Nonomuraea flavida sp. nov., a novel species of soil actinomycete isolated from Aconitum napellus rhizosphere

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A novel actinomycete strain, YN-5-1T, isolated from the rhizosphere soil of a medicinal plant, Aconitum napellus, was characterized by a polyphasic approach to determine its taxonomic position. The strain showed highest 16S rRNA gene sequence similarities of 97.3, 97.2 and 97.1 % to Nonomuraea turkmeniaca DSM 43926T, Nonomuraea ferruginea DSM 43553T and Nonomuraea candida DSM 45086T, respectively. A wide range of genotypic and phenotypic characteristics, as well as levels of DNA–DNA relatedness between strain YN-5-1T and N. turkmeniaca DSM 43926T (57.46 %), N. ferruginea DSM 43553T (53.50 %) and N. candida DSM 45086T (48.80 %), distinguished the novel isolate from its closest phylogenetic neighbours. The morphological characteristics of strain YN-5-1T were typical of the genus Nonomuraea. Chemotaxonomic characteristics, such as diagnostic diamino acid of the peptidoglycan, whole-cell sugars, phospholipid type, major menaquinone and major fatty acids, further supported the assignment of strain YN-5-1T to the genus Nonomuraea. The G+C content of the genomic DNA was 72.1 mol%. Based on the above data, strain YN-5-1T is considered to represent a novel species of the genus Nonomuraea, for which the name Nonomuraea flavida sp. nov. is proposed. The type strain is YN-5-1T (=CCTCC AB 2012909T = KCTC 29143T).

The genus Nonomura was originally proposed by Zhang et al. (1998) as a member of the family Streptosporangiaceae. The spelling of the genus name was subsequently corrected by Chiba et al. (1999) to Nonomuraea. The genus is characterized by extensively branched substrate and aerial mycelia, and the aerial hyphae differentiate into hooked, spiral or straight chains of spores, which show a folded, irregular, smooth or warty surface ornamentation (Nonomura & Ohara, 1971). At the time of writing, there were 36 species and two subspecies of the genus Nonomuraea with validly published names according to the List of Prokaryotic names with Standing in Nomenclature website (http://www.bacterio.cict.fr/index.html). In the present paper, a novel species of the genus Nonomuraea is identified based on a polyphasic taxonomic study.

Strain YN-5-1T was isolated from rhizosphere soil of a medicinal plant, Aconitum napellus, collected from Yunnan University campus of China. A 100-fold dilution of this soil suspension was prepared in sterilized distilled water and 0.1 ml was spread on modified poly(L-lactide) agar (PLA; containing 2.0 g PLA powder, 18 g agar and 1 litre of base medium; Jarerat et al. 2002). After incubation at 28 °C for 4 weeks, the pure culture was preserved on International Streptomyces Project medium 2 (ISP 2; Shirling & Gottlieb, 1966) at 4 °C and in 20 % (v/v) glycerol at −80 °C. The pure culture was preserved on International Streptomyces Project medium 2 (ISP 2; Shirling & Gottlieb, 1966) at 4 °C and in 20 % (v/v) glycerol at −80 °C.

Cultural characteristics of strain YN-5-1T were determined on ISP 2, 3, 4 and 5 (Shirling & Gottlieb, 1966), Czapek agar and potato dextrose agar (Stackebrandt, 2002). After incubation at 28 °C for up to 21 days. Colour designation of substrate and aerial mycelia was compared with colour chips from the ISCC-NBS colour charts standard sample No. 2106 (Kelly, 1964). Morphological properties were observed by scanning electron microscopy (JSM-5600LV; JEOL) after 14 days of growth on ISP 2 at 28 °C. Temperatures and

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YN-5-1T is JX467533.

One supplementary table and three supplementary figures are available with the online Supplementary Material.
pH ranges for growth and NaCl tolerance were tested in shake flasks of liquid YIM 38° medium (Xu et al., 2007) at 28 °C for 3 weeks. Utilization of compounds as sole carbon sources was carried out according to Shirling & Gottlieb (1966). Other physiological and biochemical tests, including those for H₂S production, nitrate reduction, esterase and oxidase activity, and decomposition of casein, urea, starch and gelatin, were performed as described by Smibert & Krieg (1994).

Diaminopimelic acids and whole-cell sugars of strain YN-5-1T were analysed according to the methods described by Staneck & Roberts (1974). Cells for chemotaxonomic analysis were obtained from cultures grown for 5 days in trypticase soy broth (TSB; Becton Dickinson) (for fatty acid analysis) and ISP 2 broth (for menaquinone and polar lipid analyses) at 28 °C. Analyses of menaquinones were carried out by the Identification Service of the DSMZ. Polar lipids and total lipids were analysed by the Identification Service of the Laboratory of Extremophiles, Zhejiang University, China. Menaquinones and lipids were extracted from 100 mg of freeze-dried cell material using the two-stage method described by Tindall (1990a, b). Menaquinones were analysed by HPLC; polar lipids and total lipids were examined by two-dimensional TLC and identified using published procedures (Tindall et al., 2007). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIS) (Version 6.0B; MIDI database: TSBA6) according to the method of Sasser (1990).

Chromosomal DNA of strain YN-5-1T was extracted as described by Wu et al. (2009). Amplification of the 16S rRNA gene sequence was performed as described by Carlsohn et al. (2007). The 16S rRNA gene sequence obtained in this study was compared with sequences from EzBioCloud (http://www.ezbiocloud.net/eztaxon) (Kim et al., 2012). After multiple alignment by CLUSTAL X (Thompson et al., 1997), phylogenetic analysis was performed using the software MEGA version 6.06 (Tamura et al., 2013). Genetic distances were calculated according to the Kimura two-parameter model (Kimura, 1980). A phylogenetic tree was reconstructed by using the neighbour-joining method with 1000 resamplings (Li et al., 2006). The G+C content of the genomic DNA was determined by reversed-phase HPLC of nucleosides according to the method described by Mesbah et al. (1989). DNA–DNA relatedness tests were performed in triplicate using the fluorescence micro-well method described by Christensen et al. (2000), while the hybridization experiment was carried out in 50 % formamide at 50 °C according to Xu et al. (2006) by using a FLUOstar OPTIMA microplate reader (BMG Labtech) at a wavelength of 360 nm for excitation and 460 nm for emission (He et al., 2005).

The almost-complete 16S rRNA gene sequence (1451 bp) of strain YN-5-1T showed 97.3, 97.2 and 97.1 % similarity to its closest relatives, Nonomuraea turkmenica DSM 43926T, Nonomuraea ferruginea DSM 43553T and Nonomuraea candida DSM 45086T, respectively. Levels of 16S rRNA gene sequence similarity between strain YN-5-1T and the type strains of other species in this genus were less than 97.0 %. The topology of the neighbour-joining dendrogram (Fig. 1) showed that strain YN-5-1T formed an independent sub-cluster with N. ferruginea DSM 43553T. This result was further supported in trees generated with the maximum-likelihood and maximum-parsimony methods (Figs S1 and S2, available in the online Supplementary Material). The DNA G+C content of strain YN-5-1T was 72.1 mol%. The isolate showed relatively low DNA–DNA relatedness with N. turkmenica DSM 43926T (57.46 %), N. ferruginea DSM 43553T (53.50 %) and N. candida DSM 45086T (48.80 %) (Table S1). These results are well below the 70 % cut-off point recommended for the delineation of genomic species.

The chemotaxonomic characteristics of strain YN-5-1T were consistent with the genus Nonomuraea, such as the presence of meso-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan and madurose as a whole-cell sugar, corresponding to wall chemotype III/B (Lechevalier & Lechevalier, 1970). The major menaquinones were MK-9(H₂) (43 %), MK-9(H₄) (39 %) and MK-9 (15 %), and the polar lipids comprised phosphatidylglycerol (PG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylglycol (PGM), glycolipid (GL), phosphoglycolipid (PGL) and phospholipid (PL) (Fig. S3). The predominant fatty acids were iso-C₁₆:0 (37.4 %), 10-methyl-C₁₇:0 (14.5 %), iso-C₁₅:0 (11.2 %) and iso-G-C₁₆:1 (9.2 %); other components were C₁₈:0 (5.6 %), C₁₇:0 9′c (5.5 %), C₁₇:1 9c (2.7 %), summed feature 9 (10-methyl C₁₆:0 and/or iso-C₁₇:0 9c) (2.1 %), C₁₆:0 (1.9 %), summed feature 3 (C₁₆:1 9c and/or C₁₆:1 10c(7c) 14 %), C₁₇:0 (1.1 %), iso-C₁₄:0 (1.1 %), 10-methyl C₁₈:0 (1.0 %), C₁₈:0 9c (0.9 %), iso-C₁₈:0 (0.7 %), C₁₄:0 (0.7 %), iso-C₁₇:0 (0.5 %), iso-C₁₈:1 H (0.4 %), summed feature 8 (C₁₈:1 9c and/or C₁₈:1 10c(7c) 0.4 %), anteiso-C₁₇:0 (0.3 %), iso-C₁₇:0 3-0H (0.2 %), C₁₁:0 3-0H (0.2 %), C₁₉:0 (0.2 %), C₁₃:0 (0.2 %), iso-C₁₅:1 G (0.2 %), iso-C₁₅:1 3-0H (0.1 %), anteiso-C₁₅:0 (0.1 %), C₁₅:0 2-0H (0.1 %), iso-C₁₆:0 3-0H (0.1 %) and summed feature 5 (C₁₈:0 9c, 9c and/or anteiso-C₁₈:0 9c, 9c). This matches the fatty acid profiles of N. turkmenica DSM 43926T, N. ferruginea DSM 43553T and N. candida DSM 45086T in the TSBA 6.10 library.

Strain YN-5-1T showed lichenous growth on all the tested media. Light yellow or yellow extensively branched substrate mycelia were produced on tested agar media and the aerial mycelia did not fragment. Crooked spore chains were composed of several elliptical spores (1.2 × 2.0 μm) (Fig. 2a) with a rough surface (Fig. 2b) and were born directly, singly or in clusters on the aerial mycelia. The aerial mycelia were white on ISP 2, 4 and 5, and yellow on ISP 3, Czapek agar and potato dextrose agar. No diffusible pigment was observed on any of the above media. A comparison of the physiological and biochemical characteristics of strain YN-5-1T and its closest phylogenetic neighbours is presented in Table 1.
**Nonomuraea flavida sp. nov.**

**Fig. 1.** Neighbour-joining tree based on 1451 bp 16S rRNA gene sequences showing the position of strain YN-5-1T among species of the genus *Nonomuraea*. Numbers at branch points indicate bootstrap values (1000 resamplings); only values greater than 50% are shown. Bar, 0.5% difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting any two organisms. *Thermopolyspora flexuosa* ATCC 35864T was used as the outgroup.
Although the phylogenetic data, diagnostic diamino acid, cell-wall hydrolysates, predominant menaquinones, major fatty acids, morphological characteristics and DNA G+C content were all consistent with the classification of strain YN-5-1T to the genus *Nonomuraea*, it could be distinguished from its closest phylogenetic neighbours based on physiological characteristics and DNA–DNA relatedness data (Stackebrandt & Ebers, 2006). In contrast to most strains of the genus *Nonomuraea*, which have phospholipid pattern IV (Lechevalier et al., 1977), strain YN-5-1T contained phosphatidylglycerol in the polar lipid profile, as revealed by the results of tests for total lipids (Fig. S3). A few species belonging to the genus *Nonomuraea* have phosphatidylglycerol in the polar lipid profile, such as *Nonomuraea soli* DSM 45533T (Cao et al., 2012), *Nonomuraea rhizophila* YIM 67092T (Zhao et al., 2011) and *Nonomuraea rosea* GW 12687T (Kämpfer et al., 2010). Additionally, the new isolate differed from *N. turkmeniaca* DSM 43926T, *N. ferruginea* DSM 43553T and *N. candida* DSM 45086T, mainly in the presence of large amounts of iso-C₁₅:₀ and iso-C₁₆:₁ G.

Based on data from the present study, we conclude that strain YN-5-1T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea flavida* sp. nov. is proposed.

**Description of *Nonomuraea flavida* sp. nov.**

*Nonomuraea flavida* (fla’vi.da. L. fem. adj. flavida pale yellow, referring to the colour of the colonies).

Gram-stain-positive, aerobic and non-motile actinomycete. Hyphae are extensively branched, forming light yellow or yellow substrate mycelia and white or yellow aerial mycelia. No diffusible pigment is produced. Grows at 10–37 °C (optimum 28 °C) and pH 6–8 (optimum pH 7.0). Tolerates up to 5% (w/v) NaCl in the culture medium. Casein hydrolysis and esterase (C4) activities are positive, but oxidase and urease activities are negative. Positive for gelatin liquefaction, milk coagulation and milk peptonization. Utilizes some of the tested sole carbon sources, such as D-arabinose, D-fructose, D-glucose and sucrose, but not cellobiose, myo-inositol, D-mannose, raffinose, L-rhamnose.

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**Table 1.** Differential physiological properties between strain YN-5-1T and its closest phylogenetic neighbours in the genus *Nonomuraea*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tr>
<td>H₂S production</td>
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<td>Nitrate reduction</td>
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<td>Urea</td>
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<td>Oxidase</td>
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<td>Utilization as sole carbon source:</td>
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<td>D-Arabinose</td>
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<td>1-Rhamnose</td>
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<tr>
<td>D-Xylose</td>
<td>−</td>
<td>+</td>
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Strains: 1, YN-5-1T; 2, *N. turkmeniaca* DSM 43926T; 3, *N. ferruginea* DSM 43553T; 4, *N. candida* DSM 45086T. All data are from the present study. +, Positive; w, weakly positive; −, negative.
or D-xylose. The diagnostic diamino acid of the peptido-
glycan is meso-diaminopimelic acid. Cell hydrolysates
contain madurose. The predominant menaquinones are
MK-9(H₂) and MK-9(H₄). The polar lipids include
phosphatidylglycerol (PG), diphosphatidylglycerol (DPG),
phosphatidylethanolamine (PE), phosphatidylinositol (PI),
phosphatidylinositol mannoside (PIM), glycolipid (GL),
phosphoglycolipid (PGL) and phospholipid (PL). The
major fatty acids are iso-C₁₆:₀ 10-methyl C₁₇:₀ and iso-C₁₅:₀.
The type strain is YN-5-1^T (= CCTCC AB 2012909^T
=KCTC 29143^T), isolated from the rhizosphere soil of a
medicinal plant, Aconium napellus, in Yunnan University
campus of China. The DNA G+C content of the type
strain is 72.1 mol%.

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