Micromonospora nickelidurans sp. nov., isolated from soil from a nickel-mining site

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An actinomycete, strain K55T, was isolated from a composite soil sample from a nickel mine, collected from Yuyang, Shaanxi Province, PR China. Strain K55T showed 16S rRNA gene sequence similarities of 98.73 %–98.51 % to species of the genus Micromonospora, including Micromonospora haikouensis 232617T, Micromonospora coxensis 2-30-b(28)T, Micromonospora wenchangensis 2602GPT1-05T, Micromonospora matsumotoense IMSNU 22003T, Micromonospora maoerensis NEAU-MES19T, and Micromonospora humi PO402T. This strain harboured meso-diaminopimelic acid, alanine and glycine as the major cell-wall amino acids, xylose and glucose as the characteristic whole-cell sugars, and iso-C15 : 0 (20.53 %), iso-C17 : 0 (12.74 %), iso-C16 : 0 (12.15 %), anteiso-C17 : 0 (7.97 %), C17 : 108c (7.49 %) and C17 : 0 (6.63 %) as the dominant fatty acids. The major menaquinones were MK-10(H4) and MK-10(H6). The phospholipid profile comprised phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol and unknown phosphoglycolipids. The DNA G+C content was 71.4 mol%. A comprehensive analysis of several physiological and biochemical traits and DNA–DNA relatedness indicated that strain K55T was different from closely related species. These phenotypic, genotypic and chemotaxonomic data suggest that strain K55T represents a novel species of the genus Micromonospora, for which the name Micromonospora nickelidurans sp. nov., is proposed. The type strain is K55T (=JCM 30559T=ACCC19713T).

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The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain K55T is HQ174560.

One supplementary figure is available with the online Supplementary Material.

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1CuSO4 or 0.5 mmol l−1 CuSO4 or 0.5 mmol l−1 NiSO4. The inoculated plates were incubated at 28 °C for 7 days and single colonies of actinomycetes were purified by repeatedly streaking on the same medium. The pure isolate was maintained temporally on GSM slopes at 4 °C and as glycerol kangleipakensis (Nimaichand et al., 2013). Members of the genus Micromonospora are widely distributed in different environments, but so far no species has been discovered in mine soils contaminated with heavy metals.

Mine soil contaminated with heavy metals is a special habitat for microbes and many novel microbial species have been discovered in soil from mines. The actinomycetes, Streptomyces plumbiresistens (Guo et al., 2009) and Streptomyces zinciresistens (Lin et al., 2011) were isolated from heavy metal-contaminated soil from different mines.

Strain K55T was isolated from a soil sample taken from a nickel mining zone in Huixian County (33° 54′ 10.3″ N 106° 07′ 44.3″ E; altitude 1049 m), Gansu Province, PR China using the dilution plating method on modified Gause’s synthetic medium (GSM) (Lin et al., 2011) with 0.6 mmol l−1 CuSO4 or 0.5 mmol l−1 NiSO4. The inoculated plates were incubated at 28 °C for 7 days and single colonies of actinomycetes were purified by repeatedly streaking on the same medium. The pure isolate was maintained temporally on GSM slopes at 4 °C and as glycerol
suspensions (20 %, v/v) at −80 °C for long-term preservation. Biomass for most of the chemotaxonomic and molecular systematic studies was harvested from a liquid culture of GSM with shaking at 180 r.p.m. at 28 °C for 7 days.

Morphological properties were examined by light microscopy (OLYMPUS CX31) and scanning electron microscopy (JSM 6360LV) using cultures grown on GSM for 7, 14 and 21 days, respectively. Preparation for electron micrograph observation was performed as described by Eguchi et al. (1993). The colony morphology of strain K55T and the reference strains was determined after 14 days of incubation at 28 °C on different culture media following the methods of the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966). Colours of the colony and soluble pigments were determined using methods described by Kelly (1964).

The utilization of carbon and nitrogen sources was determined by using the methods of Gordon & Mihm (1962) and Tsukamura (1966). Other phenotypic characteristics were tested by using standard procedures (Goodfellow, 1971; Williams et al., 1983). The range of temperatures (4, 10, 20, 28, 37, 45 and 50 °C), pH (from 4.0 to 12.0 with intervals of 1 pH unit), and NaCl concentrations (from 0 to 6 %, w/v, with intervals of 1 %) for growth of the strain were tested on ISP 2 agar plates after incubation by cross streaking and incubating for 7–21 days at 28 °C (except when testing temperature). Occurrence of single colonies was recorded as positive.

The detection of diamino acids in the cell wall and the analysis of the whole-cell sugars were performed as described by Lechevalier & Lechevalier (1970, 1980) and Staneck & Roberts (1974), respectively. Menaquinones and phospholipids were extracted and purified according to the protocol of Minnikin et al. (1984). Menaquinones were analysed by HPLC with an ODS-BP C18 column (4.6 x 250 mm) (Ren et al., 2013). The polar lipids were extracted and analysed as described by Tindall (1990). Fatty acids were extracted and methylated as described by Kämpfer & Kroppenstedt (1996). The fatty acid methyl esters were identified and quantified by gas chromatography following the MIS operation manual (MIDI Inc.) and using the Sherlock MIS software (version 4.5).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were carried out as described by Chun & Goodfellow (1995), with the universal forward primer P1 (5′-CGG GAT CCT GCA GAG TTT GAT CCT GCG TCA GAA CGA ACG CT-3′) and reverse primer P6 (5′-CGG GAT CCT ACG GCT ACC TTG CGA CTT CAC CCC CCG-3′). The PCR product was purified and sequenced directly with an automated DNA sequencing system (ABI 3730XL). The 16S rRNA gene sequence of strain K55T was multiply aligned with CLUSTAL X version 1.8 (Thompson et al., 1997) with the related sequences of species of the genus Micromonospora extracted from the GenBank database and sequence similarities were calculated with the EzTaxon-e server (Kim et al., 2012). The phylogenetic tree was reconstructed using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky and Nei, 1993) and maximum-parsimony (Fitch, 1971) tree-making algorithms with Kimura’s two-parameter model (Kimura, 1980) in MEGA, version 4.0 (Tamura et al., 2007). Bootstrap analysis was performed to evaluate the tree topology by performing 1000 replicates (Felsenstein, 1985). The DNA G+C content was determined using the thermal melting protocol (Marmur & Doty, 1962) with Escherichia coli K-12 as the standard. The taxonomic relationship between strain K55T and its close relatives was further examined in triplicate by DNA–DNA hybridization using the initial renaturation rate method (De Ley et al., 1970).

The morphological properties of strain K55T are consistent with the description of the genus Micromonospora (Kawamoto, 1989). It formed well-developed and branched substrate hyphae on GSM. No aerial mycelium was produced. Single spores borne from the substrate mycelium were spherical to oval, spinose and non-motile (Fig. 1). Strain K55T showed strong growth on ISP 1, ISP 2, ISP 4, ISP 6 and ISP 7 media, nutrient agar, asparagine glucose agar, while it grew moderately on Bennett’s medium and GSM, and poorly on Czapek’s agar and ISP 5. The strain produced a dark brown water soluble pigment on GSM. The substrate hyphae were black.

For strain K55T, the whole cell sugars were mainly xylose and glucose, while the cell-wall amino acids were meso-diaminopimelic acid, alanine and glycine. The characteristic polar lipids were phosphatidylethanolamine, diphasatidylglycerol, phosphatidylinositol, phosphatidylglycerol and unknown phosphoglycolipids (Fig. S1, available in the online Supplementary Material). The major fatty acids were iso-C15 : 0 (20.53 %), iso-C17 : 0 (12.74 %), iso-C16 : 0 (12.15 %), anteiso-C17 : 0 (7.97 %), C17 : 0 10MeC (7.49 %) and C17 : 0 (6.63 %). The major menaquinones of strain K55T were MK-10(H4) and MK-10(H6), which was reconstructed using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky and Nei, 1993) and maximum-parsimony (Fitch, 1971) tree-making algorithms with Kimura’s two-parameter model (Kimura, 1980) in MEGA, version 4.0 (Tamura et al., 2007). Bootstrap analysis was performed to evaluate the tree topology by performing 1000 replicates (Felsenstein, 1985). The DNA G+C content was determined using the thermal melting protocol (Marmur & Doty, 1962) with Escherichia coli K-12 as the standard. The taxonomic relationship between strain K55T and its close relatives was further examined in triplicate by DNA–DNA hybridization using the initial renaturation rate method (De Ley et al., 1970).

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Fig. 1. Scanning electron micrograph showing the cellular morphology of strain K55T after 21 days incubation at 28 °C on Gause’s synthetic medium. Bar, 2 µm.
differentiated it from the type strains of most of the closely related species in the genus *Micromonospora*.


Fig. 2. Neighbour-joining phylogenetic tree based on almost complete 16S rRNA gene sequences, showing the relationships between strain K55^T^ and related species of the genus *Micromonospora*. E indicates branches that were also found with the minimum-evolution method. Asterisks indicate branches that were recovered by the neighbour-joining, minimum-evolution and maximum-parsimony methods. Accession numbers are given in parentheses. Bootstrap values greater than 50% (based on 1000 replications) are shown at the branch points. Bar, 0.002 substitutions per nucleotide position.
Micromonospora maoerensis NEAU-MES19T and Micromonospora humi P0402T were 98.51% to 98.80%.

In the neighbour joining phylogenetic tree (Fig. 2), strain K55\(^T\) formed a distinctive lineage differing from \textit{M. coxensis} 2-30-(28)b\(^T\), which had the highest 16S rRNA gene sequence similarity and highest DNA–DNA relatedness with K55\(^T\).

The G + C content of strain K55\(^T\) was 71.4 mol%. The DNA–DNA relatedness values between strain K55\(^T\) and the phylogenetically most closely related species, \textit{M. wenchangensis} 2602GPT1-05\(^T\), \textit{M. haikouensis} 232617\(^T\), \textit{M. maoerensis} NEAU-MES19\(^T\), \textit{M. matsumotoense} IMSNU 22003\(^T\) and \textit{M. coxensis} 2-30-(28)b\(^T\), were 56.7±0.51%, 31.52±0.43%, 27.89±0.36%, 22.85±0.72% and 18.63±2.6%, respectively. These are much lower than the threshold (70%) for genomic species delineation suggested by Wayne et al. (1987). These results indicate that strain K55\(^T\) represents a distinctive and novel genomic species of the genus \textit{Micromonospora}.

Comparison with descriptions of species of the genus \textit{Micromonospora} with validly published names showed that strain K55\(^T\) could be distinguished from them based on a combination of biochemical and physiological properties, particularly the production of water-soluble pigment and the decomposition of asparagines (Table 1).

On the basis of the phenotypic and genotypic data, strain K55\(^T\) represents a novel species of the genus \textit{Micromonospora}, for which the name \textit{Micromonospora nickelidurans} sp. nov., is proposed.

### Description of \textit{Micromonospora nickelidurans} sp. nov.


Aerobic, Gram-stain-positive, mesophilic actinomycete, forming a well-developed and extensively branched sub-strate mycelium. No aerial mycelium is produced. The vegetative mycelium on Gause’s synthetic medium is black and a dark brown water-soluble pigment is produced.

### Table 1. Differential characteristics between strain K55\(^T\) and the type strains of related species of the genus \textit{Micromonospora}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
<th>Strain 5</th>
<th>Strain 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour on Gause’s synthetic agar</td>
<td>Black</td>
<td>Light yellow</td>
<td>Vivid orange</td>
<td>Orange yellow</td>
<td>Brilliant yellow</td>
<td>Melon yellow</td>
</tr>
<tr>
<td>Soluble pigment on Gause’s synthetic agar</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Assimilation of sole carbon sources (1.0%, w/v) by:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ribose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of sole nitrogen sources (0.5%, w/v) by:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Arginine</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Asparagine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Histidine</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Threonine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 45 °C:</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>5.5–9.5</td>
<td>5.0–9.0</td>
<td>5.0–10.0</td>
<td>7.0–10.0</td>
<td>6.0–8.0</td>
<td>5.0–9.0</td>
</tr>
<tr>
<td>NaCl tolerance (%)</td>
<td>2.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Spores are spherical to oval with spinose surfaces. Growth occurs at 20–37 °C, pH 5.5–9.5 and in the presence of 0–2 % (w/v) NaCl. Optimal growth occurs at 28 °C, pH 7.5 and in the presence of 1 % (w/v) NaCl. Utilizes D-glucose, glycerol and starch, but does not utilize inositol, lactose, D-mannitol, L-rhamnose, ribose, sucrose, trehalose or xylose as the sole carbon source. Metabolizes alanine, aspartate, cysteine, leucine and threonine. Positive for starch hydrolysis and catalase activity. Negative for CM-cellulose decomposition, gelatin liquefaction, melanin formation, H₂S production, catalase activity. Negative for CM-cellulose decomposition, gelatin liquefaction, melanin formation, H₂S production, catalase activity.  The type strain is K55ᵀ (=JCM 30559ᵀ=ACCC19713ᵀ), which was isolated from a soil sample from a nickel mine in Huixian, Gansu Province, PR China. The DNA G+C content of the type strain is 71.4 mol%.

Acknowledgements

This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411). This research was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), National Science Foundation of China (No.31270530) and China Postdoctoral Science Foundation on the 55th grant program (2012M511411).

References

Li, L., Tang, Y. L., Wei, B., Xie, Q. Y., Deng, Z. & Hong, K. (2013). Micromonospora sonnerata sp. nov., isolated from a soil sample from a nickel mine in Huixian, Gansu Province, PR China. The DNA G+C content of the type strain is 71.4 mol%.


