Nonomuraea soli sp. nov., an actinomycete isolated from soil

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A straight-chain, spore-forming actinobacterium, strain YIM 120770^T, was isolated from soil. Phylogenetic analysis on the basis of 16S rRNA gene sequence comparisons revealed that the isolate represents a distinct cluster within the clade comprising the genus *Nonomuraea* and is related most closely to *Nonomuraea rhizophila* YIM 67092^T (96.5 % similarity). Cells of strain YIM 120770^T grew in the presence of 0–3 % (w/v) NaCl, at 15–37 °C and at pH 7.0–8.0. The diagnostic amino acid was *meso*-diaminopimelic acid, cell hydrolysates contained madurose, glucose, mannose, ribose and galactose, the predominant cellular fatty acids were 10-methyl $C_{17:0}$ and iso-$C_{16:0}$, and the DNA G+C content was 66.4 mol%, data consistent with affiliation of strain YIM 120770^T to the genus *Nonomuraea*. Strain YIM 120770^T shared low levels of 16S rRNA gene sequence similarity (<97 %) with the type strains of recognized species of the genus *Nonomuraea* and could be differentiated from its closest phylogenetic relative based on phenotypic characteristics. These results suggested that strain YIM 120770^T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea soli* sp. nov. is proposed. The type strain is YIM 120770^T (=DSM 45533^T=JCM 17347^T).

The etymology of the genus *Nonomuraea* was corrected by Chiba *et al.* (1999) following description of the genus *Nonomuria* by Zhang *et al.* (1998). At the time of writing, the genus comprised 26 recognized species and two subspecies (http://www.bacterio.cict.fr/n/nonomuraea.html).

In an attempt to investigate the diversity of actinomycetes from Weibao Mountain in Dali, Yunnan province, China, numerous strains were obtained and characterized taxonomically. Preliminary comparative 16S rRNA gene sequence analysis showed that one of these strains, designated YIM 120770^T, formed a separate lineage within the genus *Nonomuraea*. As a consequence, it was subjected to further taxonomic study by using a polyphasic approach, which included determination of its phenotypic properties and detailed phylogenetic analysis based on 16S rRNA gene sequences.

A soil sample was collected from an altitude of 2670 m from Weibao Mountain in south-west China in April 2010. The soil suspension was diluted and spread onto mycose-proline agar [5 g mycose, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4.7H_2O$, 3.7 mg vitamin mixture (Hayakawa & Nonomura, 1987), 20 g agar, pH 7.2] after incubation at 28 °C for 30 days.

General cell morphology was studied by light microscopy (Olympus BH-2) and scanning electron microscopy (QUANTA200; FEI) of a 21-day-old culture of strain YIM 120770^T grown on ISP 2 agar medium (Shirling & Gottlieb, 1966). For cultural characterization, strain YIM 120770^T was grown for 28 days at 28 °C on ISP media 2, 3, 4 and 5 (Shirling & Gottlieb, 1966), nutrient agar (Difco), Czapek's agar (Waksman, 1967) and potato-dextrose agar. The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Color Council (Kelly, 1964). Temperature and pH ranges for growth and NaCl tolerance were determined on ISP medium 2 as described by Xu *et al.* (2005). Catalase and oxidase activity was detected by the method of Wang *et al.* (2008). Hydrolysis of starch, cellulose, gelatin, Tweens 20, 40, 60 and 80, milk coagulation and peptonization, reduction of nitrate, urease activity and H_2S production were determined as described by Smibert & Krieg (1994). Utilization of compounds as sole carbon and nitrogen sources was tested according to Gordon *et al.* (1974).

Strain YIM 120770^T for chemotaxonomic analyses was cultured in ISP 2 broth at 28 °C for 14 days. Diaminopimelic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 120770^T is JF742631.

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

acids and whole-cell sugars were analysed according to the methods described by Hasegawa *et al.* (1983) and Tang *et al.* (2009), respiratory quinones were extracted according to Collins *et al.* (1977) and identified by HPLC (Groth *et al.*, 1996), and analysis of polar lipids was performed by TLC as described by Minnikin *et al.* (1979) and Collins & Jones (1980). Biomass for fatty acid analysis was harvested from a Bacto trypticase soy broth shaker after 7 days at 28 °C. *Nonomuraea rhizophila* YIM 67092^T was used in parallel for the above experiments. Cellular fatty acid methyl esters were prepared as described by Sasser (1990) and analysed according to the standard protocol of the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6). GC was performed with an Agilent Technologies 7890A system.

Genomic DNA was extracted and purified, and PCR-mediated amplification of the 16S rRNA gene was performed as described by Li *et al.* (2007). The 16S rRNA gene sequence of strain YIM 120770^T was aligned with corresponding sequences obtained from the DDBJ/EMBL/GenBank databases by using the EzTaxon server (http://147.47.212.35:8080/) (Chun *et al.*, 2007). Multiple alignment of the data was performed by using the program CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic and molecular evolutionary analyses were conducted with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. A phylogenetic tree was constructed by using the neighbour-joining method of Saitou & Nei (1987) with MEGA version 4 (Tamura *et al.*, 2007). Evolutionary distance matrices (distance options according to the Kimura two-parameter model) were calculated as described by Kimura (1980). The tree topology was assessed by using bootstrap analysis with 1000 replicated datasets (Felsenstein, 1985). The DNA G+C content of strain YIM 120770^T was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989).

The 16S rRNA gene sequence of strain YIM 120770^T showed 94.3–96.5 % similarity to those of the type strains of all recognized species of the genus *Nonomuraea*. Strain YIM 120770^T was related most closely to the type strain of *N. rhizophila* YIM 67092^T (96.5 % 16S rRNA gene sequence similarity), and phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 120770^T formed a cluster with the type strains of *N. rhizophila* YIM 67092^T and *Nonomuraea rosea* GW 12687^T (96.1 % 16S rRNA gene sequence similarity) (Fig. 1), which was supported by a high bootstrap value (78 %). This cluster did not form any definite clusters with other closely related *Nonomuraea* strains. 16S rRNA gene sequence analysis thus suggested that strain YIM 120770^T represents a novel species of the genus *Nonomuraea*. The DNA G+C content of strain YIM 120770^T was 66.4 mol%.

With regard to chemotaxonomic characteristics, strain YIM 120770^T had *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, and madurose, mannose and galactose as diagnostic sugars. The major menaquinones were MK-9(H_4) (68.8 %), MK-9(H_6) (14.9 %) and MK-9(H_2) (14.5 %), and the polar lipids comprised diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, OH-phosphatidylethanolamine, OH-phosphatidylmonomethylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unknown aminophosphoglycolipid and three unknown phospholipids (see Fig. S1 in IJSEM Online). The predominant fatty acids were 10-methyl $C_{17:0}$ (12.8 %), iso-$C_{16:0}$ (11.9 %), $C_{17:1}\omega6c$ (9.2 %), anteiso-$C_{15:0}$ (7.4 %) and $C_{16:0}$ (7.4 %). Other components were $C_{17:1}\omega8c$ (5.8 %), 10-methyl $C_{16:0}$ (5.4 %), iso-$C_{14:0}$ (5.3 %), iso-$C_{15:0}$ (5.0 %), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; 4.6 %), $C_{14:0}$ (3.6 %), $C_{18:0}$ (2.6 %), iso-$C_{16:1}$ G (2.5 %), $C_{16:0}$ 2-OH (2.5 %), $C_{17:0}$ (2.3 %), $C_{15:0}$ 2-OH (2.2 %), anteiso-$C_{17:0}$ (2.1 %), $C_{13:0}$ (1.9 %), 10-methyl $C_{18:0}$ (1.4 %), $C_{18:1}\omega9c$ (1.3 %), iso-$C_{17:0}$ (0.8 %), $C_{17:0}$ 2-OH (0.6 %), iso-$C_{18:0}$ (0.3 %), anteiso-$C_{15:1}$ A (0.2 %), iso-$C_{15:1}$ G (0.2 %), $C_{14:0}$ 2-OH (0.2 %), anteiso-$C_{17:1}$ A (0.2 %), $C_{12:0}$ 3-OH (0.2 %) and iso-$C_{13:0}$ (0.1 %).

Strain YIM 120770^T formed extensively branched substrate mycelia and aerial mycelia on ISP 2 agar, spore chains borne on aerial mycelia were straight and the spore surfaces were smooth (Fig. 2). Good growth was observed on ISP 2 and nutrient media, and moderate growth was seen on other media tested. Orange–yellow to orange substrate mycelia and white aerial mycelia appeared on all media tested. No diffusible pigment was produced on any medium. Other physiological properties are given in the species description below and in Table 1.

16S rRNA gene sequence analysis (Fig. 1) showed that strain YIM 120770^T belongs to the genus *Nonomuraea*. It formed extensively branched substrate and aerial mycelia and straight chains of spores with a smooth ornamentation. The diagnostic diamino acid, cell hydrolysates, predominant menaquinones, polar lipids (Fig. S1), major fatty acids and DNA G+C content were all consistent with its classification in the genus *Nonomuraea*.

However, strain YIM 120770^T could be differentiated phenotypically from its closest phylogenetic neighbours, *N. rhizophila* YIM 67092^T and *N. rosea* GW 12687^T, based on morphology (Fig. 2). In addition, strain YIM 120770^T differed from *N. rhizophila* YIM 67092^T and *N. rosea* GW 12687^T in utilization of cellobiose, D-fructose, *myo*-inositol, lactose, hypoxanthine and xanthine. In the polar lipid profile, strain YIM 120770^T contained OH-phosphatidylmonomethylethanolamine, OH-phosphatidylethanolamine and an unknown aminophosphoglycolipid, which were lacking in *N. rhizophila* YIM 67092^T and *N. rosea* GW 12687^T. Strain YIM 120770^T also differed from *N. rhizophila* YIM 67092^T and *N. rosea* GW 12687^T in lacking iso-$C_{16:1}$ G as a major fatty acid ($\geqslant$5.0 %). Strain YIM 120770^T lacked MK-9, which accounts for 6 % in strain *N. rosea* GW 12687^T. Differential characteristics among strain YIM 120770^T, *N. rhizophila* YIM 67092^T and *N. rosea* GW 12687^T are given in Table 1.

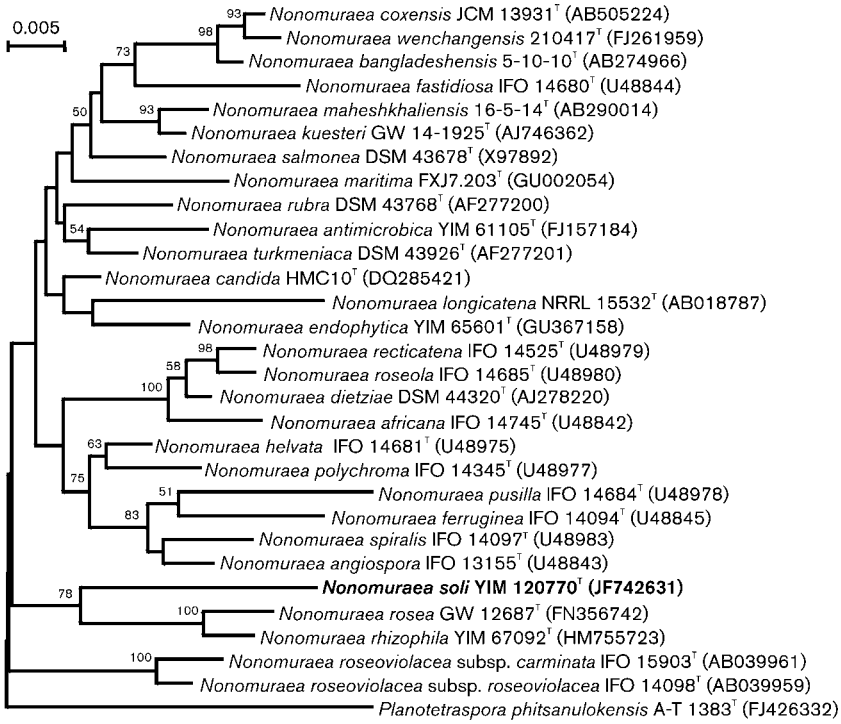

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences (1524 nt) showing the relationship between strain YIM 120770^T and members of the genus *Nonomuraea*. Numbers at branch nodes are bootstrap values based on 1000 resamplings; only values $\geqslant$50 % are shown. The sequence of *Planotetraspora phitsanulokensis* A-T 1383^T was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

On the basis of data from the present taxonomic study, we conclude that strain YIM 120770^T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea soli* sp. nov. is proposed.

Description of *Nonomuraea soli* sp. nov.

Nonomuraea soli (so′li. L. neut. gen. n. *soli* of soil, the source of the type strain).

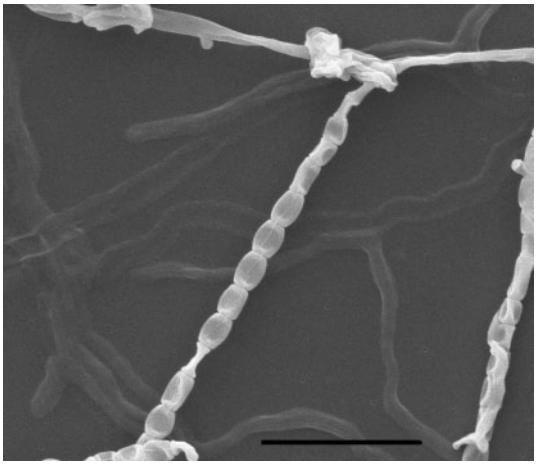

Fig. 2. Scanning electron micrograph of spiral spore chains on aerial mycelium of strain YIM 120770^T grown on ISP 2 at 28 °C for 21 days. Bar, 5 μm.

Substrate mycelia are orange–yellow to orange and aerial mycelia are white. Spore chains are straight, and show a smooth ornamentation. No diffusible pigment is produced. Growth occurs at 15–37 °C (optimum 28 °C), at pH 7.0–8.0 (optimum pH 7.0) and in the presence of 0–3 % NaCl (optimum 0 %). Positive for catalase, oxidase and nitrate reductase; negative for hydrolysis of starch, cellulose, gelatin, and Tweens 20, 40, 60 and 80, milk coagulation and peptonization, urease activity and H_2S production. Utilizes glucose, maltose, D-mannose, D-mannitol, raffinose, L-rhamnose, glycerol, D-sorbitol, D-xylose, xylitol, succinic acid and sodium DL-malate, but not cellobiose, D-fructose, *myo*-inositol, lactose, L-arabinose, D-galactose, dextrin, dulcitol, fucose or L-sorbose. Utilizes L-serine and adenine as sole nitrogen sources, but not hypoxanthine or xanthine. The diagnostic diamino acid is *meso*-diaminopimelic acid. Cell hydrolysates contain madurose, ribose, mannose, glucose and galactose. The predominant menaquinones are MK-9(H_4), MK-9(H_6) and MK-9(H_2). Polar lipids include diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, OH-phosphatidylethanolamine, OH-phosphatidylmonomethylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unknown aminophosphoglycolipid and three unknown phospholipids. Major fatty acids are 10-methyl $C_{17:0}$, iso-$C_{16:0}$, $C_{17:1}\omega6c$, anteiso-$C_{15:0}$ and $C_{16:0}$.

The type strain, YIM 120770^T (=DSM 45533^T=JCM 17347^T), was isolated from Weibao Mountain, south-west China. The DNA G+C content of the type strain is 66.4 mol%.

Table 1. Characteristics that differentiate strain YIM 120770^T from the type strains of *Nonomuraea rhizophila* and *Nonomuraea rosea*

Strains: 1, YIM 120770^T; 2, *N. rhizophila* YIM 67092^T; 3, *N. rosea* GW 12687^T. Data are from the present study except where indicated. ND, Not determined.

Characteristic	1	2	3*
Spore chain	Straight	Spiral*	Spiral
Spore ornamentation	Smooth	Rough*	ND
Max. NaCl tolerance (%)	3	7	5
Oxidase	+	−	+
Milk coagulation	−	+	ND
Milk peptonization	−	+	ND
Urea hydrolysis	−	+	−
Nitrate	+	−	ND
Tween 20	−	+	−
Carbon source utilization:			
Cellobiose	−	+	+
D-Fructose	−	+	+
myo-Inositol	−	+	+
Lactose	−	+	+
Glycerol	+	−	ND
D-Xylose	+	−	+
Nitrogen source utilization:			
Hypoxanthine	−	+	+
Xanthine	−	+	+
Polar lipids†	DPG, PG, PME, PE, OH-PME, OH-PE, APGL, PI, PIM, 3PLs	DPG, PG, PME, PE, PI, GluNu, PLs*	DPG, PG, PME, PE, OH-PME, OH-PE, APGL, PI, PIMs, PL1
Major fatty acids (⩾5 %)	10-methyl $C_{17:0}$ (12.8 %), iso-$C_{16:0}$ (11.9 %), $C_{17:1}\omega6c$ (9.2 %), anteiso-$C_{15:0}$ (7.4 %), $C_{16:0}$ (7.4 %), $C_{17:1}\omega8c$ (5.8 %), 10-methyl $C_{16:0}$ (5.4 %), iso-$C_{14:0}$ (5.3 %), iso-$C_{15:0}$ (5.0 %)	10-methyl $C_{17:0}$ (26.7 %), iso-$C_{16:0}$ (24.0 %), iso-$C_{16:1}$ G (14.1 %), $C_{17:1}\omega6c$ (5.6 %)	iso-$C_{16:0}$ (43.4 %), 10-methyl $C_{17:0}$ (19.8 %), $C_{17:1}\omega6c$ (10.2 %), iso-$C_{16:1}$ G (6.9 %)
Menaquinones	MK-9(H_4) (68.8 %), MK-9(H_6) (14.9 %), MK-9(H_2) (14.5 %)	MK-9(H_4) (82.4 %), MK-9(H_6) (12.6 %), MK-9(H_2) (5.0 %)	MK-9(H_4) (76 %), MK-9(H_2) (11 %), MK-9(H_6) (6 %), MK-9 (6 %)

*Data from Zhao *et al.* (2011) and Kämpfer *et al.* (2010).

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PL, phospholipid; PME, phosphatidylmethylethanolamine; PE, phosphatidylethanolamine; OH-PME, OH-phosphatidylmonomethylethanolamine; OH-PE, OH-phosphatidylethanolamine; APGL, unknown aminophosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; GluNu, unknown glucosamine-containing phospholipid.

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