Nonomuraea kuesteri sp. nov.Peter Kämpfer,¹ Reiner M. Kroppenstedt² and Iris Grün-Wollny³

Correspondence

Peter Kämpfer

peter.kaempfer@agrar.

uni-giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig, Germany³Labor Grün-Wollny, D-35394 Giessen, Germany

A Gram-positive, aerobic, non-motile actinomycete, strain GW 14-1925^T, that formed branched substrate and aerial mycelium was studied using a polyphasic approach. On the basis of 16S rRNA gene sequence similarity studies, strain GW 14-1925^T was shown to belong to the genus *Nonomuraea*, being most closely related to *Nonomuraea longicatena* (97.9%), *Nonomuraea turkmeniaca* (98.9%), *Nonomuraea helvata* (98.6%), *Nonomuraea polychroma* (98.5%), *Nonomuraea salmonaea* (98.3%), *Nonomuraea roseoviolacea* subsp. *roseoviolacea* (98.1%) and *Nonomuraea roseoviolacea* subsp. *carminata* (97.7%). The 16S rRNA gene sequence similarity to other *Nonomuraea* species was <97.5%. Chemotaxonomic data [major menaquinones of the MK-9 series with minor amounts of MK-8(H₄); major polar lipids of phospholipid type IV; fatty acids with major amounts of iso- and anteiso- and 10-methyl-branched fatty acids in combination with iso-branched 2-hydroxy fatty acids] supported allocation of the strain to the genus *Nonomuraea*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain GW 14-1925^T from closely related species; thus, GW 14-1925^T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea kuesteri* sp. nov. is proposed, with GW 14-1925^T (=DSM 44753^T=NRRL B-24325^T) as the type strain.

The genus *Nonomuria* [*sic*, corrected by Chiba *et al.* (1999) to *Nonomuraea*] was originally proposed by Zhang *et al.* (1998) as a member of the family *Streptosporangiaceae* that forms extensively branched substrate and aerial mycelia. On the basis of detailed phylogenetic analysis, the genus presently comprises 18 species and two subspecies, most of which were listed by Gyobu & Miyadoh (2001), Stackebrandt *et al.* (2001) and Quintana *et al.* (2003).

During the characterization of organisms from soil, strain GW 14-1925^T was recovered on oatmeal agar (ISP 3 medium; Küster, 1959) at 25 °C; it had slightly yellow-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 ×1000, using cells that had been grown for 3 days at 25 °C on R2A agar (Oxoid). The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Phylogenetic analysis was performed

by using the ARB software package (Strunk *et al.*, 2000) and also the software package MEGA, version 2.1 (Kumar *et al.*, 2001), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997).

Distances were obtained (using distance options according to the Kimura two-parameter model; Kumar *et al.*, 2001) and clustering was performed, using the neighbour-joining (Fig. 1) and maximum-parsimony methods, by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain GW 14-1925^T was a continuous stretch of 1496 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain GW 14-1925^T were *Nonomuraea turkmeniaca* (98.9%), *Nonomuraea helvata* (98.6%), *Nonomuraea salmonaea* (98.3%) and *Nonomuraea polychroma* (98.5%). Both the neighbour-joining tree (Fig. 1) and the maximum-parsimony tree (not shown) revealed that strain GW 14-1925^T clustered most closely with *Nonomuraea roseoviolacea* subsp. *roseoviolacea* (98.1%) and *Nonomuraea longicatena* (97.9%).

The results of chemotaxonomic analyses are given in the species description below. The following characteristics were analysed: menaquinones (as described by Kroppenstedt, 1985), polar lipids (as described by Lechevalier *et al.*, 1977;

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSM 44753^T is AJ746362.

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 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 K_{nuc} .

Minnikin *et al.*, 1984) and fatty acids (as described by Kämpfer & Kroppenstedt, 1996).

The quinone system found supports affiliation of strain GW 14-1925^T to the genus *Nonomuraea*. The principal menaquinones of strain GW 14-1925^T were as follows: MK-9(H₄), 71%; MK-9(H₆), 15%; MK-9(H₂), 6%; and MK-9, 2%. In addition, MK-8(H₄) was detected (6%). This is essentially in accordance with the quinone profile reported for members of the genus (Kroppenstedt & Goodfellow, 1991; Stackebrandt *et al.*, 2001; Quintana *et al.*, 2003). The polar lipids of strain GW 14-1925^T are of the PIV type, according to the phospholipid classification of Lechevalier *et al.* (1977), and include diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, methyl-phosphatidylethanolamine and the diagnostic phospholipid *N*-acetylglucosamine. Some unidentified glycolipids were also detected. 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 is as follows: 13:0 (0.4%), 14:0 iso (0.9%), 14:0 (0.9%), 15:1 iso G (0.2%), 15:0 iso (7.9%), 15:0 anteiso (0.3%), 15:0 (3.8%), 16:1 iso G (8.3%), 16:0 iso (28.9%), 16:1 *cis*9 (1.8%), 16:0 (1.8%), 15:0 2-OH (0.8%), 16:0 10-methyl (2.8%), 17:0 iso (1.8%), 17:0 anteiso (1.0%), 17:1 *cis*9 (4.2%), 16:0 iso 2-OH (10.5%), 17:0 (1.6%), 16:0 2-OH (0.5%), 17:0 10-methyl (15.5%), 18:0 iso (0.6%), 18:1 *cis*9 (0.6%), 17:0 iso 2-OH (0.4%), 18:0 (1.5%), 17:0 2-OH (0.3%) and 18:0 10-methyl (0.9%). 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). DNA–DNA hybridizations between strain GW 14-1925^T and the type strains of *N. longicatena*, *N. salmonea* and *N. turkmeniaca* were performed using the method described by Ziemke *et al.* (1998), except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. Strain GW 14-1925^T showed relatively low

DNA–DNA relatedness to *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T (15.9%, mean value of four hybridizations), *N. longicatena* NRRL 15532^T (16%, mean value of two hybridizations), *N. salmonea* DSM 43678^T (32%, mean value of four hybridizations) and *N. turkmeniaca* DSM 43926^T (40.5%, mean value of four hybridizations). 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; Poschner *et al.*, 1985; Tamura *et al.*, 2000). Stackebrandt *et al.* (2001) reported 45–48% as the highest DNA–DNA relatedness values between the type strains of *Nonomuraea africana*, *Nonomuraea dietzia* and *Nonomuraea recticatena*, strains sharing 16S rRNA gene sequence similarities between 98.9 and 99.8%. For these reasons, it is clear that strain GW 14-1925^T represents a novel species of the genus *Nonomuraea*, for which we propose the name *Nonomuraea kuesteri*. The type strain is GW 14-1925^T.

Description of *Nonomuraea kuesteri* sp. nov.

Nonomuraea kuesteri (kue'ster.i. N.L. gen. n. *kuesteri* of Küster, in honour of Eberhard Küster, a German microbiologist, in recognition of his numerous contributions to the taxonomy of actinomycetes).

Forms an extensive branched substrate mycelium. Only traces of aerial mycelium are visible on oatmeal agar. Spore chains are spiral; sporangia are not detected. Gram-positive and oxidase-positive; shows oxidative metabolism. Good growth occurs after 3 days incubation on oatmeal agar and nutrient agar (Oxoid) at 25–30 °C. DL-Diaminopimelic acid is the diagnostic amino acid in the peptidoglycan. The main menaquinones of the type strain are MK-9(H₄) (71%), MK-9(H₆) (15%), MK-9(H₂) (6%) and MK-9 (2%); MK-8(H₄) (6%) is also present. The polar lipids include diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, methyl-phosphatidylethanolamine and the diagnostic phospholipid *N*-acetylglucosamine. The fatty acids comprise mainly iso- and

Table 1. Comparison of the phenotypic properties of *N. kuesteri* sp. nov. with those of species of the genus *Nonomuraea* with validly published names

Species: 1, *N. kuesteri* sp. nov.; 2, *N. africana*; 3, *N. angiospora*; 4, *N. dietziae*; 5, *N. fastidiosa*; 6, *N. ferruginea*; 7, *N. flexuosa*; 8, *N. helvata*; 9, *N. longicatena*; 10, *N. polychroma*; 11, *N. pusilla*; 12, *N. recticatena*; 13, *N. roseola*; 14, *N. roseoviolacea* subsp. *roseoviolacea*; 15, *N. rubra*; 16, *N. salmonea*; 17, *N. spiralis*; 18, *N. turkmeniaca*; 19, *N. terrinata*. Data are taken from Meyer (1989), Holt *et al.* (1994), Chiba *et al.* (1999), Gyobu & Miyadoh (2001), Stackebrandt *et al.* (2001) and Quintana *et al.* (2003). Symbols: +, positive; –, negative; w, weak; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14*	15	16	17	18	19
Spore-chain morphology																			
Spore chains†	Sp	Str	Sp	Str, Sp	S, Sp	H, S	H, S	H, Psp	Str	ND	Psp	Str	Sp, Str	Psp	H, S, Sp	H, S	Sp	Sp	Irregular, Psp
Spore ornamentation	ND	Smooth	Ridged	Cross-ridged, smooth and rough	Irregular	Folded	Warty	Smooth	Smooth	ND	Smooth	Smooth	Folded	Smooth	Smooth	Warty	Folded	Smooth	Rugose
Number of spores	ND	4–10	4–15	≥30	4–10	4–10	4–10	4–10	10–30	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	Trace	Greyish blue	White	Beige	White/pink	White/pink	White/yellow	White	White	Trace	White/cream	White/cream	Pink	Pink/violet	Trace	Pink	White/yellow	Trace	White
Substrate mycelium	Yellow	Yellow	White/ochre	Beige	Colourless	Pink	Brown	Yellow/brown	Ochre	Colourless/brown	Grey/brown	Dark yellow/brown	Brown/red	Violet	Orange/red	Red	Yellow/brown	Violet/red	White/ochre
Soluble pigment	None	Yellowish brown	None	Yellow	None	None	None	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	+	+	–	ND	+	+	–	+	–	–	+	+	–	–	–
Gelatin	–	+	+	+	+	–	+	–	–	+	+	+	+	+	+	+	+	+	–
Hypoxanthine	–	+	+	ND	+	+	–	–	+	+	+	+	+	+	+	+	–	+	+
Starch	–	+	–	ND	–	+	+	–	+	–	–	+	–	–	+	–	–	+	+
Tyrosine	–	+	+	ND	–	+	+	–	–	–	+	–	+	–	+	+	+	–	–
Xanthine	–	–	–	ND	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Utilization of:																			
L-Arabinose	+	+	–	W	–	+	–	–	+	–	–	+	–	+	+	–	–	+	ND
Sucrose	+	+	–	–	–	+	–	+	ND	–	–	–	+	+	+	+	+	+	ND
D-Xylose	+	+	+	–	+	+	–	+	ND	–	–	–	–	–	+	–	+	+	ND
myo-Inositol	+	+	+	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	ND
D-Mannose	+	+	–	–	–	+	–	–	+	–	–	–	–	+	+	–	+	+	ND
D-Fructose	+	+	–	+	–	+	–	–	ND	–	–	–	–	+	+	–	–	+	ND
L-Rhamnose	+	+	–	+	–	+	–	–	–	–	–	+	+	+	+	+	+	+	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14*	15	16	17	18	19
D-Raffinose	ND	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	-	+	ND
Cellobiose	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	ND
Production of:																			
Gelatinase	-	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	ND
Citrate lyase	ND	+	+	+	+	+	-	+	ND	-	-	-	+	-	+	+	-	+	ND
Urease	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	ND
Arginine dihydrolase	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	ND
β-Galactosidase	-	+	+	+	+	+	-	+	+	+	-	-	+	-	+	+	-	+	ND
Lysine decarboxylase	-	+	+	+	+	+	-	+	+	+	-	-	+	-	+	+	-	+	ND
Ornithine decarboxylase	-	+	+	+	+	+	-	+	+	+	-	-	+	-	+	+	-	+	ND
Acetoin	ND	+	-	+	+	+	-	+	ND	+	-	-	+	+	+	+	-	+	ND

*The two subspecies of *N. roseoviolacea* 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).

†H, Hooks, curled; Psp, pseudosporangia; S, spirals of one or two turns; Sp, spirals of three to five turns; Str, straight.

10-heptadecanoic-branched fatty acids. Smaller amounts of unsaturated and 2-hydroxy fatty acids 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 sugars: 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 arabinol. The following carbon sources are utilized (after 7 days incubation): *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-melibiose, L-rhamnose, sucrose, D-trehalose, D-xylose, adonitol, D-mannitol, fumarate (weak), DL-lactate, malate, 2-oxoglutarate, L-aspartate (weak) and L-proline. The following carbon sources are not utilized: *N*-acetyl-D-galactosamine, *p*-arbutin, D-gluconate, D-maltose, D-ribose, salicin, inositol, maltitol, sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, glutarate, DL-3-hydroxybutyrate, itaconate, mesaconate, pyruvate, suberate, L-alanine, β-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, DL-3-hydroxybenzoate, DL-4-hydroxybenzotae and L-phenylacetate.

The type strain, GW 14-1925^T (= DSM 44753^T = NRRL B-24325^T), was isolated from a soil sample.

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