Nocardioides albertanoniae sp. nov., isolated from Roman catacombs

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A Gram-reaction-positive, aerobic, non-spore-forming, rod- or coccoid-shaped, strain, CD40127T, was isolated from a green biofilm covering the wall of the Domitilla Catacombs in Rome, Italy. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CD40127T belongs to the genus Nocardioides, closely related to Nocardioides luteus DSM 43366T and Nocardioides albus DSM 43109T with 98.86 % and 98.01 % similarity values, respectively. Strain CD40127T exhibited 16S rRNA gene sequence similarity values below 96.29 % with the rest of the species of the genus Nocardioides. The G + C content of the genomic DNA was 69.7 mol%. The predominant fatty acid was iso-C16 : 0 and the major menaquinone was MK-8(H4) in accordance with the phenotypes of other species of the genus Nocardioides. A polyphasic approach using physiological tests, fatty acid profiles, DNA base ratios and DNA–DNA hybridization showed that isolate CD40127T represents a novel species within the genus Nocardioides, for which the name Nocardioides albertanoniae is proposed. The type strain is CD40127T (=DSM 25218T = CECT 8014T).

The genus Nocardioides was established by Prauser (1976) with the type species Nocardioides albus for Gram-positive, non-acid-fast, catalase-positive, aerobic and mesophilic nocardioform actinomycetes, developing a mycelium that fragments into irregular rod- to coccus-like elements. At the time of writing, the genus Nocardioides comprised 57 species with validly published names (http://www.bacterio.cict.fr/n/nocardioides.html), isolated from a variety of habitats, including soils, sediments, sand, water, herbage, an oil shale column and glacier cryoconite, and many of these have been isolated recently from samples collected in Korea (Dastager et al., 2008, 2010; Yoon et al., 1997, 2009, 2010). In addition, strains of the genus Nocardioides have been isolated from caves in Spain and Italy (Groth et al., 1999, 2001), but the authors were unable to assign the isolates to defined species.

During investigations on the microbial biodiversity from Roman catacombs (http://www2.bio.uniroma2.it/biologia/laboratori/lab-botanica/Algae/CATS.htm) a large number of new species of actinobacteria were found. In this study, we describe strain CD40127T isolated from a green biofilm, mainly composed of phototrophic micro-organisms, covering a wall of the Domitilla Catacombs in Rome, Italy.

Strain CD40127T was isolated on casein agar media (Küster & Williams, 1964) after 1 week of incubation at 28 °C. Morphological, physiological and chemotaxonomic studies were carried out using cultures on trypticase soy agar (TSA; Oxoid) at 28 °C unless indicated otherwise. Cell morphology and dimensions were observed by a stereo microscope and phase-contrast microscope. Media such as oatmeal agar (Prauser, 1976), nutrient agar (Difco) and R2A agar (Difco) were used for testing mycelial production. Oxidase activity was determined by monitoring the oxidation of dryslide oxidase (Becton Dickinson). Catalase production was indicated by the production of bubbles after mixing a cell suspension with a drop of 3 % hydrogen peroxide solution on a slide. Acid production from a variety of substrates was tested using the API 50 CH system and API 50 CH B/E kit (bioMérieux). Assimilation tests were carried out using the API 20NE kit (bioMérieux) and enzymic activities were detected with API ZYM galleries (bioMérieux). All API tests were performed according to the manufacturer’s instructions. For the Gram reaction, a 3 % solution of potassium hydroxide was used (Halebian et al., 1981). Growth temperature was tested over the range of 3 to 40 °C. These authors contributed equally to this work.

This paper is dedicated to the memory of Professor Patrizia Albertano who died on March 14th, 2012.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence from strain CD40127T is HE801966.

A supplementary table is available with the online version of this paper.
4–45°C. Tolerance to NaCl was studied on TSA supplemented with 0–15% (w/v) NaCl.

Standard procedures for the analyses of fatty acids by gas chromatography were adopted with the Microbial Identification System (MIDI) for automated GC analyses (Kroppenstedt, 1985) using TSA after 3 days at 28°C. Analysis of respiratory quinones and G+C content of genomic DNA were determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany.

Genomic DNA extraction was performed as described by Marmur (1961). The 16S rRNA gene was amplified by PCR using the primers 27F (5′-AGAGTTTGATCCTGGCTC-AG) and 1522R (5′-AAGGAGGTGATCCAGCCGCA). PCR thermal conditions were as follows: 95°C for 60 s; 35 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 120 s; and a final extension cycle at 72°C for 10 min. Forward and reverse strands of the amplified DNA fragment were sequenced using an ABI 3700 sequencer (Applied Biosystems). The identification of phylogenetic neighbours was carried out by submitting the sequence of strain CD40127T to BLAST (Altschul et al., 1990) and by using the GenBank database and the EzTaxon-e database (Kim et al., 2012). Pairwise 16S rRNA gene sequence similarities among the most closely related strains were determined using the global alignment algorithm on the EzTaxon server (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012).

Table 1. Phenotypic characteristics of strain CD40127T and related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Rods, cocci</td>
<td>Hyphae</td>
<td>Hyphae</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>1.0–1.6</td>
<td>0.5–1.0</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>Cell width (μm)</td>
<td>0.6–0.8</td>
<td>0.5–1.0</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Cream</td>
<td>Cream</td>
<td>Yellow to cream</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acid produced from L-rhamnose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activities:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatinase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Major fatty acids (&gt;5%)</td>
<td>iso-C16:0</td>
<td>iso-C16:0</td>
<td>iso-C16:0</td>
</tr>
<tr>
<td>Predominant menaquinone</td>
<td>MK-8(H4)</td>
<td>MK-8(H4)</td>
<td>MK-8(H4)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69.7</td>
<td>66.5–68.6</td>
<td>67.5</td>
</tr>
<tr>
<td>Isolation source</td>
<td>Green biofilm</td>
<td>Soil</td>
<td>Soil</td>
</tr>
</tbody>
</table>

For phylogenetic analyses, the nearly complete 16S rRNA gene sequence (1379 nt) of strain CD40127T was aligned and compared with corresponding sequences of members of the genus Nocardioides using the multiple sequence alignment program MUSCLE (Edgar, 2004) integrated in MEGA 5 software. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011) and by applying the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA 5 software. Tree robustness was assessed by bootstrap resampling (1000 replicates each). The degree of genomic relatedness among strain CD40127T, N. albus DSM 43109T and Nocardioides luteus DSM 43366T, which shared high similarity values for their 16S rRNA gene sequences, was determined by DNA–DNA hybridization as described by De Ley et al. (1970) and Rosselló-Mora & Amann (2001).

Cells of strain CD40127T were aerobic, Gram-positive, catalase-positive and oxidase-negative, non-spore-forming and rod-shaped or coccoid. Growth of strain CD40127T occurred in the temperature range of 10–30°C, with an optimum at 25°C. Strain CD40127T grew at NaCl concentrations of 0–10% (w/v) (optimum 0–4%). Table 1 shows other physiological characteristics of strain CD40127T, as well as numerous phenotypic differences from the phylogenetically closest species of the Nocardioides genus. Several physiological and chemotaxonomic differences...
Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships between *Nocardioides albertanoniae* sp. nov. CD40127\(^7\) and all species of the genus *Nocardioides* with validly published names at the time of writing. The tree was constructed using the neighbour-joining method based on comparison of 1379 nt. Bootstrap values are expressed as percentages of 1000 replicates; values <50\% are not shown. Asterisks indicate that the corresponding branches were also recovered by the maximum-parsimony and maximum-likelihood treeing algorithms. Bar, 0.01 nt substitutions per site. *Terrabacter tumescens* KCTC 9133\(^7\) (AF005023) was used as outgroup.

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*Fig. 1.* Phylogenetic tree based on 16S rRNA gene sequences showing the relationships between *Nocardioides albertanoniae* sp. nov. CD40127\(^7\) and all species of the genus *Nocardioides* with validly published names at the time of writing. The tree was constructed using the neighbour-joining method based on comparison of 1379 nt. Bootstrap values are expressed as percentages of 1000 replicates; values <50\% are not shown. Asterisks indicate that the corresponding branches were also recovered by the maximum-parsimony and maximum-likelihood treeing algorithms. Bar, 0.01 nt substitutions per site. *Terrabacter tumescens* KCTC 9133\(^7\) (AF005023) was used as outgroup.
were noted among strains CD40127T, \textit{N. albus} DSM 43109T and \textit{N. luteus} DSM 43366T. These differences included growth at 37°C and spore production. Strain CD40127T did not grow at 37°C and also did not produce spores on the tested culture media, while \textit{N. albus} and \textit{N. luteus} grew well at this temperature and produced spores. Other differences were the production of acid from \textit{L}-rhamnose and the presence or absence of gelatinase, \textit{z}-mannosidase and trypsin activities. Assimilation of \textit{N}-acetylglucosamine and maltose differed among the three strains. Further dissimilarities were noticed in fatty acid composition. Although 14-methyl pentadecanoic acid (iso-C16:0) is the predominant fatty acid in all three \textit{Nocardioides} species, there were differences in the abundance of fatty acids: C18:0 10-methyl, C18:1 \textit{cis}-9c and C17:0 10-methyl (Table S1 available in IJSEM Online). The menaquinone pattern revealed that MK-8(H4) was the predominant isoprenoid quinone (76\%) in accordance with the phenotypes of other species of the genus \textit{Nocardioides}; MK-8(H2) was present as a minor component (24\%).

Phylogenetic analysis showed that strain CD40127T was related to the genus \textit{Nocardioides}. According to the 16S rRNA gene sequence similarity, strain CD40127T was most closely related to \textit{N. luteus} DSM 43366T (GenBank accession number AF005007) and \textit{N. albus} DSM 43109T (AF004988) with similarity values of 98.86\% and 98.01\%, respectively. In the phylogenetic tree based on the 16S rRNA gene sequence (Fig. 1), strain CD40127T formed a well defined clade with the type strains of \textit{N. luteus} and \textit{N. albus} that is supported by a bootstrap value of 100\% in the neighbour-joining analysis. Strain CD40127T showed DNA–DNA relatedness of 48.90\% with \textit{N. luteus} DSM 43366T and 58.79\% with \textit{N. albus} DSM 43109T. These results indicate that strain CD40127T shows sufficient genomic coherence and hybridization differences from its closest relatives to be considered as a single species (Rossello-Mora & Amann, 2001; Stackebrandt et al., 2002).

Phenotypic and genotypic characteristics described above and in the species description below, together with the differences observed between strain CD40127T and previously described species of the genus \textit{Nocardioides} reveal that strain CD40127T is a novel species within the genus \textit{Nocardioides}. The name \textit{Nocardioides albertanoniae} sp. nov. is proposed for this novel species.

**Description of \textit{Nocardioides albertanoniae} sp. nov.**

\textit{Nocardioides albertanoniae} (al.be.r.ta.no.ni’a.e. N.L. gen. n. \textit{albertanoniae} of Albertano, named in honour of Professor Patrizia Albertano).

Cells are Gram-reaction-positive, aerobic, non-spore-forming, non-motile and rod-shape or coccoid (0.6–0.8 µm wide and 1.0–1.6 µm long after 2 days on R2A agar). Colonies on R2A agar are cream-coloured, smooth, circular and 0.1 mm in diameter after 2 days growth at 28°C on R2A agar. Neither substrate nor aerial mycelium is formed. Catalase-positive and oxidase-negative. Does not reduce nitrate to nitrite. Growth occurs between 10 and 30°C, optimum at 25°C. Cells grow at NaCl concentrations of 0–10\% (w/v) (optimum 0–4\%). Does not produce indole. Produces acid from aesculin. Assimilates arabinose, glucose, mannose, mannitol, \textit{N}-acetylglucosamine, maltose, potassium gluconate and malate, but does not assimilate capric acid, adipic acid, trisodium citrate and phenylacetic acid. Produces acid phosphatase, alkaline phosphatase, cystine aroylamidase, esterase (C4), esterase lipase (C8), \textit{a}-galactosidase, \textit{z}-galactosidase, \textit{z}-mannosidase, leucine aroylamidase, naphthol-AS-BI-phosphohydrolase and valine aroylamidase but not arginine dihydrolase, \textit{z}-chymotrypsin, \textit{z}-fucosidase, \textit{z}-galactosidase, \textit{\beta}-glucuronidase, \textit{N}-acytetyl-\textit{\beta}-glucosaminidase, lipase (C14), trypsin and urease. Displays variable glucose fermentation activity. The predominant fatty acid is iso-C16:0. The major menaquinone is MK-8(H4).

The type strain, CD40127T (=DSM 25218T =CECT 8014T) was isolated from a green biofilm covering the wall of the Domitilla Catacombs, Rome, Italy. The DNA G+C content of the type strain is 69.7 mol%.

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**References**


