Nonomuraea muscovyensis sp. nov., isolated from soil

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A novel actinomycete, strain FMN03^T, was isolated from a soil sample collected from Yuga Zapadnaya South-West Forest Park, Moscow, Russia. The isolate had chemical and morphological properties typical of members of the genus *Nonomuraea* and formed a distinct 16S rRNA gene subclade with the type strains *Nonomuraea roseoviolacea* subsp. *carminata* NBRC 15903^T and *Nonomuraea roseoviolacea* subsp. *roseoviolacea* NBRC 14098^T. The organism formed extensively branched substrate and aerial hyphae, which generated spiral chains of spores with smooth surfaces. The cell wall contained *meso*-diaminopimelic acid and the whole cell sugars were glucose, galactose and trace amounts of madurose, mannose and xylose. The polar lipids were phosphatidylethanolamine, hydroxyphosphatidylethanolamine, four unidentified phospholipids, four unidentified glycolipids and one unidentified lipid. The predominant menaquinone was MK-9(H_4). The major fatty acids were iso-$C_{16:0}$ 2-OH, $C_{17:0}$ 10-methyl, $C_{17:1}$ *cis*9 and iso-$C_{16:0}$. Analyses of its morphological, physiological and biochemical characteristics, together with DNA–DNA relatedness data, confirmed that strain FMN03^T is a representative of a novel species of the genus *Nonomuraea*, which is distinct from closely related reference strains. Strain FMN03^T (=DSM 45913^T=KCTC 29233^T) is proposed as the type strain of a novel species, for which the name *Nonomuraea muscovyensis* sp. nov. is proposed.

The genus *Nonomuria* was originally proposed by Zhang *et al.* (1998) as a member of the family *Streptosporangiaceae*, but subsequently the spelling was corrected to *Nonomuraea* by Chiba *et al.*, (1999). The genus is characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid in the cell wall, madurose as a characteristic sugar in whole-cell hydrolysates, and di-, tetra- and hexa-hydrogenated menaquinones with nine isoprene units as the predominant isoprenologues (Nonomura & Ohara, 1971; Zhang *et al.*, 1998; Quintana *et al.*, 2003). Members of the genus *Nonomuraea* are widespread in nature, and are found in soil, the rhizosphere, marine and river sediments, caves, leaves, and in many more locations (Ara *et al.*, 2007; Qin *et al.*, 2009; Li *et al.*, 2011; Wang *et al.*, 2011; Xi *et al.*, 2011; Zhao *et al.*, 2011; Cao *et al.*, 2012; Nakaew *et al.*, 2012; Camas *et al.*, 2013). There is evidence that the genus *Nonomuraea* is underspeciated (Wang *et al.*, 1999). At the time of writing the genus comprises 32 species with validly published names and two subspecies, all identified on the basis of a polyphasic approach (http://www.bacterio.net/index.html); most of these have been described in the last decade. The type species of the genus is *Nonomuraea pusilla* (Nonomura & Ohara, 1971; Zhang *et al.*, 1998). As part of a programme to discover actinomycetes from diverse habitats an aerobic actinomycete strain, FMN03^T, was isolated. In this study, we performed a polyphasic taxonomic investigation of this strain, and propose that strain FMN03^T is assigned to a novel species of the genus *Nonomuraea*.

Strain FMN03^{T}was isolated from soil collected from the Yuga Zapadnaya South-West Forest Park, Moscow, Russia. The soil sample was passed through a 2 mm mesh sieve, air-dried at room temperature for 14 days, and triturated using a sterile pestle. One gram of soil was suspended in 9 ml sterile strength Ringer's solution (Merck) and shaken for 30 min on a tumble shaker. Suspensions were then placed in a pre-warmed water bath (60 °C for 20 min). These 10^{-1} dilutions were serially diluted down with Ringer's solution to dilutions of 10^{-4}. The diluted soil suspension was inoculated on modified tryptone-yeast extract-glucose (TYG) with vitamin agar (Bowers & Hucker, 1935), supplemented with filter-sterilized cycloheximide (50 μg ml^{-1}), nalidixic acid (10 μg ml^{-1}) and rifampicin (0.5 μg ml^{-1}), and incubated at 28 °C for 21 days. The strain was isolated as a pure culture and maintained on yeast extract-malt extract

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of FMN03^T is JN896617.

Three supplementary figures and one supplementary table are available with the online version of this paper.

agar [International Streptomyces Project medium 2 (ISP 2)] (Shirling & Gottlieb, 1966) slopes at room temperature and stored in glycerol suspensions (20 %, v/v) at −20 °C.

Genomic DNA was extracted from biomass obtained from shaking incubations in ISP 2 broth at 28 °C for 14 days, using the method described for the modified guanidine thiocyanate DNA extraction procedure (Pitcher *et al.*, 1989). The 16S rRNA genes were amplified using the universal primers, 27f (5′-AGAGTTTGATCMTGGCTCAG-3′ (Lane, 1991) and 1525r (5′-AAGGAGGTGWTCCARCC-3′ (Lane, 1991). The almost-complete 16S rRNA gene sequence of the novel strain (1495 nt) was determined and aligned with corresponding sequences of representative type strains of the genus *Nonomuraea* (retrieved from the EzTaxon-e server; Kim *et al.*, 2012) by using CLUSTAL W in MEGA5 (Tamura *et al.*, 2011). Phylogenetic analysis was carried out using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distances were calculated using the model of Jukes & Cantor (1969). The topography of the reconstructed trees was evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985).

DNA–DNA hybridization was employed to clarify further the relatedness of strain FMN03^T to its closest neighbours (*Nonomuraea salmonea* DSM 43678^T, *Nonomuraea maheshkhaliensis* DSM 45163^T and *Nonomuraea roseoviolacea* subsp. *carminata* DSM 44170^T) based on 16S rRNA gene sequence similarities, and this was performed by the Identification Service at the Deutsche Sammlung von Mikroorganismen und Zelkulturen (DSMZ, 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 Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian).

Biomass for chemotaxonomic studies was prepared by growing strain FMN03^T in ISP 2 broth (Shirling & Gottlieb, 1966) at 160 r.p.m. for 10 days at 28 °C; cells were harvested by centrifugation, washed twice in distilled water, recentrifuged and freeze-dried. Whole-cell amino acids and sugars were prepared according to the methods of Lechevalier & Lechevalier (1970) and analysed by TLC (Staneck & Roberts, 1974). Analyses of polar lipids and respiratory quinones were carried out by the Identification Service of the DSMZ. Respiratory quinones were extracted from 100 mg of freeze-dried cells based on the two-stage method described by Tindall (1990a; b). Respiratory quinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (Macherey-Nagel; art. no. 805 023), using hexane/tert-butyl methyl ether (9 : 1, v/v) as solvent. UV absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and analysed further by HPLC. This step was carried out using an 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 eluent. Respiratory lipoquinones were detected at 269 nm. For the extraction of whole-cell fatty acids, cells were grown in 20 ml trypticase soy broth (TSB; Merck) at 28 °C with shaking at 150 r.p.m. After 5 days of incubation, 5 ml of seed culture was inoculated into 50 ml TSB. The inoculated flask was incubated as before for a further 5 days. After harvesting by cellulose membrane filtration (0.45 μm), wet cells (200 mg) were placed in an extraction tube. Cellular fatty acids were extracted and used to prepare fatty acid methyl esters, which were separated by the Microbial Identification System (MIDI; Microbial ID), utilizing an Agilent Technologies 6890N gas chromatograph with a G2614A autosampler and a 6783 injector (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Fatty acid methyl ester peaks were analysed using the TSBA database, version 5.0. The DNA G + C content of strain FMN03^T was determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

Cultural characteristics were determined after incubation at 28 for 14 days on various media as described by Shirling & Gottlieb (1966): yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), modified Bennett's agar (MBA; Jones, 1949), nutrient agar (NA; Difco) and trypticase soy agar (TSA; Merck). The National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1964) were used for designation of colours and names. Growth at different temperatures (4, 10, 20, 28, 30, 37, 40, 45 and 50 °C), and pH (4–11) (at intervals of 1.0 pH unit), and in the presence of NaCl (0–10 %,w/v, at 1.0 % intervals) was determined on ISP 2. Established methods were used to determine whether the strains degraded Tweens 40 and 80 (Nash & Krent, 1991); the remaining degradation tests employed the 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 % (w/v) of the carbon sources tested (0.1 % for succinic acid). Nitrogen source utilization was examined using the basal medium recommended by Williams *et al.* (1983) supplemented with a final concentration of 0.1 % (w/v) of the nitrogen sources tested. The antimicrobial activity of strain FMN03^T in inhibiting the growth of twenty-four micro-organisms including Gram-positive and Gram-negative bacteria and fungi was observed using an overlay technique, as described by Williams *et al.* (1983). The test organisms were provided by the American Type Culture Collection (ATCC) and the National Center for Agricultural Utilization Research (NRRL). Spot-inoculated colonies on modified Bennett's agar plates were inverted over 2 ml chloroform for 40 min. Killed colonies were overlaid with 5–7 ml sloppy modified Bennett's broth (Jones, 1949) inoculated with test organisms.

Zones of inhibition were scored as positive results after 24 h at 37 °C. With the exception of those for antimicrobial activity, the type strains *N. salmonea* DSM 43678^T, *N. maheshkhaliensis* DSM 45163^T, *N. roseoviolacea* subsp. *carminata* DSM 44170^T and *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T were included for comparison in all tests. The colony morphology and micromorphological properties of isolate FMN03^T were determined by examining gold-coated dehydrated specimens of 21-day-old cultures grown on ISP 2 medium at 28 °C using a scanning electron microscope (JEOL; JSM-6060).

The 1495 bp sequence corresponding to the 16S rRNA gene region of strain FMN03^T was compared with sequences deposited in public databases. Comparison of the almost-complete 16S rRNA gene sequence of the isolate with corresponding sequences of phylogenetically related species with validly published names showed that it formed a branch in the 16S rRNA gene tree of the genus *Nonomuraea* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain FMN03^T formed a distinct branch with the type strains *N. roseoviolacea* subsp. *roseoviolacea* NBRC 14098^T and *N. roseoviolacea* subsp. *carminata* NBRC 15903^T. This relationship was supported by all the tree-making algorithms used in this study (Figs S1 and S2, available in the online Supplementary Material). The highest 16S rRNA sequence similarities between the novel isolate and type strains of recognized species in the databases were: 98.07 % (28 nt differences at 1449 sites) to *N. salmonea* DSM 43678^T; 97.79 % (32 nt differences at 1448 sites) to *N. maheshkhaliensis* 16-5-14^T; 97.75 % (33 nt differences at 1465 sites) to *Nonomuraea kuesteri* GW 14-1925^T; 97.62 % (34 nt differences at 1430 sites) to *Nonomuraea turkmeniaca* DSM 43926^T; 97.61 % (35 nt differences at 1466 sites) to *Nonomuraea endophytica* YIM 65601^T; 97.58 % (34 nt differences at 1404 sites) to *Nonomuraea candida* HMC10^T; 97.42 % (36 nt differences at 1398 sites) to *N. roseoviolacea* subsp. *carminata* NBRC 15903^T; 97.37 % (37 nt differences at 1409 sites) to Nonomuraea angiospora NBRC 13155^T; 97.33 % (39 nt

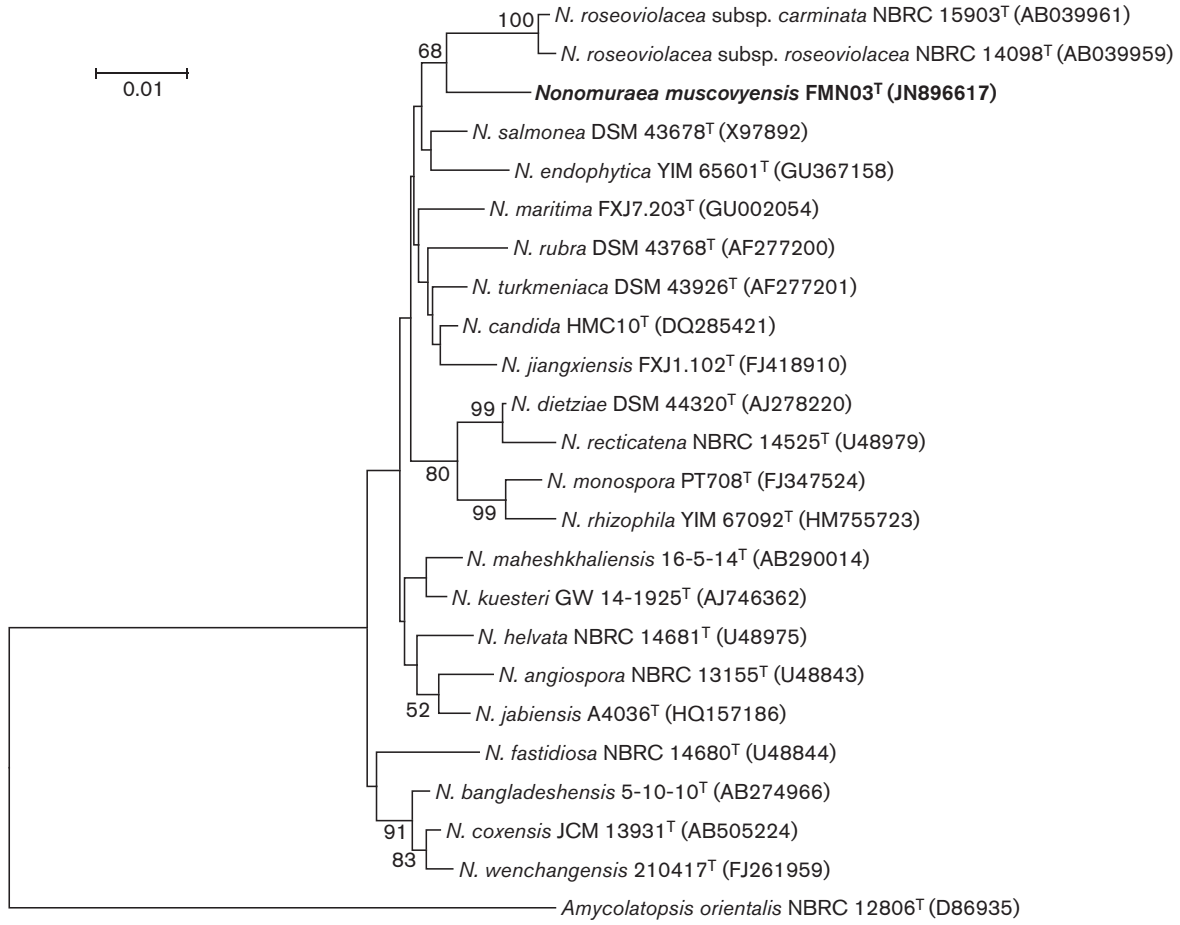

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences (1495 nt) showing the relationship between strain FMN03^T and recognized species of the genus *Nonomuraea* . *Amycolatopsis orientalis* NBRC 12806^T was used as an outgroup. Numbers at the nodes indicate the levels of bootstrap support (%); only values $\geqslant$50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per site.

differences at 1463 sites) to *Nonomuraea maritima* FXJ7.203^T; 97.30 % (38 nt differences at 1410 sites) to *Nonomuraea helvata* NBRC 14681^T; 97.27 % (40 nt differences at 1464 sites) to *Nonomuraea jabiensis* A4036^T; and 97.04 % (43 nt differences at 1451 sites) to *N. roseoviolacea* subsp. *roseoviolacea* NBRC 14098^T.

Strain FMN03^T showed DNA–DNA relatedness values of 19.9 ± 3.5 % to *N. salmonea* DSM 43678^T, 17.4 ± 0.5 % to *N. maheshkhaliensis* DSM 45163^T and 26.3 ± 5 % to *N. roseoviolacea* subsp. *carminata* DSM 44170^T (based on a mean of duplicate determinations in 2 × SSC and 10 % (v/v) formamide at 70 °C); these are well below the 70 % threshold value for the definition of a bacterial genomic species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). It has been pointed out that 16S rRNA gene nucleotide similarities within the range 97.6–99.4 % have been recorded for members of several species of the genus *Nonomuraea* with validly published names that exhibit low DNA–DNA relatedness values (Fischer *et al.*, 1983; Poschner *et al.*, 1985; Tamura *et al.*, 2000; Stackebrandt *et al.*, 2001; Quintana *et al.*, 2003; Kämpfer *et al.*, 2005). For this reason DNA–DNA relatedness experiments were not carried out with strain FMN03^T and the remaining related type species of the genus *Nonomuraea*.

Strain FMN03^T contained *meso*-diaminopimelic acid (cell wall type III; Lechevalier & Lechevalier, 1970) as the cell-wall diamino acid and the whole-cell sugars were glucose, galactose (major components), mannose, madurose and xylose (Type B; madurose was the diagnostic sugar). The polar lipids were phosphatidylethanolamine, hydroxyphosphatidylethanolamine, four unidentified phospholipids, four unidentified glycolipids and one unidentified lipid (Fig S3). The predominant menaquinone of strain FMN03^T was MK-9(H_4) (71.0 %); MK-9(H_2) (10.0 %), MK-9(H_6) (9.0 %), MK-9 (5.0 %) and MK-9(H_8) (1.0 %) were also detected. The major cellular fatty acids were iso-$C_{16:0}$ 2-OH (19.0 %), $C_{17:0}$ 10-methyl (16.4 %), $C_{17:1}$ *cis*9 (14.0 %) and iso-$C_{16:0}$ (13.4 %). The comparative cellular fatty acid compositions of strain FMN03^T, *N. salmonea* DSM 43678^T, *N. maheshkhaliensis* DSM 45163^T, *N. roseoviolacea* subsp. *carminata* DSM 44170^T and *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T are shown in Table S1. The DNA G+C content of strain FMN03^T was 69.6 mol%.

The morphological characteristics of strain FMN03^T were consistent with those of members of the genus *Nonomuraea*. Strain FMN03^T showed good growth on modified Bennett's, ISP 2–5 and ISP 7, and moderate growth on tryptic soy, Czapek's, nutrient and ISP 6 media. Aerial hyphae were light orange on ISP 2, yellowish-brown on ISP 3, pale orange-yellow on ISP 4, pale pink on ISP 6, and light yellowish-pink on ISP 7. The colour of the substrate mycelium was yellowish-brown to brownish-orange. Diffusible pigments were not produced. Melanoid pigments were not produced on ISP 6 or ISP 7 media. Strain FMN03^T formed extensively branched substrate and aerial hyphae that often formed spiral chains of spores with smooth surfaces (Fig. 2). The

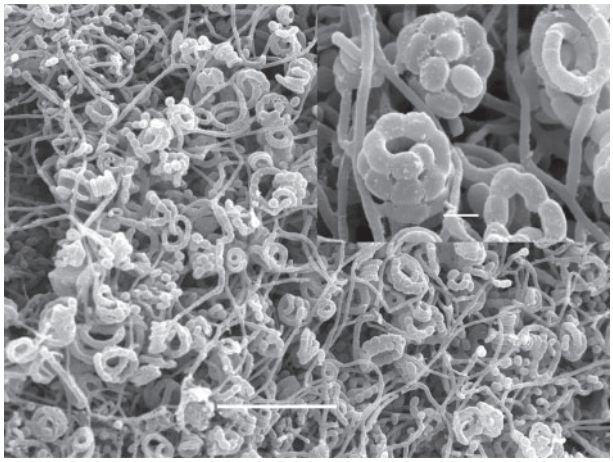

Fig. 2. Scanning electron micrographs of strain FMN03^T grown on yeast extract-malt extract agar (ISP 2) at 28 °C for 21 days. Bar, 10 μm in main micrograph and 1 μm in the inset micrograph.

physiological properties that distinguish strain FMN03^T from closely related species of the genus *Nonomuraea* are presented in Table 1.

It is evident that strain FMN03^T can be distinguished from its close phylogenetic relatives *N. salmonea* DSM 43678^T, *N. maheshkhaliensis* DSM 45163^T, *N. roseoviolacea* subsp. *carminata* DSM 44170^T and *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T based on 16S rRNA gene sequence analysis, DNA–DNA relatedness and phenotypic data. It is, therefore, proposed that the micro-organism should be recognized as a representative of a novel species, with the name *Nonomuraea muscovyensis* sp. nov.

Description of *Nonomuraea muscovyensis* sp. nov.

Nonomuraea muscovyensis (mus.co.vy.en′sis. N.L. fem. adj. *muscovyensis* of or belonging to Moscow, Russia, the source of the type strain).

Aerobic, Gram-reaction-positive, non-motile actinomycete, which forms extensively branched, brownish-orange substrate mycelia that bear light-orange aerial hyphae on yeast extract-malt extract agar. Aerial mycelia bear spiral chains of spores with smooth surfaces. No diffusible pigment or melanin is detected on any media tested. Growth occurs at pH 6.0–11, and at 28–40 °C, but not at pH 4.0 or 5.0 or at temperatures of 4, 10, 20 or 45 °C. Optimal growth occurs at 28–30 °C and pH 7.0. Growth is observed in the presence of 0–1 % (w/v) NaCl. Positive for arbutin, aesculin and nitrate reduction, but negative for allantoin and urea hydrolysis. Tween 80 is degraded but not adenine, casein, xanthine, gelatin, guanine, hypoxanthine, xylan, starch or Tween 40. Utilizes adonitol, cellobiose, D-sorbitol, D-mannitol, inulin, L-rhamnose, sucrose and xylose as sole carbon sources, but not L-arabinose, D-arabinose, D-fructose, D-galactose, D-mannose, D-ribose, dextrin, dextran, L-sorbose, lactose,

Table 1. Phenotypic properties of strains FMN03^T and closely related type strains

Strains: 1, FMN03^T; 2, *N. salmonea* DSM 43678^T; 3, *N. maheshkhaliensis* DSM 45163^T; 4, *N. roseoviolacea* subsp. *carminata* DSM 44170^T; 5, *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T. +, Positive; − negative. Strains were positive for arbutin, aesculin hydrolysis, and the ability to grow with adonitol, inulin and L-rhamnose as sole carbon sources (1.0 %). Strains grew at pH 6–11, 28–37 °C and with 0–1 % (w/v) NaCl. Strains were negative for urea hydrolysis, degradation of adenine (0.5 %; w/v), casein (1 %; w/v), guanine (0.5 %; w/v), xylan (0.4 %; w/v), hypoxanthine (0.4 %; w/v), xanthine (0.4 %; w/v) and gelatin (0.4 %; w/v), and the ability to grow with D-arabinose, dextrin, L-glutamic acid, maltose, xylitol or D-ribose as sole carbon sources (1.0 %; w/v). Growth occurred at pH 4, 4, 10, 50 and 55 °C, and with 4–10 % (w/v) NaCl. All data were obtained in this study.

Properties	1	2	3	4	5
Nitrate reduction	+	+	+	−	+
Tolerance of pH 5.0	−	−	+	+	−
Temperature for growth (°C)					
20	−	−	+	−	−
40	+	−	+	+	+
45	−	−	−	+	−
NaCl tolerance (%, w/v)					
2.0	−	+	+	+	+
3.0	−	+	+	+	−
Degradation of (1 %):					
Starch (1 %; w/v)	−	−	−	+	−
Tween 40 (1 %; v/v)	−	+	+	+	−
Tween 80 (1 %; v/v)	+	−	−	−	−
Growth on sole carbon sources (1.0 %, w/v)					
L-Arabinose	−	−	+	+	−
Cellobiose	+	−	+	−	+
D-Fructose	−	−	+	+	−
D-Sorbitol	+	+	−	−	+
D-Galactose	−	+	+	+	+
D-Mannose	−	−	+	+	−
D-Mannitol	+	+	+	−	+
Dextran	−	−	+	−	+
L-Sorbose	−	+	+	−	−
Lactose	−	−	−	−	+
myo-Inositol	−	−	−	−	+
Succinic acid (0.1 %)	−	+	−	+	+
Sucrose	+	+	+	+	−
Xylose	+	−	+	+	+
Use of sole nitrogen sources (0.1 %, w/v)					
α-Isoleucine	−	−	+	−	+
D-Phenylalanine	−	+	+	−	−
Glycine	−	+	+	+	−
L-Alanine	−	+	+	+	+
L-Histidine	−	+	+	+	+
Hydroxy-L-proline	−	+	+	−	−
L-Methionine	−	+	+	+	+
L-Phenylalanine	−	+	+	−	+
L-Proline	−	+	+	+	+
L-Valine	−	+	+	−	+
L-Tyrosine	−	+	+	+	+

L-glutamic acid, maltose, *myo*-inositol, xylitol or succinic acid. Utilizes L-arginine, L-cysteine, L-serine, L-threonine and L-asparagine as sole nitrogen sources, but not α-isoleucine, D-phenylalanine, glycine, L-alanine, L-histidine, hydroxy-L-proline, L-methionine, L-phenylalanine, L-proline, L-valine or L-tyrosine. Antimicrobial activity is shown against *Bacillus subtilis* NRRL B-209 and *Enterobacter aerogenes* NRRL B-427, but not against *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* NRRL B-767, *Staphylococcus aureus* ATCC 33862, *Listeria monocytogenes* ATCC 19117, *Enterobacter aerogenes* NRRL B-3567, *Providencia stuartii*, *Aspergillus parasiticus* NRRL-465^T, *Aspergillus flavus* NRRL-1957^T, *Aspergillus niger*, *Candida albicans* ATCC 10231^T, *Candida utilis* NRRL Y-900, *Escherichia coli* ATCC 25922, *Escherichia coli* MC4100, *Citrobacter freundii* NRRL B-2643, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* NRRL B-2679, *Proteus vulgaris* NRRL B-123, *Bacillus licheniformis* NRRL B-1001, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213 or *Micrococcus luteus* NRRL B-1018. The predominant menaquinone is MK-9(H_4), with minor amounts of MK-9(H_2), MK-9(H_6), MK-9 and MK-9 (H_8) also detected. The polar lipid profile contains phosphatidylethanolamine, hydroxyphosphatidylethanolamine, four unidentified phospholipids, four unidentified glycolipids and one unidentified lipid. Major fatty acids are iso-$C_{16:0}$ 2-OH, $C_{17:0}$ 10-methyl, $C_{17:1}$ *cis*9 and iso-$C_{16:0}$.

The type strain, FMN03^T (=DSM 45913^T=KCTC 29233^T), was isolated from soil from the Yuga Zapadnaya South-West Forest Park, Moscow, Russia. The G+C content of the genomic DNA of the type strain is 69.6 mol%.

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References

Ara, I., Kudo, T., Matsumoto, A., Takahashi, Y. & Omura, S. (2007). *Nonomuraea maheshkhaliensis* sp. nov., a novel actinomycete isolated from mangrove rhizosphere mud. *J Gen Appl Microbiol* **53**, 159–166.

Bowers & Hucker (1935). Tech. Bull. 228. NY State Agar. Exp. Sta.

Camas, M., Sazak, A., Spröer, C., Klenk, H. P., Cetin, D., Guven, K. & Sahin, N. (2013). *Nonomuraea jabiensis* sp. nov., isolated from arid soil. *Int J Syst Evol Microbiol* **63**, 212–218.

Cao, Y. R., Jin, R. X., Jiang, Y., He, W. X. & Jiang, C. L. (2012). *Nonomuraea soli* sp. nov., an actinomycete isolated from soil. *Int J Syst Evol Microbiol* **62**, 1587–1591.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Chiba, S., Suzuki, M. & Ando, K. (1999). Taxonomic re-evaluation of '*Nocardiopsis*' sp. K-252^T (=NRRL 15532^T): a proposal to transfer this strain to the genus *Nonomuraea* as *Nonomuraea longicatena* sp. nov. *Int J Syst Bacteriol* **49**, 1623–1630.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement 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. (1985). Confidence limits on phylogeny: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fischer, A., Kroppenstedt, R. M. & Stackebrandt, E. (1983). Molecular-genetic and chemotaxonomic studies on *Actinomadura* and *Nocardiopsis*. *J Gen Microbiol* **129**, 3433–3446.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gonzalez, J. M. & Saiz-Jimenez, C. (2005). A simple fluorimetric method for the estimation of DNA–DNA relatedness between closely related microorganisms by thermal denaturation temperatures. *Extremophiles* **9**, 75–79.

Huss, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

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.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Kämpfer, P., Kroppenstedt, R. M. & Grün-Wollny, I. (2005). *Nonomuraea kuesteri* sp. nov. *Int J Syst Evol Microbiol* **55**, 847–851.

Kelly, K. L. (1964). Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors. Washington, DC: US Government Printing Office.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.

Li, J., Zhao, G. Z., Huang, H. Y., Zhu, W. Y., Lee, J. C., Xu, L. H., Kim, C. J. & Li, W. J. (2011). *Nonomuraea endophytica* sp. nov., an endophytic actinomycete isolated from *Artemisia annua* L. *Int J Syst Evol Microbiol* **61**, 757–761.

Nakaew, N., Sungthong, R., Yokota, A. & Lumyong, S. (2012). *Nonomuraea monospora* sp. nov., an actinomycete isolated from cave soil in Thailand, and emended description of the genus *Nonomuraea*. *Int J Syst Evol Microbiol* **62**, 3007–3012.

Nash, P. & Krent, M. M. (1991). Culture media. In *Manual of Clinical Microbiology*, 5th edn, pp. 1268–1270. Edited by A. Ballows, W. J. Hauser, K. L. Herrmann, H. D. Isenberg & H. J. Shadomy. Washington, DC: American Society for Microbiology.

Nonomura, H. & Ohara, Y. (1971). Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier et al. *J Ferment Technol.* **49**, 904–912.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Poschner, J., Kroppenstedt, R. M., Fischer, A. & Stackebrandt, E. (1985). DNA-DNA reassociation and chemotaxonomic studies on *Actinomadura, Microbispora, Microtetraspora, Micropolyspora* and *Nocardiopsis*. *Syst Appl Microbiol* **6**, 264–270.

Qin, S., Zhao, G. Z., Klenk, H. P., Li, J., Zhu, W. Y., Xu, L. H. & Li, W. J. (2009). *Nonomuraea antimicrobica* sp. nov., an endophytic actinomycete isolated from a leaf of *Maytenus austroyunnanensis*. *Int J Syst Evol Microbiol* **59**, 2747–2751.

Quintana, E., Maldonado, L. & Goodfellow, M. (2003). *Nonomuraea terrinata* sp. nov., a novel soil actinomycete. *Antonie van Leeuwenhoek* **84**, 1–6.

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.

Stackebrandt, E., Wink, J., Steiner, U. & Kroppenstedt, R. M. (2001). *Nonomuraea dietzii* sp. nov. *Int J Syst Evol Microbiol* **51**, 1437–1441.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Tamura, T., Suzuki, S. & Hatano, K. (2000). *Acrocarpospora* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Evol Microbiol* **50**, 1163–1171.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Wang, Y., Zhang, Z., Ruan, J., Wang, Y. & Ali, S. (1999). Investigation of actinomycete diversity in the tropical rainforests of Singapore. *J Ind Microbiol Biotechnol* **23**, 178–187.

Wang, F., Xu, X. X., Qu, Z., Wang, C., Lin, H. P., Xie, Q. Y., Ruan, J. S., Sun, M. & Hong, K. (2011). *Nonomuraea wenchangensis* sp. nov., isolated from mangrove rhizosphere soil. *Int J Syst Evol Microbiol* **61**, 1304–1308.

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). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.

Xi, L., Zhang, L., Ruan, J. & Huang, Y. (2011). *Nonomuraea maritima* sp. nov., isolated from coastal sediment. *Int J Syst Evol Microbiol* **61**, 2740–2744.

Zhang, Z., Wang, Y. & Ruan, J. (1998). Reclassification of *Thermomonospora* and *Microtetraspora*. *Int J Syst Bacteriol* **48**, 411–422.

Zhao, G. Z., Li, J., Huang, H. Y., Zhu, W. Y., Xu, L. H. & Li, W. J. (2011). *Nonomuraea rhizophila* sp. nov., an actinomycete isolated from rhizosphere soil. *Int J Syst Evol Microbiol* **61**, 2141–2145.