Nonomurea jiangxiensis sp. nov., isolated from acidic soil

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An actinomycete, designated FXJ1.102T, was isolated from acidic soil collected in Jiangxi Province, south-east China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain FXJ1.102T belonged to the genus Nonomurea and was most closely related to Nonomurea candida HMC10T, Nonomurea turkmeniaca DSM 43926T, Nonomurea antimonica YIM 61105T and ‘Nonomurea aegyptia’ S136 (98.9, 98.3, 97.9 and 97.5 % 16S rRNA gene sequence similarities, respectively). The morphological characteristics were typical of the genus Nonomurea. The chemotaxonomic properties, such as cell-wall chemotype III B, phospholipid type IV, MK-9(H4) as the major menaquinone and iso-C16 : 0 (22.2 %) as the major fatty acid, supported the assignment of the strain to the genus Nonomurea. DNA–DNA relatedness and physiological tests allowed genotypic and phenotypic differentiation of strain FXJ1.102T from its closest phylogenetic relatives. The isolate therefore represents a novel species, for which the name Nonomurea jiangxiensis sp. nov. is proposed. The type strain is FXJ1.102T (=CGMCC 4.6533T = NBRC 106679T).

The genus Nonomurea, the spelling of which was corrected by Chiba et al. (1999), was originally proposed by Zhang et al. (1998) as a member of the family Streptosporangiaceae, which forms extensively branched substrate and aerial mycelia. The type species is Nonomurea pusilla. At the time of writing, the genus Nonomurea comprised 27 species and two subspecies with validly published names (http://www.bacterio.cict.fr/index.html), which were established on the basis of a polyphasic approach (Gyobu & Miyadoh, 2001; Stackebrandt et al., 2001; Quintana et al., 2003; Ara et al., 2007a, b; Le Roes & Meyers, 2008; Kämpfer et al., 2010; Wang et al., 2011; Zhao et al., 2011; Xi et al., 2011).

Strain FXJ1.102T 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 incubated on humic acid-vitamin agar (pH 5.0; Hayakawa & Nonomura, 1987) supplemented with (mg l−1) cycloheximide (50), nystatin (50) and nalidixic acid (20) at 28 °C for 3 weeks. The strain was maintained on glucose-yeast extract-malt extract agar [International Streptomycetes Project medium 2 (ISP 2), pH 5.0; Shirling & Gottlieb, 1966] at 4 °C and as suspensions of mycelial fragments in 20 % (v/v) glycerol at −20 °C.

The morphological properties of the isolate were examined by light microscopy (Zeiss Axioskop) and scanning electron microscopy (model FEI Quanta 200) using cultures grown on ISP 2 at 28 °C for 14 days. For cultural characterization, the isolate was grown at 28 °C for 14–21 days on various agar media as described by Waksman (1961) and Shirling & Gottlieb (1966). Growth at pH 4.0–11.0 (at intervals of one pH unit) was determined on Bennett’s medium at 28 °C for 14 days. Growth at 4, 10, 15, 20, 28, 37, 45 and 55 °C and with 0–10 % (w/v) NaCl (at intervals of 1 %) at 28 °C was determined on ISP 2 for 14 days. Decomposition of adenine, guanine, arbutin, hypoxanthine, L-tyrosine, xanthine, casein, aesculin, urea and allantoin was evaluated using the media of Gordon et al. (1974). Utilization of various compounds as sole carbon sources was tested using ISP 9 as the basal medium, according to the method of Pridham & Gottlieb (1948).

Strain FXJ1.102T showed good growth on ISP 2, ISP 3, nutrient agar, tryptic soy agar (TSA; Difco, Becton Dickinson) and glucose-asparagine agar, moderate growth on ISP 4, ISP 6 and ISP 7 and poor growth on ISP 5 and Czapek’s agar. The isolate produced an ivory to dark yellow substrate mycelium.
on various agar media and a white aerial mycelium that differentiated into spiral spore chains on ISP 2, ISP 3 and ISP 4 (Fig. S1, available in IJSEM Online). No soluble pigments were produced on any of the above media. The physiological and biochemical characteristics that serve to distinguish between strain FXJ1.102T and closely related strains of species Nonomuraea jiangxiensis are shown in Table 1.

Biomass for fatty acid analysis was prepared by scraping colonies from TSA plates that had been incubated for 14 days at 28 °C. For other chemotaxonomic analyses, freeze-dried cells were obtained from cultures grown in ISP 2 broth on a rotary shaker at 180–200 r.p.m. and 28 °C for 7 days. The isomer of the diaminopimelic acid and whole cell sugars were determined using standard procedures (Lechevalier & Lechevalier, 1980; Hasegawa et al. 1983). Menaquinones were extracted and purified according to the method of Collins (1985) and analysed by HPLC (Kroppenstedt, 1985). Polar lipids were extracted, examined and identified using the two-dimensional TLC procedure described by Minnikin et al. (1984). Fatty acids were analysed using the standard MIDI procedure (Sherlock version 6.0; Microbial Identification; Sasser, 1990) and a gas chromatograph (Agilent GC 6890). The fatty acids were identified using the database library TSBA6 version 6.0.

Strain FXJ1.102T exhibited chemotaxonomic characteristics that are typical for members of the genus Nonomuraea, such as the presence of meso-diaminopimelic acid in the peptidoglycan and galactose, glucose, mannose, ribose and madurose as whole-cell sugars (cell-wall chemotype IIIB) (Lechevalier & Lechevalier, 1970). The predominant menaquinone was MK-9(H4) (72.9 %) and minor amounts of MK-9(H6) (12.2 %), MK-9(H2) (10.7 %) and MK-9 (4.2 %) were also present. The detected phospholipids were diphosphatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylmethyl ethanolamine, two unknown glucosamine-containing phospholipids, phosphatidylinositol, phosphatidylglycerol and an unknown phospholipid (Fig. S2), which corresponds to phospholipid type IV (Lechevalier et al., 1977). The detailed fatty acid composition was iso-C16 : 0 (22.2 %), iso-C16 : 1 G (20.4 %), C17 : 0 6f6c (16.6 %), 10-methyl C17 : 0 (7.8 %), iso-C15 : 0 (7.3 %), anteiso-C17 : 0 (4.0 %), 10-methyl C16 : 0 (3.6 %), 10-methyl C19 : 0 (2.9 %), C16 : 1 07c (2.6 %), C14 : 0 (2.0 %), C17 : 1 08c (1.9 %), C16 : 0 (1.6 %), iso-C14 : 0 (1.5 %), C18 : 0 (0.9 %), C15 : 0 2-OH (0.9 %), C16 : 0 2-OH (0.8 %), C17 : 0 (0.6 %), iso-C15 : 0 G (0.6 %), C13 : 0 (0.5 %), iso-C17 : 0 (0.4 %), 10-methyl C18 : 0 (0.4 %), C12 : 0 (0.2 %), anteiso-C15 : 0 (0.2 %), iso-C18 : 0 (0.1 %), anteiso-C17 : 0 9c (0.1 %) and C18 : 1 9c (0.1 %).

Extraction of genomic DNA and amplification and sequencing of the 16S rRNA gene were carried out using the method of Ara & Kudo (2006). An almost full-length 16S rRNA gene sequence (1486 bp) was aligned with related sequences obtained from public databases 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 constructed using MEGA version 4.0 (Tamura et al., 2007). The reliability

### Table 1. Comparison of the phenotypic properties of strain FXJ1.102T and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>–</td>
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<td>+</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Cascin</td>
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<td>+</td>
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<tr>
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</tr>
<tr>
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<td>+</td>
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<td>–</td>
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<tr>
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<td>6</td>
<td>7</td>
<td>6</td>
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<tr>
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<td>15–45</td>
<td>15–37</td>
<td>15–37</td>
<td>10–37</td>
<td>10–45</td>
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</table>

Strains: 1, Nonomuraea jiangxiensis sp. nov. FXJ1.102T; 2, N. candida HMC10T; 3, N. antimicrobica YIM 61105T; 4, ‘N. aegyptia’ S136; 5, N. turkeenica DSM 43926T; 6, N. rubra JCM 3389T. All data were taken from this study. +, Positive; w, weakly positive; –, negative.
of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain FXJ1.102T belonged to the genus Nonomuraea. The isolate formed a subcluster with Nonomuraea antimicrobica YIM 61105T and ‘Nonomuraea aegyptia’ S136, recovered by both the neighbour-joining and the maximum-parsimony algorithms (Fig. 1), with which it shared 97.9 and 97.5% 16S rRNA gene sequence similarity, respectively. The isolate also shared high 16S rRNA gene sequence similarity with other members of the cluster to which the subcluster belonged (Nonomuraea candida HMC107, 98.9%; Nonomuraea turkmeniaca DSM 43926T, 98.3%; Nonomuraea rubra DSM 43768T, 98.0%) and a relatively distant cluster (Nonomuraea kuesteri GW 14-1925T, 98.5%; Nonomuraea maheshkhalensis 16-5-14T, 98.1%).

The DNA G+C content of strain FXJ1.102T was determined by HPLC and found to be 72.6 mol% (Tamaoka & Komagata, 1984). Using the method described by Rong & Huang (2010), DNA–DNA hybridization was carried out between the isolate and the type strains that were most closely related in terms of both 16S rRNA gene sequence similarity or phylogenetic position, i.e. N. candida DSM 45086T, N. turkmeniaca DSM 43926T, N. antimicrobica YIM 61105T and ‘N. aegyptia’ S136. Strain FXJ1.102T showed low DNA–DNA relatedness with the reference strains (22.4±4.2, 21.0±4.0, 19.4±2.2 and 16.0±2.6%, respectively). These values are well below the 70% cut-off point for assigning strains to the same species (Stackebrandt & Goebel, 1994). Previous studies have shown that members of the genus Nonomuraea that exhibit high 16S rRNA gene sequence similarity (ranging from 97.6 to 99.4%) can exhibit low DNA–DNA relatedness (Fischer et al., 1983; Poschner et al., 1985; Stackebrandt et al., 2001; Kämpfer et al., 2005); therefore, DNA–DNA hybridizations were not carried out between strain FXJ1.102T and other members of the genus Nonomuraea.

The morphological, chemotaxonomic and phylogenetic data clearly support the assignment of strain FXJ1.102T to the genus Nonomuraea, but the physiological characters and DNA–DNA relatedness demonstrate that the strain can be differentiated from its closest relatives in the genus Nonomuraea. Therefore, on the basis of genotypic and phenotypic evidence, strain FXJ1.102T represents a novel species of the genus Nonomuraea, for which the name Nonomuraea jiangxiensis sp. nov. is proposed.

**Description of Nonomuraea jiangxiensis sp. nov.**

Nonomuraea 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 3 and Czapek’s agar, dark yellow on ISP 2 and yellow on ISP 4, ISP 5, ISP 6, ISP 7, respectively). These values are well below the 70% cut-off point for assigning strains to the same species (Stackebrandt & Goebel, 1994). Previous studies have shown that members of the genus Nonomuraea that exhibit high 16S rRNA gene sequence similarity (ranging from 97.6 to 99.4%) can exhibit low DNA–DNA relatedness (Fischer et al., 1983; Poschner et al., 1985; Stackebrandt et al., 2001; Kämpfer et al., 2005); therefore, DNA–DNA hybridizations were not carried out between strain FXJ1.102T and other members of the genus Nonomuraea.

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The micromorphological characteristics are typical for the genus Nonomuraea. Casein, aesculin, allantoin and arbutin are hydrolysed and L-tyrosine and guanine are weakly hydrolysed, but adenine, hypoxanthine, gelatin, starch, xanthine and urea are not hydrolysed. Catalase-positive. Nitrate is reduced. H₂S is not produced. Utilizes L-arabinose, celllobiose, dextrin, D-galactose, D-glucose, glycerol, lactose, maltose, mannitol, D-mannose, melibiose, raffinose, rhamnose, salicin, adonitol and methyl 2-D-glucoside as sole carbon sources, but not myo-inositol, sucrose, xylose, ribose, D-fructose, trehalose, D-sorbitol or meso-erythritol. Grows with up to 6% (w/v) NaCl (optimum 0% NaCl), at pH 5.0–9.0 (optimum pH 6.0) and at 20–45°C (optimum 25–35°C). The diagnostic diaminoc acid of the peptidoglycan is meso-diaminopimelic acid. Cell hydrolysates contain madurose, glucose, mannose, ribose and galactose. The polar lipids include diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylmethyl-ethanolamine, two unknown glucosamine-containing phospholipids, phosphatidylinositol, phosphatidylglycerol and an unknown phospholipid. The predominant menaquinone of the type strain is MK-9(H₂). The major fatty acids (>10%) are iso-C₁₆:0, iso-C₁₆:1 G and C₁₇:0 3OH.

The type strain, FXJ1.102ᵀ (=CGMCC 4.6533ᵀ=NBRC 106679ᵀ), was isolated from acidic soil in Nanchang city, Jiangxi Province, China. The DNA G+C content of the type strain is 72.6 mol%.

Acknowledgements

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References


