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Nonomuraea rosea sp. nov.
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Nonomuraea rosea sp. nov.

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A Gram-positively staining, aerobic, non-motile actinomycete, strain GW 12687^T, that formed rose-pigmented colonies and branched substrate and aerial mycelia was studied in detail for its taxonomic position. On the basis of 16S rRNA gene sequence similarity studies, strain GW 12687^T was grouped into the genus *Nonomuraea*, being most closely related to *Nonomuraea dietziae* (97.6 %), *Nonomuraea africana* (97.1 %), and *Nonomuraea kuesteri* (97.1 %). The 16S rRNA gene sequence similarity to other species of the genus *Nonomuraea* was $\leqslant$97 %. The chemotaxonomic characterization supported allocation of the strain to the genus *Nonomuraea*. The major menaquinone was MK-9(H_4) with minor amounts of MK-9(H_2), MK-9(H_6), MK-9(H_0) and MK-8(H_4). The polar lipid profile contained the major compound diphosphatidylglycerol, moderate amounts of phosphatidylmonomethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, and an unknown aminophosphoglycolipid. Phosphatidylinositol mannosides and phosphatidylinositol were also present. The major fatty acids were iso- and anteiso- and 10-methyl-branched fatty acids. The results of physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain GW 12687^T from closely related species. Thus, GW 12687^T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea rosea* sp. nov. is proposed, with GW 12687^T (=DSM 45177^T =CCUG 56107^T) as the type strain.

The genus *Nonomuraea* (originally validly published as *Nonomuria*, but corrected by Chiba *et al.*, 1999) was proposed by Zhang *et al.* (1998) and is characterized by organisms that form extensively branched substrate and aerial mycelia. At the time of writing, the genus comprises 21 species and two subspecies, most of which were listed by Gyobu & Miyadoh (2001), Stackebrandt *et al.* (2001), Quintana *et al.* (2003), Ara *et al.* (2007a, b) and Le Roes & Meyers (2008).

During the characterization of organisms from soil, strain GW 12687^T was recovered on oatmeal agar [International Streptomyces Project (ISP) 3 medium; Küster, 1959] at 25 °C; it had a slightly rose-coloured vegetative mycelium with white aerial mycelium. Subcultivation was done on tryptone soy agar (Oxoid) at 25 °C for 24 h. Gram-staining was performed as described by Gerhardt *et al.* (1994). The cell morphology was observed under a Zeiss light microscope at a magnification of ×1000, using cells that had been grown for 3 days at 25 °C on R2A agar (Oxoid). The 16S rRNA gene sequence was analysed as described previously (Kämpfer *et al.*, 2003). Phylogenetic analysis was performed by using the software package MEGA, version 2PT1 (Kumar *et al.*, 2001), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). A distance matrix method (distance options according to the Kimura two-parameter model), including clustering by the neighbour-joining method (Fig. 1), and a discrete character-based maximum-parsimony method, were used. In each case bootstrap values were calculated based on 1000 replications. The 16S rRNA gene sequence of strain GW 12687^T was a continuous stretch of 1376 bp. Sequence

Abbreviations: CID, collision induced dissociation; OH-PE, hydroxy-phosphatidylethanolamine; OH-PME, hydroxy-phosphatidylmonomethyl-ethanolamine; PE, phosphatidylethanolamine; PME, phosphatidylmono-methylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GW 12687^T is FN356742.

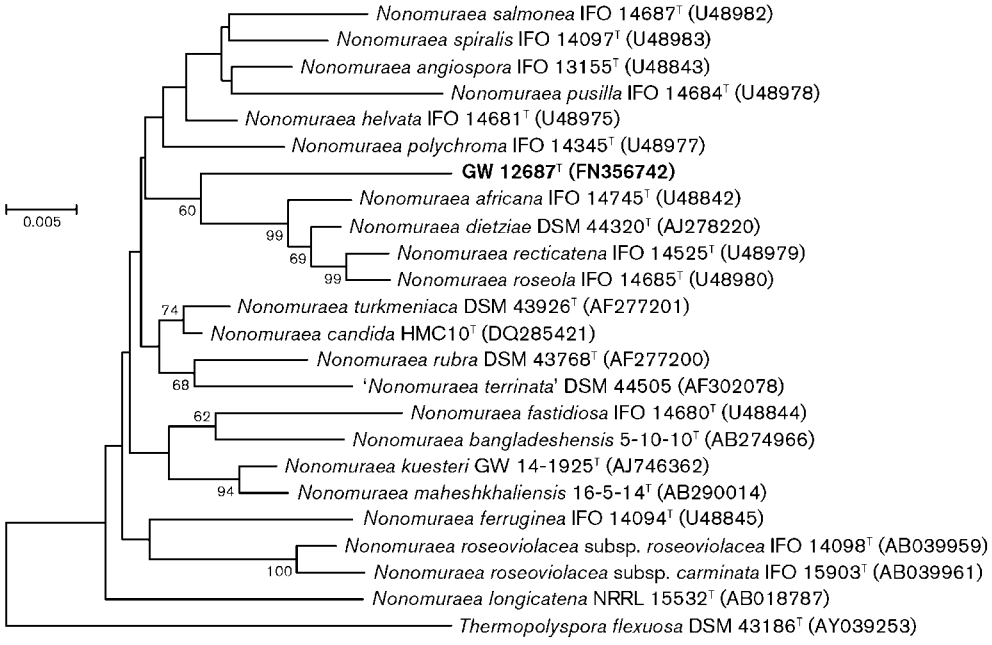

Fig. 1. Phylogenetic analysis, based on 16S rRNA gene sequences available from the EMBL database (accession nos are given in parentheses), constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained (using distance options according to the Kimura-2 model) and clustering was performed, using the neighbour-joining method, by using the software package MEGA, version 2.0 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 K_{nuc}.

similarity calculations indicated that the closest relatives of strain GW 12687^T were the type strains of *Nonomuraea dietziae* (97.6 %), *Nonomuraea africana* (97.1 %) and *Nonomuraea kuesteri* (97.1 %). Both the neighbour-joining tree (Fig. 1) and the maximum-parsimony tree (not shown) showed that strain GW 12687^T formed a clade with *N. dietziae and N. africana.*

The quinone system and polar lipids were extracted and analysed by applying the integrated procedure reported by Tindall (1990a, b) and Altenburger *et al.* (1996). HPLC analysis was carried out using the equipment described by Stolz *et al.* (2007). Fatty acids were extracted and analysed as described by Kämpfer & Kroppenstedt (1996).

The quinone system found supported the affiliation of strain GW 12687^T to the genus *Nonomuraea.* The principal menaquinones of strain GW 12687^T were: menaquinone MK-9(H_4) (76 %) with minor amounts of MK-9(H_2) (11 %), MK-9(H_6) (6 %), MK-9 (6 %) and MK-8(H_4) (2 %). This is essentially in accordance with the quinone system reported for members of this genus (Kroppenstedt & Goodfellow, 1991; Stackebrandt *et al.*, 2001; Quintana *et al.*, 2003). The polar lipid profile of strain GW 12687^T contained diphosphatidylglycerol as the major component, moderate amounts of phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE), hydroxy-phosphatidylethanolamine (OH-PE), hydroxy-phosphatidylmonomethylethanolamine (OH-PME) and an unknown aminophosphoglycolipid. Phosphatidylinositol mannosides and phosphatidylinositol were also present, as were small amounts of an unknown phospholipid (Fig. 2). The presence of PME has been reported so far only for *N. kuesteri* and *N. dietziae* (Kämpfer *et al.*, 2005; Stackebrandt *et al.*, 2001) within the genus *Nonomuraea.* OH-PE has been found in *Nonomuraea longicatena* (Chiba *et al.*, 1999), *Nonomuraea bangladeshensis*, *Nonomuraea coxensis* (Ara *et al.*, 2007b), and *Nonomuraea maheshkhaliensis* (Ara *et al.*, 2007a), and in all species described by Zhang *et al.* (1998) which had been examined by Kroppenstedt *et al.* (1990). However, the presence of OH-PME is reported here for the first time.

Based on its similar chromatographic behaviour, the unknown aminophosphoglycolipid most likely corresponds to the *N*-acetylglucosamine-containing phospholipid reported to be present in the lipid profile of *N. kuesteri* (Kroppenstedt *et al.*, 1990; Kämpfer *et al.*, 2005). Hence, the phospholipid type PIV is suggested, according to the classification of Lechevalier *et al.* (1977).

In order to confirm the identity of the lipids identified by TLC as PME, PE, OH-PME and OH-PE, spots were scraped from unstained TLC plates, extracted with methanol and evaporated to dryness. The extracts were redissolved in methanol (20 % v/v) in water and 3 µl was applied to a gold-coated nanospray glass capillary tube (Protana), whose tip was placed orthogonally in front of

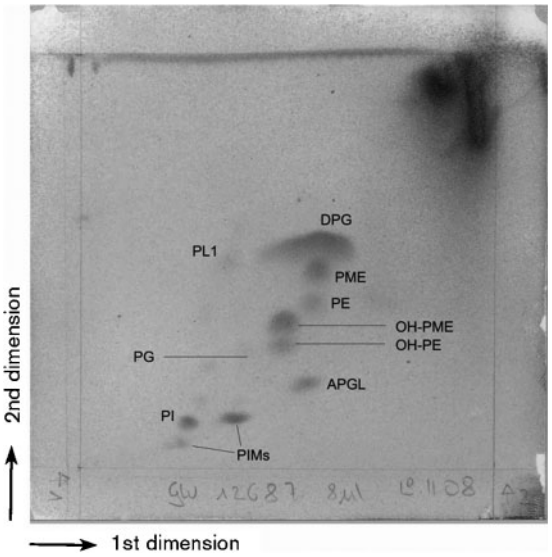

Fig. 2. Polar lipid profile of GW 12687^T after two-dimensional TLC and detection with molybdatophosphoric acid. PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; OH-PME, hydroxy-phosphatidylmonomethylethanolamine; OH-PE, hydroxy-phosphatidylethanolamine; PL1, unknown phospholipid; APGL, aminophosphoglycolipid supposed to be phospholipid *N*-acetylglucosamine.

the entrance hole of a quadrupole time-of-flight (Q-TOF 2) mass spectrometer (Micromass) equipped with a nanospray ion source, and spectra were recorded in the positive and negative ion modes leading to the detection of sodium adducts or deprotonated molecular ions, respectively. In collision induced dissociation (CID) experiments, parent ions were selectively transmitted from the quadrupole mass analyser into the collision cell. Argon was used as the collision gas. The resulting daughter ions were then separated by the orthogonal time-of-flight (TOF) mass analyser. To obtain accurate mass measurements (<3 p.p.m.) in the MS and MS^2 modes, samples were analysed using the nanospray ion source of an Orbitrap instrument (Thermo Scientific) equipped with an external collision cell at a resolution of 100 000.

The presence of PE and PME was confirmed by the presence of fragments in the mass spectra at 164 (phosphoethanolamine$-H_2O+Na^+$) and 178 (phosphomonomethylethanolamine$-H_2O+Na^+$) in CID experiments in the positive mode.

CID experiments were carried out in the negative mode in order to determine the nature of the fatty acids attached to the glycerol backbone. Under CID conditions it has been documented that fatty acids attached to the C2 (i.e. middle, *sn*-2) hydroxyl group of the glycerol molecule are cleaved preferentially (Hsu & Turk, 2000). Examination of all samples presumed to contain hydroxyl fatty acids based on the molecular mass of the parent mole peak were shown to contain hydroxylated C16 derivatives at position C2 of the glycerol molecule. The CID mass spectrum alone did not allow one to determine the position of the hydroxyl group, or whether the fatty acid was straight or branched chain. Different fatty acids attached to C1 (*sn*-1) of the glycerol molecule gave rise to a homologous series differing in mass by 14 Da. The accurate mass measurement of the compound (calculated mass in parentheses) with a nominal mass of 772 Da was shown to be 772.545 (722.546), corresponding to $C_{40}H_{80}O_9NNaP$ in accordance with a monomethylphosphatidylethanolamine containing a hydroxylated C16 fatty acid (may include a branched chain fatty acid) and a C18 fatty acid (may include a branched chain fatty acid, such as 17 : 0 10-methyl). The fragment ions obtained by CID of this parent ion in the external collision cell afforded daughter ions fully in agreement with the proposed structure; in particular the fragmentation pattern unequivocally identified an *N*-methylethanolamine building block and not an isomeric structure containing aminoethanol and a longer chain fatty acid: *m*/*z* 715.488 (715.488; $C_{37}H_{73}O_9NaP$) corresponding to the elimination of *N*-methyl-ethene, 617.512 (617.512; $C_{37}H_{70}O_5Na$) further elimination of phosphoric acid, 443.253 (443.253; $C_{21}H_{41}O_6NaP$) elimination of *N*-methyl-ethene, OH-C16 and 178.024 (178.024; $C_3H_{10}O_3NNaP$) *N*-methylethanolaminephosphate minus water. All samples presumed to contain normal fatty acids based on the molecular mass of the parent mole peak were shown to contain normal C16 derivatives at the C2 position (i.e. middle, *sn*-2) hydroxyl group of the glycerol molecule.

These results confirmed the presence of PE, PME, OH-PE and OH-PME. While many members of the genus *Nonomuraea* have been shown to produce PE and OH-PE, *N. kuesteri* and *N. dietziae* have been reported to produce PE and PME. This is the first report of the presence of OH-PME, and the first example of an organism producing PE, PME, OH-PE and OH-PME.

The fatty acids comprised mainly iso- and 10-heptadecanoic-branched fatty acids. Smaller amounts of unbranched saturated and 2-hydroxy fatty acids were detected (fatty acid type 3c of Kroppenstedt, 1985). The detailed fatty acid profile obtained from cells grown on TS medium after 72 h of incubation at 28 °C is as follows: 13 : 0 (0.3 %), 14 : 0 iso (1.3 %), 14 : 0 (0.5 %), 15 : 0 iso (4.3 %), 15 : 0 anteiso (0.4 %), 15 : 0 (3.1 %), 16 : 1 iso G (6.9 %), 16 : 0 iso (43.4 %), 16 : 1ω7*c* (0.6 %), 16 : 0 (1.1 %), 15 : 0 2-OH (0.4 %), 16 : 0 10-methyl (2.6 %), 17 : 0 iso (0.5 %), 17 : 0 anteiso (0.7 %), 17 : 1ω8*c* (2.1 %), 17 : 1ω6*c* (10.2 %), 17 : 0 (1.0 %), 17 : 0 10-methyl (19.8 %) and 18 : 0 10-methyl (0.5 %). However, when grown on medium 65 (http://www.dsmz.de) for 72 h at 28 °C, strain GW 12687^T produced also mainly iso- and 10-heptadecanoic-branched fatty acids but showed higher amounts of hydroxylated C16 fatty acids. The detailed fatty acid profile was: 12 : 0 (0.2 %), 13 : 0 (0.4 %), 14 : 0 iso (1.2 %), 14 : 0 (3.3 %), 15 : 0

Table 1. Comparison of the phenotypic properties of strain GW 12687^T with those of species of the genus *Nonomuraea* with validly published names

Species: 1, *Nonomuraea rosea* sp. nov.; 2, *N. africana*; 3, *N. angiospora*; 4, *N. bangladeshensis*; 5, *N. candida*; 6, *N. coxensis*; 7, *N. dietziae*; 8, *N. fastidiosa*; 9, *N. ferruginea*; 10, *N. helvata*; 11, *N. kuesteri;* 12, *N. longicatena*; 13, *N. maheshkhaliensis;* 14, *N. polychroma*; 15, *N. pusilla*; 16, *N. recticatena*; 17, *N. roseola*; 18, *N. roseoviolacea* subsp. *roseoviolacea*; 19, *N. rubra*; 20, *N. salmonea*; 21, *N. spiralis*; 22, *N. turkmeniaca*; 23, '*N. terrinata*'. Data are taken from Meyer (1989), Holt *et al.* (1994), Chiba *et al.* (1999), Gyobu & Miyadoh (2001), Stackebrandt *et al.* (2001), Quintana *et al.* (2003), Ara *et al.* (2007a, b) and Le Roes & Meyers (2008). +, Positive; −, negative; +/−, doubtful positive reaction; W, weak; ND, not determined. H, Hooks, curled; Psp, pseudosporangia; S, spirals of one or two turns; Sp, spirals of three to five turns; Str, straight; I, irregular.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Spore-chain morphology												
Spore chains	S	Str	Sp	S	H	Str, H	Str, Sp	S, Sp	H, S	H, Psp	Sp	Str
Spore ornamentation	ND	Smooth	Ridged	Smooth	Smooth	Smooth	Cross-ridged, smooth or rough	Irregular	Folded	Smooth	ND	Smooth
Number of spores	4–10	4–10	4–15	8–12	ND	12–17	⩾30	4–10	4–10	4–10	ND	10–30
Growth on ISP 3 medium												
Aerial mycelium	White	Greyish blue	White	Pale brown	White (sparse)	Pink to white	Beige	White/pink	White/pink	White	Trace	White
Substrate mycelium	Pink to violet	Yellow	White/ ochre	Pale brown	Cream	Orange	Beige	Colourless	Pink	Yellow/ brown	Yellow	Ochre
Soluble pigment	None	Yellowish brown	None	None	None	None	Yellow	None	None	None	None	None
Biochemical tests												
Aesculin hydrolysis	+	+	+	ND	+	ND	ND	+	−	+	+	+
Nitrate reductase	ND	+	−	ND	−	ND	ND	+	+	+	ND	−
Degradation of:												
Casein	+	+	+	ND	+	ND	ND	+	+	−	−	+
DNA	ND	+	+	ND	ND	ND	ND	+	+	−	ND	−
Elastin	ND	−	+	ND	ND	ND	ND	+	+	ND	ND	+
Gelatin	+	+	+	ND	W	ND	+	+	−	−	−	−
Hypoxanthine	+	+	+	ND	−	ND	ND	+	+	−	−	+
Starch	+	+	−	ND	−	ND	ND	−	+	−	−	+
Tyrosine	+	+	+	ND	−	ND	ND	−	+	−	−	−
Xanthine	+	−	−	ND	−	ND	ND	−	−	−	−	−
Utilization of:												
L-Arabinose	+	+	−	+/−	W	+/−	W	−	+	−	+	+
Sucrose	−	+	−	+/−	+	+	−	−	+	+	+	ND
D-Xylose	+	+	+	+/−	+	+	−	+	+	+	+	ND
myo-Inositol	+	+	+	+/−	+	+/−	−	+	−	+	+	−
D-Mannose	+	+	−	+	+	+	−	−	+	−	+	+
D-Fructose	+	+	−	+/−	+	+/−	+	−	+	−	+	ND
L-Rhamnose	+	+	−	+	+	+	+	−	+	−	+	−
D-Raffinose	ND	+	+	+	+	+/−	−	+	−	+	ND	+
Cellobiose	+	+	−	+/−	W	+	−	−	−	−	+	−

Table 1. cont.

Characteristic	1	13	14	15	16	17	18*	19	20	21	22	23
Spore-chain morphology												
Spore chains	S	Sp	ND	Psp	Str	Sp, Str	Psp	H, S, Sp	H, S	Sp	Sp	I, Psp
Spore ornamentation	ND	Rough	ND	Smooth	Smooth	Folded	Smooth	Smooth	Warty	Folded	Smooth	Rugose
Number of spores	4–10	17-20	ND	>10	4–20	6–20	4–20	4–20	4–30	4–20	10–20	8–15
Growth on ISP 3 medium												
Aerial mycelium	White	White	Trace	White/cream	White/cream	Pink	Pink/violet	Trace	Pink	White/ yellow	Trace	White
Substrate mycelium	Pink to violet	Light wheat	Colourless/ brown	Grey/brown	Dark yellow/ brown	Brown/ red	Violet	Orange/red	Red	Yellow/ brown	Violet/red	White/ ochre
Soluble pigment	None	None	None	None	None	None	Violet	Red	None	None	Pink/ violet	None
Biochemical tests												
Aesculin hydrolysis	+	ND	+	+	+	+	+	−	+	+	+	+
Nitrate reductase	ND	ND	−	+	+	+	+	+	+	+	+	−
Degradation of:												
Casein	+	ND	−	−	−	−	−	−	+	−	+	+
DNA	ND	ND	−	+	−	−	+	−	+	−	−	+
Elastin	ND	ND	+	−	+	−	−	+	+	−	−	−
Gelatin	+	ND	+	+	+	+	+	+	+	+	+	−
Hypoxanthine	+	ND	+	+	+	+	+	+	+	−	+	+
Starch	+	ND	−	−	+	−	−	+	−	−	+	+
Tyrosine	+	ND	−	+	−	+	−	+	+	+	−	−
Xanthine	+	ND	−	−	−	−	−	−	−	−	−	−
Utilization of:												
L-Arabinose	+	+/−	−	−	+	−	+	+	−	−	+	ND
Sucrose	−	+	−	−	−	+	+	+	+	+	+	ND
D-Xylose	+	+	−	−	−	−	−	+	−	+	+	ND
myo-Inositol	+	+/−	−	−	+	+	+	+	−	−	+	ND
D-Mannose	+	+	−	−	−	−	+	+	−	+	+	ND
D-Fructose	+	+	−	−	−	−	+	+	−	−	+	ND
L-Rhamnose	+	+	−	−	+	+	+	+	+	+	+	ND
D-Raffinose	ND	ND	−	−	−	−	+	+	−	−	+	ND
Cellobiose	+	+	−	−	−	−	−	+	−	−	+	ND

*Both subspecies of *N. roseoviolacea*, *N. roseoviolacea* subsp. *roseoviolacea* and *N. roseoviolacea* subsp. *carminata*, are phenotypically very similar. *N. roseoviolacea* subsp. *carminata* is negative for gelatin liquefaction, reduction of nitrate, and *myo*-inositol utilization, and positive for D-xylose utilization (Gyobu & Miyadoh, 2001).

iso (4.0 %), 15 : 0 anteiso (3.5 %), 15 : 0 (4.2 %), 16 : 1 iso G (1.3 %), summed feature 3 (16 : 1ω7*c*/15 : 0 iso 2-OH; 4.2 %), 16 : 0 iso (11.4 %), 16 : 0 (21.0 %), 15 : 0 2-OH (0.8 %), 16 : 0 10-methyl (5.6 %), 17 : 0 iso (1.4 %), 17 : 0 anteiso (1.9 %), 17 : 1ω8*c* (3.0 %), 17 : 1ω6*c* (4.0 %), 17 : 0 (6.5 %), 16 : 0 2-OH (2.7 %), 17 : 0 10-methyl (8.1 %), summed feature 5 (18 : 0 anteiso/18 : 2ω6,9*c*; 2.0 %), 18 : 0 (2.1 %) and 18 : 0 10-methyl (3.8 %). These results confirmed those of Kroppenstedt *et al.* (1990), who detected hydroxylated C16 fatty acids for members of this genus.

It should also be noted that, although the description of the genus *Nonomuraea* (Zhang *et al.*, 1998) mentions the presence of OH-PE among the polar lipids, no mention is made of the presence and characteristic feature of 2-hydroxy fatty acids, which are a pre-requisite for the synthesis of such polar lipids. Typically the major 2-hydroxy fatty acids in members of the genus *Nonomuraea* producing OH-PE (and OH-PME) are 16 : 0 2-OH and/or 16 : 0 iso 2-OH, with smaller amounts of the corresponding C15 and C17 derivatives.

Based on the chemotaxonomic results presented here and in previous work, attention should be given in the future to re-examining the value of the 2-OH fatty acids and the stereospecific location of the double bonds in the unsaturated fatty acids in further defining the genus. Previous work (Kroppenstedt *et al.*, 1990; Collins *et al.*, 1988) has also shown that the major quinones in members of the genus *Nonomuraea* and related taxa are based on MK-9 (III, VIII-H_4), and routine methods need to be developed in order to distinguish this characteristic compound from other isomers of MK-9(H_4).

The results of the physiological characterization, performed using methods described previously (Kämpfer, 1990; Kämpfer *et al.*, 1991), are given in Table 1 and in the species description. In addition, degradative tests were performed according to Williams *et al.* (1983). It has been shown that *Nonomuraea* species have high 16S rRNA gene sequence similarities (within the range 97.6–99.4 %) and have low DNA–DNA relatedness values (Fischer *et al.*, 1983; Poscher *et al.*, 1985; Tamura *et al.*, 2000; Kämpfer *et al.*, 2005). Stackebrandt *et al.* (2001) reported 45–48 % as the highest DNA–DNA relatedness values between the type strains of *N. africana*, *N. dietziae* and *Nonomuraea recticatena*, strains sharing 16S rRNA gene sequence similarities between 98.9 and 99.8 %. Based on these findings, DNA–DNA hybridizations were not performed. It is clear that strain GW 12687^T represents a novel species of the genus *Nonomuraea*, for which we propose the name *Nonomuraea rosea* sp. nov. The type strain is GW 12687^T.

Description of *Nonomuraea rosea* sp. nov.

Nonomuraea rosea (ro.se′a. L. fem. adj. *rosea* rose-coloured or rosy, referring to the pinkish colour of the colonies).

Forms an extensive branched substrate mycelium. A white aerial mycelium is visible on oatmeal agar. Spore chains are spiral; sporangia are not detected. Gram-stain-positive and oxidase-positive; shows an aerobic respiratory metabolism. Good growth occurs after 3 days of incubation on oatmeal agar or nutrient agar (Oxoid) at 25–30 °C. The polar lipids include the major compound diphosphatidylglycerol, moderate amounts of PME, PE, OH-PE, OH-PME and an unknown aminophosphoglycolipid. Phosphatidylinositol-mannosides and phosphatidylinositol are present and small amounts of an unknown phospholipid. The fatty acids comprise mainly iso- and 10-heptadecanoic-branched fatty acids. Smaller amounts of unsaturated fatty acids and hydroxylated fatty acids (15 : 0 2-OH, 16 : 0 2-OH) are also detected. Results of carbon-source utilization and degradation tests (including differentiating characteristics) are shown in Table 1. The type strain does not produce acids from the following carbon sources: glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose and arabitol. The following carbon sources are utilized (after 7 days of incubation): *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, L-rhamnose, D-trehalose, D-xylose, adonitol, inositol, 4-aminobutyrate, citrate, fumarate (weak), DL-malate, L-aspartate (weak) and L-proline. The following carbon sources are not utilized: *p*-arbutin, D-gluconate, melibiose, D-ribose, sucrose, salicin, maltitol, D-mannitol, sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, mesaconate, pyruvate, DL-lactate, suberate, 2-oxoglutarate, L-alanine, β-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, DL-3-hydroxybenzoate, DL-4-hydroxybenzoate and L-phenylacetate.

The type strain, GW 12687^T (=DSM 45177^T =CCUG 56107^T), was isolated from a soil sample.

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References

Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.

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

Ara, I., Kudo, T., Matsumoto, A., Takahashi, Y. & Omura, S. (2007b). *Nonomuraea bangladeshensis* sp. nov. and *Nonomuraea coxensis* sp. nov. *Int J Syst Evol Microbiol* **57**, 1504–1509.

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.

Collins, M. D., Kroppenstedt, R. M., Tamaoka, J., Komagata, K. & Kinoshita, T. (1988). Structures of the tetrahydrogenated menaqui-

nones from *Actinomadura angiospora*, *Faenia rectivirgula*, and *Saccharothrix australiensis*. *Curr Microbiol* **17**, 275–279.

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

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

Gyobu, Y. & Miyadoh, S. (2001). Proposal to transfer *Actinomadura carminata* to a new subspecies of the genus *Nonomuraea* as *Nonomuraea roseoviolacea* subsp. *carminata* comb. nov. *Int J Syst Evol Microbiol* **51**, 881–889.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.

Hsu, F.-F. & Turk, J. (2000). Charge-remote and charge-driven fragmentation processes in diacyl glycerophosphoethanolamine upon low-energy collisional activation: a mechanistic proposal. *J Am Soc Mass Spectrom* **11**, 892–899.

Kämpfer, P. (1990). Evaluation of the Titertek-Enterobac-Automated system (TTE-AS) for identification of *Enterobacteriaceae*. *Zentralbl Bakteriol* **273**, 164–172.

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., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.

Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.

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

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.

Kroppenstedt, R. M. & Goodfellow, M. (1991). The family *Thermomonosporaceae*. In *The Prokaryotes*, 2nd edn, pp. 1085–1114. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Kroppenstedt, R. M., Stackebrandt, E. & Goodfellow, M. (1990). Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetraspora*. *Syst Appl Microbiol* **13**, 148–160.

Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

Küster, E. (1959). Outline of a comparative study of criteria used in characterisation of the actinomycetes. *Int Bull Bacteriol Nomencl Taxon* **9**, 97–104.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Le Roes, M. & Meyers, P. P. (2008). *Nonomuraea candida* sp. nov., a new species from South African soil. *Antonie Van Leeuwenhoek* **93**, 133–139.

Meyer, J. (1989). Genus *Actinomadura* Lechevalier and Lechevalier 1970a, 400^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2511–2526. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Poscher, 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.

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

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

Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.

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.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

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.

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.

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