Pseudonocardia ammonioxydans sp. nov., isolated from coastal sediment

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Actinomycete strain H9^T was isolated from coastal sediment of the Jiao-Dong peninsula (near Tsingdao city) in China, and was identified by means of polyphasic taxonomy. The strain grew autotrophically on modified nitrifying medium and heterotrophically on Luria–Bertani medium, with NaCl ranging from 0 to 8% (w/v) (optimal growth at 3.5%). The 16S rRNA gene sequence similarities of strain H9^T to members of the genus *Pseudonocardia* ranged from 93.0 to 97.5%, indicating that strain H9^T was phylogenetically related to members of the genus *Pseudonocardia*. Strain H9^T had type IV cell wall and type PIII phospholipid, and its major menaquinone was MK-8 (H₄). DNA–DNA relatedness values between strain H9^T and *Pseudonocardia kongjuensis*, *Pseudonocardia autotrophica* and *Pseudonocardia compacta* were 42, 13 and 11%, respectively. These results support the conclusion that H9^T represents a novel species within the genus *Pseudonocardia*, for which the name *Pseudonocardia ammonioxydans* sp. nov. is proposed, with the type strain H9^T (=CGMCC 4.1877^T=JCM 12462^T).

The genus *Pseudonocardia* was first established by Henssen (1957) and its description has since been subjected to repeated emendations by Warwick *et al.* (1994), McVeigh *et al.* (1994), Reichert *et al.* (1998) and Huang *et al.* (2002). The current membership of the genus *Pseudonocardia* includes 16 species with validly published names that form a phylogenetically coherent cluster and exhibit physiological versatility: psychrotolerant (Prabakar *et al.*, 2004), thermophilic (Henssen, 1957) and autotrophic and heterotrophic (Takamiya & Tubaki, 1956). The physiological versatility of the species of *Pseudonocardia* renders them environmentally important in the removal of hazardous compounds and in biogeochemical cycles of elements. *Pseudonocardia benzenivorans* (Kämpfer & Kroppenstedt, 2004) and *Pseudonocardia chloroethenivorans* (Lee *et al.*, 2004) were enriched for degradation of 1,2,3,5-tetrachlorobenzene and trichloroethene, respectively. *Pseudonocardia asaccharolytica* and *Pseudonocardia sulfidoxydans* were isolated from biofilters and they were reported to use dimethyl disulfide as an energy source (Reichert *et al.*, 1998). In this paper, we describe the newly isolated actinomycete strain H9^T.

Strain H9^T was isolated from coastal sediment (20 °C and

3.3% salinity of the overlying sea water, GPS location of sampling site was 120° 14' 12" E 35° 58' 48" N) from Jiao-Dong peninsula near Tsingdao city, Shandong province, China, with a modified nitrifying medium [MNM; 2.0 g (NH₄)₂SO₄, 0.25 g NaH₂PO₄, 0.75 g K₂HPO₄, 0.01 g MnSO₄·4H₂O, 0.03 g MgSO₄·7H₂O, 5.0 g CaCO₃ and 33 g NaCl in 1000 ml deionized water (pH 8.0)]. For preparation of silica plates, silica (GF 254; Tsingdao marine chemical factory) was used as the solid matrix and was washed with 5 vols deionized water. After being washed twice with deionized water and autoclaved, 30 ml silica suspension [50% (w/v) in deionized water] was poured into each plate (9 cm in diameter) and dried at 50 °C for 5–7 days, and 4 ml fivefold MNM was then added to the top of each plate and evaporated at 50 °C. The plates were inoculated with a loopful of sediment sample (0.1 g ml⁻¹, suspended in saline) and were incubated at 30 °C for 1 month. One or two colonies were observed on each plate. Strain H9^T was purified after several transfers and streaking onto MNM silica plates. For the growth assay of strain H9^T in MNM, 20 ml of the cultural liquid was sampled and centrifuged, and the supernatant was used for analysis of ammonium (Slawyk & MacIsaac, 1972). The cell pellet was resuspended in 2 ml sterile deionized water and cells were broken by sonication. Nitrite and nitrate were determined according to van't Riet *et al.* (1968) and Rand *et al.* (1975), respectively. Results indicated that strain H9^T grew autotrophically (uses CaCO₃ as carbon source) and heterotrophically, and oxidized ammonia to nitrate in both MNM (nitrification) and blends of MNM and Luria–Bertani (LB) media (dissimilatory, heterotrophic ammonium oxidation; Supplementary Table S1 available in IJSEM Online). Strain

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H9^T is AY500143.

A figure showing an electron micrograph of strain H9^T and tables showing ammonia oxidation of nitrite and nitrate and cellular fatty acid composition of strain H9^T are available as supplementary material in IJSEM Online.

H9^T showed typical morphology of the genus *Pseudonocardia*. Chains of spores formed by acropetal budding from branched substrate mycelium. Both substrate and aerial mycelia fragmented into rod-shaped elements on trypticase soy broth (TSB; BBL) agar. The diameter of the aerial mycelium was about 0.5 µm (Supplementary Fig. S1 available in IJSEM Online).

Physiological and biochemical tests were carried out by following the procedures of Gordon *et al.* (1974) and Reichert *et al.* (1998), respectively, using Biolog GP2 plates (MicroStation), with reference strains in parallel. Nitrate reduction test was carried out according to Dong & Cai (2001). Strain H9^T grew at NaCl concentrations ranging from 0 to 8% with an optimum NaCl concentration of 3.5%. Detailed physiological and biochemical properties of strain H9^T are provided in the species description. Some characteristic and differential properties from phylogenetically closely related *Pseudonocardia* species are given in Table 1.

Cell-wall amino acids and whole-cell sugars were analysed by following the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Strain H9^T contained *meso*-diaminopimelic acid and arabinose and galactose, thus giving a cell wall type IV, according to Lechevalier & Lechevalier (1970, 1980). Cells of strain H9^T had type PIII phospholipid (Lechevalier *et al.*, 1977; Lechevalier & Lechevalier, 1980). No glucosamine-containing phospholipids were detected. Menaquinones were extracted and purified from freeze-dried biomass according to Collins (1985), and were determined by using an HPLC procedure (Wu *et al.*, 1989). The major menaquinone of strain H9^T was MK-8 (H₄). Cellular fatty acids were determined as described previously (Hu *et al.*, 2004). The results (Supplementary Table S2 available in IJSEM Online) indicated that the major fatty acids of strain H9^T were 16:0 (41.1%), iso-16:1 (15.7%) and 17:1ω8c (12.1%).

The 16S rRNA gene of strain H9^T was amplified and sequenced as described previously (Zhang *et al.*, 2003), and the sequence was aligned by using the CLUSTAL X program (Thompson *et al.*, 1997). The 16S rRNA gene sequence similarities of strain H9^T to members of the genus *Pseudonocardia* ranged from 93.0 to 97.5%. The closest relatives were *Pseudonocardia kongjuensis* (97.5%), *Pseudonocardia autotrophica* (97.1%) and *Pseudonocardia compacta* (96.8%), according to 16S rRNA gene similarity. Phylogenetic trees were constructed with neighbour-joining and maximum-parsimony methods, all trees showed similar topology. The tree constructed with the neighbour-joining method (Saitou & Nei, 1987) using Kimura's two-parameter calculation model in TREECON W version 1.3b (Van de Peer & De Wachter, 1994) is shown in Fig. 1. Strain H9^T, together with *P. kongjuensis*, *P. autotrophica* and *P. compacta*, formed a phyletic clade with 100% support.

DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962) and genomic DNA from *Escherichia coli* DH5α was analysed in parallel for calibration

Table 1. Differential properties of strain H9^T from closely related *Pseudonocardia* species

Strains: 1, *P. ammonioxydans* H9^T; 2, *P. kongjuensis* DSM 44525^T (data from Prabakar *et al.*, 2004); 3, *P. autotrophica* IMSNU 20050^T (for columns 3–5, data from Lee *et al.*, 2001); 4, *P. compacta* IMSNU 20111^T; 5, *P. alni* IMSNU 20049^T; 6, *P. antarctica* DVS5a1^T (data from Prabakar *et al.*, 2004). +, Positive or present; W, weakly positive; –, negative or absent; ND, not done.

Characteristic	1	2	3	4	5	6
Acid production from:						
L-Arabinose	+	–	+	–	+	–
D-Cellobiose	–	+	+	+	–	–
D-Galactose	+	+	+	–	+	+
Maltose	+	+	+	–	+	+
D-Mannose	+	+	+	–	–	+
D-Melezitose	–	+	+	–	+	–
Methyl α-D-glucoside	–	–	+	–	–	ND
Adonitol	+	+	+	–	+	–
myo-Inositol	–	+	+	–	–	–
1,2-Propanediol	+	–	+	–	+	ND
D-Sorbitol	–	–	+	–	+	–
Sucrose	–	+	+	–	+	–
D-Trehalose	+	+	+	–	+	–
D-Xylose	W	+	+	–	+	+
Decomposition of:						
Hypoxanthine	–	+	–	–	+	+
Tyrosine	+	+	–	–	+	+
Xanthine	–	–	–	–	+	–
Hydrolysis of:						
Gelatin	W	–	–	–	+	ND
Starch	W	–	–	–	+	+
Casein	+	+	–	–	–	–
Urease activity	+	+	+	–	+	+
NO ₃ [–] reduction to NO ₂ [–]	+	–	–	+	–	+
H ₂ S production	+	+	+	–	+	ND
Oxidation of ammonia	+	–	–	+	–	–
Growth at/on:						
4 °C	+	+	–	+	–	–
37 °C	+	+	+	–	+	+
7% NaCl	+	+	+	–	+	+

of the *T_m* value. The result showed that the G + C content of strain H9^T was 69.6 mol% (*T_m*). DNA–DNA hybridizations were carried out according to De Ley *et al.* (1970). Renaturation rates and relatedness values were calculated as described by Jahnke (1992). DNA–DNA relatedness values between strain H9^T and *P. kongjuensis* DSM 44525^T, *P. autotrophica* IMSNU 20050^T and *P. compacta* IMSNU 20111^T were 42, 13 and 11%, respectively.

Description of *Pseudonocardia ammonioxydans* sp. nov.

Pseudonocardia ammonioxydans (N.L. n. *ammonia* ammonia; N.L. part. adj. *oxydans* oxidizing; N.L. part. adj. *ammonioxydans* oxidizing ammonia).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain H9^T in the genus *Pseudonocardia*. Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values over 50 % are shown. *Nocardia puris* IFM 0583^T was used as the outgroup. Bar, evolutionary distance (K_{nuc}) of 0.1.

Aerobic, Gram-positive. Forms branched, brown substrate mycelium and white aerial mycelium on TSB and LB agar. The mycelium fragments into rod-shaped elements. Smooth spores are borne in short chains by acropetal budding from the substrate mycelium. No pigment is produced. Growth occurs at 10–40 °C. Growth occurs on MNM and ammonia is oxidized to nitrate as the sole energy source. Ammonia is also oxidized to nitrate during growth on a blend of MNM and LB media. Growth occurs at NaCl concentrations ranging from 0 to 8 % with an optimum at 3.5 % NaCl. Catalase-positive. Acid is produced from D-fructose, D-glucose, N-acetyl-D-glucosamine, D-ribose, D-arabitol, D-galacturonic acid, D-gluconic acid and glycerol, but not from N-acetyl-β-D-mannosamine, arbutin, L-fucose, gentiobiose, α-D-lactose, lactulose, maltotriose, methyl α-D-galactoside, methyl β-D-galactoside, methyl α-D-glucoside, methyl α-D-mannoside, palatinose, D-psicose, D-raffinose, L-rhamnose, D-galactose, turanose or xylitol. Uses acetic acid, β-hydroxybutyric acid, α-ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, L-malic acid, pyruvic acid methyl ester, succinic acid mono-methyl ester, propionic acid, succinamic acid, succinic acid, N-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglytamic acid, L-serine, putrescine, but not α-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketovaleric acid, D-malic acid or pyruvic acid. Other physiological properties are listed in Table 1. The cell-wall chemotype is type IV. Predominant menaquinone is MK-8 (H₄). It contains phosphatidylcholine, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidyl-inositol mannosides and diphosphatidylglycerol, but does not contain glucosamine-containing phospholipids or mycolic acids. The major fatty acids are iso-hexadecanoic acid (16:0-iso, 41.1 %), iso-hexadecenoic acid (16:1-iso, 15.7 %) and heptadecenoic acid (17:1ω8c, 12.1 %). The G + C content of the DNA is 69.6 mol% (T_m).

The type strain, H9^T (=CGMCC 4.1877^T=JCM 12462^T), was isolated from coastal sediment collected from Jiao-Dong peninsula near Qingdao, Shandong province, China.

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