Pseudonocardia carboxydivorans sp. nov., a carbon monoxide-oxidizing actinomycete, and an emended description of the genus *Pseudonocardia*

Sae W. Park, Sang T. Park,[†] Jung E. Lee and Young M. Kim

Correspondence

Young M. Kim
young547@yonsei.ac.kr

Molecular Microbiology Laboratory, Department of Biology, Yonsei University, Seoul 120-749, Republic of Korea

A bacterial strain, Y8^T, capable of oxidizing carbon monoxide, was isolated from a soil sample collected from a roadside in Seoul, Korea. On the basis of 16S rRNA gene sequence similarity analyses, strain Y8^T was shown to belong to the genus *Pseudonocardia* and was related most closely to the type strain of *Pseudonocardia alni* (99.6% similarity). The cells were aerobic and stained Gram-positive, with white aerial mycelium and brown substrate mycelium. The predominant fatty acids were 16:0 iso and 16:1 iso. The cell-wall peptidoglycan of strain Y8^T contained *meso*-diaminopimelic acid. The DNA G+C content was 77 mol%. Strain Y8^T contained MK-9 as the major menaquinone, which is different from the major menaquinone reported previously in the genus *Pseudonocardia*, MK-8(H₄). DNA–DNA relatedness between strain Y8^T and the type strains of *P. alni* and *Pseudonocardia antarctica* was respectively 10 and 63%. Based on phylogenetic, morphological and chemotaxonomic evidence, it is proposed that strain Y8^T (=KCCM 42678^T =JCM 14827^T) be classified as the type strain of a novel species, *Pseudonocardia carboxydivorans* sp. nov. An emended description of the genus *Pseudonocardia* is also presented.

The genus *Pseudonocardia* was first established by Henssen (1957). The genus presently comprises 27 species and has the following characteristics: vegetative and aerial mycelia with spore chains produced by acropetal budding or fragmentation, *meso*-diaminopimelic acid, arabinose and galactose in the peptidoglycan (type IV cell-wall composition), MK-8(H₄) as the major menaquinone, iso-branched hexadecanoic acid as the predominant fatty acid, phospholipid type PII (phosphatidylethanolamine or its derivatives as a diagnostic phospholipid) or PIII (phosphatidylcholine) pattern, depending on the species, and a DNA G+C content of 68–79 mol% (Lee *et al.*, 2001; Huang *et al.*, 2002).

Carbon monoxide (CO) is produced in large quantities by the incomplete combustion of carbonaceous materials used as fuels and is the most abundant and widely distributed air pollutant found in the lower atmosphere (Samoli *et al.*,

2007; Sohn *et al.*, 1999). CO occurs in non-urban and urban atmospheres at about 45–250 p.p.b. (Novelli *et al.*, 1994) and 2.2–4.5 p.p.m. (Holmes *et al.*, 2005), respectively. CO is oxidized by micro-organisms living in soil and water, for example carboxydobacteria (Ferry, 1995; Kim & Hegeman, 1983; Meyer & Schlegel, 1983), homoacetogenic bacteria (Conrad, 1996; Ferry, 1995), phototrophic bacteria (Conrad, 1996; Ferry, 1995), methanotrophic bacteria (Bedard & Knowles, 1989) and methanogenic bacteria (Zeikus *et al.*, 1985) (see Supplementary Table S1, available in IJSEM Online), as well as algae (Chappelle, 1962) and fungi (Inman & Ingersoll, 1971).

Carboxydobacteria are a group of aerobic bacteria that can grow aerobically on CO as the sole source of carbon and energy (Kim & Hegeman, 1983; Meyer & Schlegel, 1983). Our previous studies have focused on carboxydobacteria able to grow at high CO concentrations (300 000 p.p.m.), but, in this study, we have isolated and characterized a novel carboxydobacterium able to grow at a low CO concentration (400 p.p.m.).

Strain Y8^T was isolated from soil collected from a roadside in Seoul, Korea. For the enrichment of aerobic bacteria growing on a low concentration of CO, a primary enrichment was established using 20 g (fresh weight) samples of soil that were transferred into 500 ml flasks and incubated under 400 p.p.m. CO in air for 2 weeks at 30 °C. Secondary enrichment was initiated by transferring

[†]Present address: Division of Infectious Diseases, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA.

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A list of CO-utilizing bacteria, an HPLC trace of the menaquinones of strain Y8^T, a scanning electron micrograph of aerial mycelium of strain Y8^T and a comparison of its fatty acid profile with those of related strains are available as supplementary material with the online version of this paper.

2 g of the primary enrichment to a 500 ml flask containing 200 ml liquid mineral medium (Kim & Hegeman, 1981) supplemented with 400 p.p.m. CO in air and 0.005 % (w/v) yeast extract. The flask was incubated for 2 weeks at 30 °C with shaking at 200 r.p.m. Portions (1 ml) of the enrichments were then spread on solid mineral medium supplemented with 0.2 % (w/v) glucose and incubated for 2 weeks at 30 °C under 400 p.p.m. CO in air (Hardy & King, 2001). Strain Y8^T grew slowly on mineral medium supplemented with 0.2 % (w/v) glucose at 30 °C under 400 p.p.m. CO in air, but did not grow under a high CO concentration (300 000 p.p.m.). The CO dehydrogenase (CO-DH) enzyme assay was carried out according to Kraut *et al.* (1989) and revealed that strain Y8^T was able to oxidize CO [1.2 nmol 2-(4-indophenyl)-3-(4-nitrophenyl)-2H-tetrazolium chloride (ϵ_{496} = 17.981 mM⁻¹ cm⁻¹) reduced per mg protein per minute].

The morphological characteristics of strain Y8^T were observed by scanning electron microscopy of a 14-day-old culture grown on Bennett's medium. An LEO 1455VP scanning electron microscope was used. Members of the genus *Pseudonocardia* are characterized morphologically by the formation of well-developed vegetative and aerial mycelium, from which spore chains are produced by acropetal budding or fragmentation (Lee *et al.*, 2001; Huang *et al.*, 2002). Strain Y8^T showed morphology typical of the genus *Pseudonocardia*. Strain Y8^T formed brown substrate and white aerial mycelia. The aerial mycelium fragmented into rod-shaped spores. The spore surface was smooth (Supplementary Fig. S1). Gram-staining and physiological tests were carried out by following the procedures of Gerhardt *et al.* (1994) and Gordon *et al.* (1974), respectively. Carbon utilization of a variety of substrates was examined by MicroID (Daejeon, Korea) using a Biolog GP2 MicroPlate. Strain Y8^T was aerobic and stained Gram-positive. Detailed physiological properties of strain Y8^T are provided in the species description. Some characteristic and differential properties from phylogenetically closely related *Pseudonocardia* species are shown in Table 1.

Total genomic DNA from strain Y8^T was isolated by using cetyltrimethylammonium bromide (Ausubel *et al.*, 1995). PCR amplification of the 16S rRNA gene using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3') was carried out as described by Lane (1991). Amplified DNA fragments were purified from agarose gels using a gel extraction kit (iNtRON) and sequenced. Phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). 16S rRNA gene sequence studies indicated that strain Y8^T is related to members of the genus *Pseudonocardia* (Fig. 1). The nearly complete 16S rRNA gene sequence of strain Y8^T was a continuous stretch of 1322 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain Y8^T are *Pseudonocardia alni* IMSNU 20049^T (99.6 %

Table 1. Phenotypic differences between strain Y8^T and type strains of closely related species of the genus *Pseudonocardia*

Strains: 1, strain Y8^T; 2, *P. alni* DSM 44104^T (data from Prabahar *et al.*, 2004); 3, *P. antarctica* DSM 44749^T (Prabahar *et al.*, 2004); 4, *P. autotrophica* IMSNU 20050^T (Lee *et al.*, 2001); 5, *P. compacta* IMSNU 20111^T (Lee *et al.*, 2001). +, Positive or present; (+), weakly positive; -, negative or absent; ND, no data available. All strains were negative for hydrolysis of casein and did not grow at 4 °C.

Test	1	2	3	4	5
Acid produced from:					
Cellobiose	-	-	-	+	+
D-Fructose	-	+	-	+	+
D-Galactose	-	-	+	+	-
myo-Inositol	-	-	-	+	-
Inulin	+	-	+	ND	ND
Maltose	-	-	+	+	-
D-Mannitol	-	-	(+)	+	+
D-Mannose	-	-	+	ND	-
Melezitose	-	-	-	+	-
L-Rhamnose	-	(+)	+	-	-
D-Ribose	-	-	+	ND	ND
Sorbitol	-	+	-	+	-
Sucrose	-	+	-	+	-
Trehalose	-	+	-	+	-
D-Xylose	-	-	+	+	-
Hydrolysis of:					
Gelatin	-	+	+	-	-
Starch	-	+	+	-	-
Urease activity	+	+	+	+	-
Growth at/on:					
37 °C	+	+	+	+	-
3 % NaCl	+	+	+	+	-
4 % NaCl	+	+	+	+	-
5 % NaCl	-	+	+	+	-

similarity) and *Pseudonocardia antarctica* DVS 5a1^T (99.5 %) (Fig. 1).

Whole-cell fatty acid methyl ester analysis and menaquinone composition analysis were performed according to Sasser (1990) and Wu *et al.* (1989), respectively, by the Korean Culture Center of Micro-organisms (KCCM, Seoul, Korea). The diaminopimelic acid composition of strain Y8^T was determined according to Stanek & Roberts (1974) by MicroID. The most abundant fatty acids found in Y8^T were 16:0 iso (47.2 %) and 16:1 iso (22.8 %). Analysis of the fatty acid profile also revealed the presence of smaller amounts of 15:0 iso (5.0 %), 16:1 *cis*9 (5.9 %), 16:0 10-methyl (4.6 %), 17:0 iso (3.0 %), 17:0 anteiso (2.6 %) and 17:1 *cis*9 (2.4 %). The fatty acid 16:0 iso is also found in the highest concentrations in *P. alni*, *P. antarctica*, *P. asaccharolytica*, *P. autotrophica*, *P. compacta*, *P. halophobica*, *P. hydrocarbonoxydans*, *P. kongjuensis* and *P. sulfidoxydans* (Lee *et al.*, 2001; Prabahar *et al.*, 2004; Reichert *et al.*, 1998) (Supplementary Table S2). The major menaquinone of strain Y8^T was MK-9 (Supplementary Fig. S2), which is

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain Y8^T in the genus *Pseudonocardia*. The tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) based on a comparison of 1322 unambiguous nucleotide positions. Numbers at branching points are percentages of occurrence in 1000 bootstrapped trees. Bar, 1 substitution per 100 nucleotide positions.

different from the major menaquinone reported for the genus *Pseudonocardia*, MK-8(H₄) (Embley *et al.*, 1988; Huang *et al.*, 2002). Strain Y8^T contained *meso*-diaminopimelic acid as a cell-wall diamino acid.

DNA–DNA hybridization experiments were performed between strain Y8^T and the type strains of *P. alni* and *P. antarctica*, using the method described by Nicholas *et al.* (2002). DNA–DNA hybridization showed that strain Y8^T exhibited low levels of DNA relatedness to its phylogenetic neighbours *P. alni* DSM 44104^T (10 ± 0.6 %, mean ± SD of three hybridizations) and *P. antarctica* DSM 44749^T (63 ± 7.0 %, mean ± SD of five hybridizations). The DNA base composition was determined according to Mesbah *et al.* (1989) by the KCCM. The G + C content of strain Y8^T was 77 mol%.

The observation that strain Y8^T contains MK-9 as the major menaquinone necessitates a further emendation of the description of the genus *Pseudonocardia*.

Description of *Pseudonocardia carboxydivorans* sp. nov.

Pseudonocardia carboxydivorans (car.bo.xy'di.vo'rans. N.L. neut. n. *carboxydum* carbon monoxide; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *carboxydivorans* digesting carbon monoxide).

Aerobic, Gram-positive, non-motile actinomycete. Forms brown substrate mycelium and white aerial mycelium. The aerial mycelium fragments into rod-shaped spores. Grows optimally at 25 °C and tolerates up to 4 % (w/v) NaCl. Positive for catalase and urease, but negative for oxidase. Utilizes *N*-acetyl-D-glucosamine, inulin and mannan but not cellobiose, D-fructose, D-galactose, *myo*-inositol, maltose, D-mannitol, D-mannose, melezitose, raffinose,

L-rhamnose, D-ribose, sorbitol, sucrose, trehalose, xylitol or D-xylose as sole carbon sources. *meso*-Diaminopimelic acid is present in the cell wall and MK-9 is the major menaquinone. Predominant fatty acids are 16:0 iso and 16:1 iso. The G + C content of the type strain is 77 mol%.

The type strain, Y8^T (=KCCM 42678^T =JCM 14827^T), was isolated from soil collected at a roadside in Seoul, Korea.

Emended description of the genus *Pseudonocardia* Henssen 1957 emend. Huang *et al.* 2002

The description of the genus is as emended by Huang *et al.* (2002), with the exception that the major menaquinone is MK-8(H₄) or MK-9(H₀).

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