Lechevalieria nigeriaca sp. nov., isolated from arid soil

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A novel actinobacterium, designated strain NJ2035T, was isolated from soil collected from Abuja, Nigeria and was characterized to determine its taxonomic position. The isolate was found to have chemical and morphological properties associated with members of the genus Lechevalieria. Phylogenetic analyses based on almost-complete 16S rRNA gene sequences indicated that the isolate was closely related to members of the genus Lechevalieria, and was shown to form a distinct phyletic line in the Lechevalieria phylogenetic tree. Strain NJ2035T was most closely related to Lechevalieria roselyniae C81T, Lechevalieria atacamensis C61T and Lechevalieria deserti C68T (98.5% 16S rRNA gene sequence similarity). Sequence similarities with other members of the genus Lechevalieria were less than 98.2%. The cell wall of the novel strain contained meso-diaminopimelic acid, and galactose, mannose and rhamnose as the diagnostic sugars. The predominant menaquinone was MK-9(H4). The polar lipids detected were diphasatidyglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol. DNA-DNA relatedness and phenotypic data showed that the novel isolate and L. roselyniae C81T, L. atacamensis C61T and L. deserti C68T belong to distinct genomic species. On the basis of data from this taxonomic study using a polyphasic approach, strain NJ2035T represents a novel species of the genus Lechevalieria, for which the name Lechevalieria nigeriaca sp. nov. is proposed. The type strain is NJ2035T (=DSM 45680T=KCTC 29057T=NRRL B-24881T).

The genus Lechevalieria was proposed by Labeda et al. (2001) for aerobic, Gram-reaction-positive actinobacteria that form an extensively branched vegetative mycelium and scant aerial hyphae on some media. Members of the genus are also characterized by a number of chemical markers, including the presence of meso-diaminopimelic acid in the whole-cell hydrolysate peptidoglycan (wall chemotype III sensu Lechevalier & Lechevalier, 1970), galactose, mannose and rhamnose as the diagnostic sugars, MK-9(H4) as the predominant menaquinone, phosphatidylethanolamine as the main polar lipid (type II sensu Lechevalier et al., 1977), and a fatty acid profile mainly consisting of iso- and anteiso-branched fatty acids. Members of the genus Lechevalieria form a distinct branch in the phylogenetic tree based on 16S rRNA gene sequences of the family Pseudonocardiaceae (Labeda et al., 2001) and can be distinguished from one another by a combination of phenotypic properties (Zhang et al., 2007; Wang et al., 2007; Okoro et al., 2009, 2010). At the time of writing, the genus Lechevalieria contains seven species with validly published names isolated from environmental samples, Lechevalieria aerocolonigenes (Labeda, 1986, emended Labeda et al., 2001), Lechevalieria flava (Gauze et al., 1974, emended Labeda et al., 2001), Lechevalieria fradiae (Zhang et al., 2007), Lechevalieria xinjiangensis (Wang et al., 2007), and Lechevalieria atacamensis, Lechevalieria deserti and Lechevalieria roselyniae (Okoro et al., 2010).

The present investigation was designed to determine the taxonomic position of the isolate NJ2035T by using a polyphasic approach. Strain NJ2035T was isolated from SM2 agar plates (Tan et al., 2006), supplemented with (+)-d-melezitose (1%, w/v), cycloheximide (50 μg ml⁻¹), neomycin sulphate (4 μg ml⁻¹) and nystatin (50 μg ml⁻¹), after incubation at 28 °C for 21 days, following inoculation with a suspension of a soil sample collected from Abuja, Nigeria. The organism was maintained on modified Bennett’s agar slopes (modified after Jones, 1949) at 4 °C and as suspensions of mycelial fragments in 20% (v/v) glycerol at 37 °C.
–20 °C. Biomass for chemotaxonomic and molecular systematic analyses was prepared by growing strain NJ2035T in ISP 2 broth cultures, at 160 r.p.m. for 10 days at 28 °C; cells were harvested by centrifugation, washed twice in distilled water, recenterfuged and freeze-dried.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product were carried out following Chun & Goodfellow (1995). The almost-complete 16S rRNA gene sequence of strain NJ2035T (1473 bp) was determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012). Multiple alignments with sequences from closely related species was performed by using the program CLUSTAL W in the MEGA5 software package (Tamura et al., 2011). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA5 (Tamura et al., 2011). Evolutionary distance matrices were prepared according to Jukes & Cantor (1969). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The almost-complete 16S rRNA gene sequence of strain NJ2035T (1473 bp) was used for phylogenetic analysis. The phylogenetic tree based on the neighbour-joining algorithm showed that strain NJ2035T was a member of the genus *Lechevaleria* and formed a distinct branch from other species of the genus *Lechevaleria*, which was also supported by a high bootstrap value (Fig. 1). The other two tree-making algorithms (maximum-likelihood and maximum-parsimony) resulted in trees showing similar topologies (Figs S1 and S2 available in IJSEM Online). Strain NJ2035T shared 16S rRNA gene sequence similarities of 98.5 % (22 nt differences at 1471 locations), 98.5 % (22 nt differences at 1464 locations) and 98.49 % (22 nt differences at 1453 locations), with its nearest relatives, *L. roselyniae* C81T, *L. deserti* C68T and *L. deserti* C61T, respectively. Sequence similarities with all other members of the genus *Lechevaleria* were <98.2 %.

DNA–DNA relatedness values between strain NJ2035T and related type strains, *L. roselyniae* DSM 45481T, *L. atacamensis* DSM 45479T and *L. deserti* DSM 45480T were determined by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zelkulturen (DSMZ; Braunschweig, Germany). DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970), incorporating the modifications described by Huß et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

The DNA–DNA relatedness values between strain NJ2035T and *L. roselyniae* DSM 45481T, *L. atacamensis* DSM 45479T and *L. deserti* DSM 45480T were 51.5 ± 1.6, 49.0 ± 2.3 and 46.6 ± 0.8 % (values are means of duplicate determinations), respectively. These values are well below the 70 % cut-off point recommended by Wayne et al. (1987) for the delineation of genomic species. DNA–DNA hybridization was not carried out between strain NJ2035T and other species of the genus *Lechevaleria* because they were positioned in different clusters in the phylogenetic tree and shared relatively low 16S rRNA gene sequence similarities (98.2–97.7 %).

Amino acid and sugar analyses of whole-cell hydrolysates were performed according to the procedures described by Hasegawa et al. (1983). Polar lipid and respiratory quinones analyses were carried out by the Identification Service of the DSMZ. Respiratory quinones were extracted from 100 mg freeze-dried cells based on the two-stage method described by Tindall (1990a; 1990b). Respiratory quinones were separated into their different classes (menaquinones and ubiquinones) by thin layer chromatography on silica gel (Macherey-Nagel Art. no. 805 023), using hexane/tert-butylmethylether (9:1, v/v) as the solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on a LDC Analytical HPLC (Thermo Separation Products) fitted with a reverse-phase column (Macherey-Nagel, 2 mm × 125 mm, 3 μm, RP18) using methanol as the eluant. Respiratory quinones were detected at 269 nm.

Cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890N instrument, fitted with an auto sampler and a 6783 injector, according to the standard protocol of the Sherlock Microbial identification (MIDI) system (Sasser, 1990; Kämpfer & Kroppestedt, 1996), and the fatty acid methyl ester peaks were identified and quantified using TSBA 5.0 software. The DNA G+C content of the isolate was determined following the procedure of Gonzalez & Saiz-Jimenez (2005).

Chemotaxonomic analyses revealed that strain NJ2035T displayed chemical characteristics that were consistent with those of the genus *Lechevaleria*. The cell-wall diamino acid in the peptidoglycan layer of strain NJ2035T was meso-diaminopimelic acid, and the whole-cell hydrolysates contained galactose, mannose and rhamnose (major components), glucose and small amounts of ribose (cell-wall chemotype III sensu Lechevalier & Lechevalier, 1970). The polar lipids of strain NJ2035T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, two unidentified phospholipids, one aminophospholipid and three glycolipids (Fig. S3). The predominant aminoaqueous of strain NJ2035T was MK-9(H4) (80.0 %); MK-9(H2) (11.0 %), MK-11(H4) (5.0 %) and MK-9(H6) (1.0 %) were also detected. The major cellular fatty acids were iso-C16:0 (36.1 %), anteiso-C15:0 (8.7 %), iso-C14:0 (8.6 %), iso-C15:0 (7.5 %), C16:0 (7.2 %)
and anteiso-C17:0 (6.8%). Comparative cellular fatty acid compositions of strain NJ2035T and L. roselyniae C81T, L. atacamensis C61T and L. deserti C68T are shown in Table S1. The DNA G+C content of strain NJ2035T was 68.4 mol%.

Cultural characteristics were investigated on media from the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966), modified Bennett’s agar (MBA; Jones, 1949), Czapek’s and tryptic soy agar (TSA; Difco). The degree of growth, aerial mycelium and pigmentation were recorded after 14 days of incubation at 28 °C. The National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1964) was used for determining colour designation and names. Colony morphology and micro-morphological properties of strain NJ2035T were determined by examining gold-coated dehydrated specimens of 14-day-old cultures from ISP 3 medium using a JSM 6060 instrument (JEOL). Growth at different temperatures (4, 10, 20, 28, 30, 37, 45, 50 and 55 °C), at pH 4.0–11.0 (at intervals of 1.0 pH unit), and in the presence of NaCl (0–10 %; w/v) (at intervals of 1.0 pH unit) was determined on ISP 2. Established methods were used to determine whether the strains degraded chitin (Hsu & Lockwood, 1975), RNA (Goodfellow et al. 1979) and Tweens 20 and 80 (Nash & Krent, 1991); the remaining degradation tests were examined using methods described by Williams et al. (1983). Carbon source utilization was tested using carbon source utilization (ISP 9) medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the tested carbon sources. Nitrogen source utilization was examined using the basal medium recommended by Williams et al. (1983) supplemented with a final concentration of 0.1 % of the tested nitrogen sources. The type strains L. roselyniae DSM 45481T, L. atacamensis DSM 45479T and L. deserti DSM 45480T were included for comparison in all tests. Strain NJ2035T exhibited very good growth on tested media. The aerial mycelium was not produced on ISP 2, ISP 5, ISP 6, Czapek’s or nutrient agar, while grey aerial mycelium was produced on ISP 3, ISP 4 and ISP 7 media, and light yellow aerial mycelium was produced on modified Bennett’s agar medium. The strain did not produce melanoid pigments on ISP 6 or ISP 7 media. The aerial mycelium was fragmented into rod-shaped spores (0.7–1.1 μm wide × 0.8–1.7 μm long) (Fig. 2). The detailed results of the morphological and physiological tests are given in Table 1 and the species description.
It is evident from these genotypic and phenotypic data that strain NJ2035<sup>T</sup> can be distinguished from its closest phylogenetic neighbours, *L. roselyniae* C81<sup>T</sup>, *L. atacamensis* C61<sup>T</sup> and *L. deserti* C68<sup>T</sup>. We therefore suggest that strain NJ2035<sup>T</sup> represents a novel species of the genus *Lechevalieria*, for which the name *Lechevalieria nigeriaca* sp. nov., is proposed.

**Description of *Lechevalieria nigeriaca* sp. nov.**

*Lechevalieria nigeriaca* (ni.ge.ri'a.ca. N.L. fem. adj. nigeriaca of or belonging to Nigeria).

Aerobic, Gram-reaction-positive, catalase-positive, non-motile actinomycete which forms an extensively branched substrate mycelium that fragments into rod-shaped elements. Growth occurs at 20–45 °C (optimum, 28–30 °C), pH 5.0–10.0 (optimum, pH 7.2) and in the presence of 1.0–5.0 % NaCl. Positive for aesculin hydrolysis, but negative for allantoin, arbutin and urea hydrolysis, and nitrate reduction. Elastin, hypoxanthine and Tweens 20 and 80 are degraded, but not guanine or L-tyrosine. Dextrin, cellobiose, D-galactose, D-mannitol, D-mannose, inulin, L-arabinose, L-rhamnose, lactose, maltose, starch and sucrose are used as sole carbon sources for energy and growth but adonitol, D-sorbitol and melezitose are not. Utilizes α-isoleucine, D-phenylalanine, L-alanine, L-arginine, L-cysteine, L-hydroxyproline, L-methionine, L-phenylalanine, L-valine, L-proline, L-serine and L-threonine as sole nitrogen sources, but not glycine. The predominant menaquinone is MK-9(H<sub>4</sub>). The polar lipid profile contains diphosphatidyleglycerol, phosphatidylycerol and phosphatidylinositol. The major fatty acids of the type strain are iso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub>, iso-C<sub>14:0</sub>, iso-C<sub>15:0</sub>, C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>. The type strain, NJ2035<sup>T</sup> (=DSM 45680<sup>T</sup> = KCTC 29057<sup>T</sup> = NRRLB-24881<sup>T</sup>), was isolated from an arid soil sample collected in Abuja, Nigeria. The DNA G+C content of the type strain is 68.4 mol%.

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**References**


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**Table 1. Phenotypic properties of strain NJ2035<sup>T</sup> and type strains of closely related species of the genus *Lechevalieria***

<table>
<thead>
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<th>Characteristic</th>
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<td>Hydrolysis of:</td>
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<td>Aesculin</td>
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<td>Arbutin</td>
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<td>Allantoin</td>
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<td>Nitrate reduction</td>
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<td>Hypoxanthine (0.4%)</td>
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<td>Carbon source utilization (1.0%)</td>
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<td>Adonitol</td>
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<td>D-Sorbitol</td>
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<td>Nitrogen source utilization (0.1%)</td>
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