^T was isolated from acidic soil (pH 5.0) collected from Jiangxi Agricultural University, Nanchang city, Jiangxi Province, China. The sample was dried at room temperature, ground into powder and then suspended in sterile distilled water and serially diluted. The diluted soil suspension was inoculated on modified GTV agar (Busti *et al.*, 2006) containing 500 ml l⁻¹ soil extract (prepared by autoclaving 100 g fresh soil resuspended in 500 ml H₂O, followed by filtering through sterile gauze), 25.0 g l⁻¹ agar and 3 mM CaCl₂ and supplemented with 0.1 % (v/v) B-vitamin solution (0.05 g thiamine–HCl, 0.05 g riboflavin, 0.05 g niacin, 0.05 g pyridoxin–HCl, 0.05 g inositol, 0.05 g calcium pantothenate, 0.025 g biotin, 0.05 g *p*-aminobenzoic acid and 100 ml distilled water; pH 5.0), cycloheximide (50 mg l⁻¹), nystatin (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹), followed by incubation at 28 °C for 2 weeks. The strain was maintained on glucose–yeast extract–malt extract agar (ISP 2 medium; International *Streptomyces* project; Shirling & Gottlieb, 1966; pH 5.0) at 4 °C and as suspensions of mycelial fragments in 20 % (v/v) glycerol at –20 °C.

The micromorphological properties of strain FXJ1.034^T were examined by light microscopy (Zeiss Axioskop) and scanning electron microscopy (model FEI Quanta 200) using cultures grown on ISP 2 medium at 28 °C for 14 days. For cultural characterization, the isolate was grown at 28 °C for 14–21 days on various ISP agar media

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; OH-PE, hydroxyphosphatidylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FXJ1.034^T is FJ938354.

A supplementary figure is available with the online version of this paper.

(Shirling & Gottlieb, 1966), Bennett's agar (Jones, 1949), tryptic soy agar (TSA, Difco, BD), Czapek agar and nutrient agar (Waksman, 1967). The growth at pH 4–11 (at intervals of 1 pH unit) was determined by using both solid and liquid Bennett's medium and ISP 2 medium and incubating at 28 °C for 14 days. Growth at 4, 10, 15, 20, 28, 37, 45 and 55 °C and in 0–10 % (w/v) NaCl (at intervals of 1 %) was determined on ISP 2 agar at 28 °C for 14 days. The production of acid from carbohydrates and the decomposition of adenine, guanine, arbutin, hypoxanthine, tyrosine, xanthine, casein, aesculin, urea and allantoin were tested by using the media of Gordon *et al.* (1974). The utilization of carbohydrates as sole carbon sources was tested by using ISP 9 medium as a basal medium according to the method of Pridham & Gottlieb (1948) and the results were recorded weekly for a period of 3 weeks.

Strain FXJ1.034^T showed good growth on ISP 2, ISP 3, ISP 4 and Bennett's agars, moderate growth on TSA, nutrient agar and Czapek agar and poor growth on ISP 5 agar. No soluble pigments were produced when grown on the media tested and melanin pigments were not produced on ISP 6 or ISP 7 agars. The cultural and morphological characteristics of strain FXJ1.034^T were typical of those of the genus *Lentzea*. The novel strain produced ivory–yellow substrate mycelium on various agar media and white–yellowish white aerial mycelium that fragmented into spiral spore chains on ISP 2 and ISP 3 agars (Fig. 1). The strain also shared several physiological and biochemical characteristics with all recognized species of the genus *Lentzea*, namely growth at 37 °C, hydrolysis of starch and tyrosine but not adenine, and acid production from fructose, galactose and glucose. The phenotypic characteristics of strain FXJ1.034^T are shown in Table 1 and in the species description.

Biomass of stationary-phase cells was prepared for fatty acid analysis by scraping colonies grown at 28 °C for 14 days from TSA plates. Fatty acids were analysed by CG (Agilent GC 6890) using the standard Sherlock Microbial Identification System version 6.0 (MIDI) procedure

Table 1. Phenotypic properties of strain FXJ1.034^T and phylogenetically closely related species

Strains: 1, FXJ1.034^T; 2, *L. kentuckyensis* JCM 14913^T (=NRRL B-24416^T); 3, *L. albida* JCM 10670^T (=NBRC 16102^T). +, Positive; w, weakly positive; –, negative. All data are from this study and all tests were performed in parallel.

Characteristic	1	2	3
Nitrate reductase	+	–	–
Degradation of:			
Allantoin	+	–	–
Arbutin	+	–	+
Gelatin	–	+	+
Urea	–	+	w
Hypoxanthine	–	+	+
Xanthine	–	+	–
Utilization of:			
Rhamnose	w	+	+
Raffinose	+	w	–
Adonitol	–	–	+
Methyl- α -D-glucoside	–	w	+
Acid production from:			
Rhamnose	w	+	+
Raffinose	+	+	–
Maltose	w	+	+
Adonitol	–	–	+
Melezitose	w	–	+
NaCl tolerance (w/v)	6 %	7 %	4 %

(Sasser, 1990). The resulting profiles were identified using the database library TSBA6 version 6.0. For other analyses of chemotaxonomic characteristics, biomass was obtained from cultures grown in ISP 2 broth on a rotary shaker at 28 °C for 7 days. The isomers of diaminopimelic acid and whole cell sugars were examined using standard procedures (Lechevalier & Lechevalier, 1980; Hasegawa *et al.*, 1983). Menaquinones were extracted and purified according to the method of Collins (1985) and then analysed by HPLC (Kroppenstedt, 1985). Polar lipids were extracted, examined and identified using the two-dimensional TLC procedure described by Minnikin *et al.* (1984).

Strain FXJ1.034^T exhibited chemotaxonomic characteristics that were typical of members of the genus *Lentzea*, such as the presence of *meso*-diaminopimelic acid in the cell-wall and galactose, mannose and ribose as the whole-cell sugars. The major menaquinone, phospholipid pattern type and fatty acid profile of strain FXJ1.034^T were also in line with those of the genus *Lentzea*. The predominant menaquinone was MK-9(H₄) (83.8 %); minor amounts of MK-9 (H₂) (6.2 %) and MK-9 (H₁) (5.1 %) were also present. The major polar lipids detected were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and hydroxyphosphatidylethanolamine (OH-PE) with trace amounts of phosphatidylinositol (PI) and phosphatidylinositol mannosides (PIM), three unknown phospholipids and one unknown

Fig. 1. Scanning electron micrograph of strain FXJ1.034^T, showing aerial mycelium fragmenting into spore chains after growth on ISP 2 agar at 28 °C for 2 weeks. Bar, 5 μ m.

glycolipid (Fig. S1a, b, available in IJSEM Online). The phospholipid profile of strain FXJ1.034^T was very similar to that of *Lentzea kentuckyensis* NRRL B-24416^T, both containing DPG, PE, OH-PE, PI and PIM (Fig. S1c, d). The detailed fatty acid profile of strain FXJ1.034^T was as follows: C_{16:1}ω7c (21.19 %), iso-C_{16:0} (18.66 %), iso-C_{15:0} (11.68 %), anteiso-C_{15:0} (11.65 %), C_{17:1}ω6c (5.07 %), iso-C_{14:0} (5.05 %), C_{16:0} (4.32 %), iso-C_{16:1}H (4.16 %), anteiso-C_{17:0} (3.96 %), C_{17:1}ω8c (2.23 %), iso-C_{17:1}ω9c (1.92 %), C_{14:0} (1.38 %), anteiso-C_{17:1}ω9c (0.93 %), C_{19:0}10-methyl (0.39 %), iso-C_{17:0} (0.79 %), C_{18:1}ω9c (0.52 %), C_{17:0} (0.43 %), anteiso-C_{13:0} (0.41 %), iso-C_{15:1} G (0.39 %), anteiso-C_{16:0} (0.36 %), C_{16:0} 2-OH (0.35 %), iso-C_{13:0} (0.34 %), C_{13:0} 3-OH (0.27 %), iso-C_{12:0} (0.24 %), C_{18:1}ω7c (0.20 %), C_{12:0} (0.16 %), anteiso-C_{11:0} (0.15 %), iso-C_{10:0} (0.14 %), C_{14:1}ω5c (0.12 %), iso-C_{18:0} (0.11 %).

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out using the method of Chun & Goodfellow (1995), except that an ABI Prism 3730 automated DNA sequencer (Applied Biosystems) was used instead of a 373A sequencer. The resultant almost full-length 16S rRNA gene sequence (1474 bp) was aligned with related sequences obtained from the GenBank/EMBL/DDBJ database using CLUSTAL_X version 1.8 (Thompson *et al.*, 1997) and the calculation of pairwise 16S rRNA gene sequence similarities was achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) trees were reconstructed using MEGA version 4.0 (Tamura *et al.*, 2007). The robustness of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985). As shown in Fig. 2, phylogenetic analysis based on 16S rRNA gene sequences revealed that strain FXJ1.034^T fell within the genus *Lentzea*, supported by both neighbour-joining and maximum-parsimony algorithms. The novel strain showed the highest 16S rRNA gene sequence similarity with *Lentzea kentuckyensis* NRRL B-24416^T (98.5 %) and formed a monophyletic clade with this type strain in the neighbour-joining tree, supported by a bootstrap value of 68 %. Sequence similarities between strain FXJ1.034^T and other members of the genus *Lentzea* ranged from 96.0 (*Lentzea californiensis*) to 98.2 % (*Lentzea albida*).

The DNA G + C content of strain FXJ1.034^T was 69.6 mol% as determined by the HPLC method of Mesbah *et al.* (1989). DNA–DNA relatedness studies were carried out between the isolate and the most closely related type strains, *L. kentuckyensis* NRRL B-24416^T and *L. albida* NBRC 16102^T, using the method described by Rong & Huang (2010). Strain FXJ1.034^T showed low DNA–DNA relatedness values of 35.6 ± 6.8 % and 28.6 ± 5.2 % with *L. kentuckyensis* NRRL B-24416^T and *L. albida* NBRC 16102^T, respectively. These values are well below the 70 % cut-off point for assigning strains to the same species (Stackebrandt & Goebel, 1994) and confirm the separation of strain FXJ1.034^T from its closest phylogenetic neighbours. Further relatedness determinations were not considered necessary.

Strain FXJ1.034^T could also be distinguished from closely related strains by its phenotypic characteristics. There were a series of physiological and biochemical features that differed between strain FXJ1.034^T and its close relatives, including nitrate reduction, degradation of allantoin, gelatin, urea and hypoxanthine, utilization of methyl-α-D-glucoside and tolerance of NaCl (Table 1).

In summary, the morphological, chemotaxonomic and phylogenetic data clearly support the assignment of strain FXJ1.034^T to the genus *Lentzea* and the physiological and DNA–DNA relatedness data demonstrate that the strain can be differentiated from the most closely related type strains of species in this genus. Based on the genotypic and phenotypic evidence presented here, strain FXJ1.034^T represents a novel species of the genus *Lentzea*, for which the name *Lentzea jiangxiensis* sp. nov. is proposed.

Description of *Lentzea jiangxiensis* sp. nov.

Lentzea jiangxiensis (ji.ang.xi.en'sis. N.L. fem. adj. *jiangxiensis* of or belonging to Jiangxi Province, South-east China, the source of the type strain).

Substrate mycelium is ivory on ISP 5 agar, light yellow on Bennett's, tryptic soy, nutrient and Czapek's agars, and yellow on ISP 2, ISP 3 and ISP 4 agars. Yellowish white aerial mycelium that fragments into short spore chains is produced on ISP 2 and ISP 3 agars. No soluble pigments are produced on the above media. No melanin pigments

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain FXJ1.034^T and species of the genus *Lentzea*. Numbers at nodes indicate the levels of bootstrap support (%) based on a neighbour-joining analysis of 2000 resampled datasets; asterisks denote nodes that were also recovered using the maximum-parsimony method. Bar, 0.005 substitutions per nucleotide position.

are produced on ISP 6 or ISP 7 media. Casein, aesculin, tyrosine, allantoin and starch are hydrolysed but guanine, arbutin, gelatin, adenine, hypoxanthine, xanthine and urea are not. Catalase-positive. Nitrate is reduced. H₂S is not produced. Arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, mannitol, D-mannose, melibiose, raffinose, rhamnose, salicin, trehalose, ribose, sucrose and xylose are used as sole carbon sources but methyl α -D-glucoside, D-sorbitol, adonitol and *meso*-erythritol are not. Acid is produced from arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, mannitol, D-mannose, raffinose, sucrose and xylose, and weakly produced from rhamnose, melezitose and maltose but not produced from adonitol, D-sorbitol, *meso*-erythritol or methyl α -D-glucoside. Growth occurs at 10–37 °C (optimum at 20–28 °C) at pH 5–10 (optimum pH 6), and in the presence of up to 6 % NaCl (w/v) (optimum 0 %). The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. Cell hydrolysates contain galactose, mannose and ribose. The polar lipids include DPG, PE, OH-PE and PI. The predominant menaquinone is MK-9(H₄). The major fatty acids (>10 %) are C_{16:1} ω 7c, iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{15:0} and anteiso-C_{15:0} 2-OH.

The type strain, FXJ1.034^T (=CGMCC 4.6609^T =NBRC 106680^T), was isolated from acidic soil in Nanchang, Jiangxi Province, China. The DNA G+C content of the type strain is 69.6 mol%.

Acknowledgements

We thank the international culture collections CGMCC and JCM for providing type strains. This research was supported by the National Natural Science Foundation of China (grant no. 31170010) and the Opening Project of the State Key Laboratory of Microbial Resources.

References

- Busti, E., Cavaletti, L., Monciardini, P., Schumann, P., Rohde, M., Sosio, M. & Donadio, S. (2006). *Catenulispora acidiphila* gen. nov., sp. nov., a novel, mycelium-forming actinomycete, and proposal of *Catenulisporaceae* fam. nov. *Int J Syst Evol Microbiol* **56**, 1741–1746.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D. (1985). Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- 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.
- 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.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*. No. 20 SAB Technical Series, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- 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.
- Labeda, D. P., Goodfellow, M., Chun, J., Zhi, X. Y. & Li, W. J. (2011). Reassessment of the systematics of the suborder *Pseudonocardineae*: transfer of the genera within the family *Actinosynnemataceae* Labeda and Kroppenstedt 2000 emend. Zhi *et al.* 2009 into an emended family *Pseudonocardaceae* Embley *et al.* 1989 emend. Zhi *et al.* 2009. *Int J Syst Evol Microbiol* **61**, 1259–1264.
- Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, pp. 22–291. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society for Industrial Microbiology.
- Lee, S. D., Kim, E. S., Roe, J. H., Kim, J. H., Kang, S. O. & Hah, Y. C. (2000). *Saccharothrix violacea* sp. nov., isolated from a gold mine cave, and *Saccharothrix albidocapillata* comb. nov. *Int J Syst Evol Microbiol* **50**, 1315–1323.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- 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.
- Pridham, T. G. & Gottlieb, D. (1948). The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. *J Bacteriol* **56**, 107–114.
- Rong, X. Y. & Huang, Y. (2010). Taxonomic evaluation of the *Streptomyces griseus* clade using multilocus sequence analysis and DNA–DNA hybridization, with proposal to combine 29 species and three subspecies as 11 genomic species. *Int J Syst Evol Microbiol* **60**, 696–703.
- Saitou, N. & Nei, M. (1987). The neighbor-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. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- 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.

Waksman, S. A. (1967). *The Actinomycetes. A Summary of Current Knowledge*. New York: Ronald Press.

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.