Corynebacterium deserti sp. nov., isolated from desert sand

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A novel coryneform bacterium, designated strain GIMN1.010^T, was isolated from a sand sample collected in the desert in the west of China. Cells were Gram-stain-positive, non-spore-forming, catalase-positive, irregular rods. Comparative 16S rRNA gene sequence analysis demonstrated that strain GIMN1.010^T belonged to the genus *Corynebacterium* and was related closely to *Corynebacterium glutamicum* ATCC 13032^T (98.4 % similarity). However, the level of DNA–DNA relatedness between strain GIMN1.010^T and *C. glutamicum* ATCC 13032^T was only 22.4 ± 1.72 %, showing that strain GIMN1.010^T represented a genomic species distinct from *C. glutamicum*. On the basis of phenotypic and phylogenetic data, strain GIMN1.010^T is considered to represent a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium deserti* sp. nov. is proposed. The type strain is GIMN1.010^T (=CCTCC AB 2010341^T =NRRL B-59552).

The genus *Corynebacterium* was created by Lehmann and Neumann in 1896 and represents a major group of rod-shaped bacteria within the Gram-positive, high-G+C content *Actinobacteria* (Collins & Cummins, 1986; Liebl, 1992). At the time of writing, the genus comprises over 100 recognized species, many of which have been described since 2000. The recognition of large numbers of novel species in recent years has been made possible by the use of improved taxonomic methods, such as chemotaxonomic and molecular-based approaches, especially 16S rRNA gene sequencing. *Corynebacteria* have been isolated from a wide range of environments. Most described species of the genus *Corynebacterium* were isolated from clinical samples and animals, but some non-clinical species have been isolated from soil or plant sources. Here, we have characterized a novel *Corynebacterium*-like organism from a sand sample from the desert in the west of China. On the basis of both phenotypic and molecular genetic criteria, we consider this to represent a novel species of the genus *Corynebacterium*.

Strain GIMN1.010^T was isolated from a mixed sand sample from the desert of western China. It was enriched in TGY medium (0.5 % peptone, 0.5 % yeast extract, 0.1 % glucose; pH adjusted to 6.8 prior to autoclaving) at 30 °C with shaking at 200 r.p.m. for 2 days, followed by isolation of lemon yellow colony-forming bacteria on TGY agar plates (TGY medium with 1.5 % agar). The strain was cultivated

aerobically at 30 °C for 24–48 h. Cells for biochemical and molecular systematic analysis were grown in shaken flasks (about 150 r.p.m.) of TGY medium broth. Stock cultures were maintained at 4 °C, by using modified TGY (1.0 % peptone, 0.5 % yeast extract, 0.1 % glucose, pH adjusted to 6.8 prior to autoclaving), and as glycerol suspensions (20 %, v/v) at –80 °C. Gram staining was carried out by using a modification of the method described by Cowan *et al.* (2003). Physiological characterization and additional biochemical tests were performed at the Institute of Microbiology, Guangdong Province, China. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum and re-extracted in n-hexane/water (1 : 1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as described by Hiraishi *et al.* (1996). Cellular fatty acids were determined for strains grown on TGY medium at 30 °C for 3 days. Fatty acid methyl esters were prepared according to Kämpfer & Kroppenstedt (1996) by using the classical method of the Sherlock Microbial Identification System version 6.1 (MIDI) and were analysed by GC (6890; Hewlett Packard) using the Microbial Identification standard MIS database TSBA6. Polar lipids, extracted from freeze-dried biomass of strain GIMN1.010^T, were examined by two-dimensional TLC and were identified according to the procedures of Minnikin *et al.* (1984). The presence of mycolic acids was determined by the TLC method of Minnikin *et al.* (1980).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GIMN1.010^T is HQ612241.

Genomic DNA of strain GIMN1.010^T was extracted by using a TIANamp bacterial DNA kit (Tiangen), according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR with bacterial universal primers F27 and R1492, which were adapted from primers fD1 and rP1 (Weisburg *et al.*, 1991), and then ligated into the pGEM-T vector (Promega), followed by sequencing by Takala Co. (Dalian, China). Multiple alignments with sequences of a broad selection of actinobacteria and calculations of levels of 16S rRNA gene sequence similarity were carried out by using MEGA version 4 (Tamura *et al.*, 2007). Phylogenetic dendrograms, which showed slightly different topologies, were reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Felsenstein, 1985) with bootstrap values based on 1000 resamplings. The DNA G+C content was measured by the thermal denaturation method (Marmur & Doty, 1962) and was calculated by using the equation of Owen & Lapage (1976). DNA-DNA hybridization studies were carried out by using the fluorometric microdilution plate method (Yuan *et al.*, 2009).

Cells of strain GIMN1.010^T were Gram-stain-positive, non-spore-forming, irregular short rods. On TGY agar, colonies were lemon yellow, circular, opaque, convex with regular edges and approximately 0.5 mm in diameter after 24 h at 30 °C. The isolate was catalase- and urease-positive, but oxidase- and tyrosinase-negative. Furthermore, it was negative for nitrate reduction and the Voges-Proskauer test. Strain GIMN1.010^T was able to utilize a number of substrates (such as glucose, D-xyllose, sucrose and salicin) as

sole carbon sources for growth, but not gelatin or casein. The optimum pH and cultivation temperature for growth were pH 7 and 30 °C. HPLC analysis of the respiratory quinones showed that strain GIMN1.010^T possessed menaquinones MK9 (H₂) (66.04 %) and MK8 (H₂) (27.13 %) as predominant components. Cellular fatty acids found in strain GIMN1.010^T were straight-chain saturated components, namely C_{16:0} (45.5 %), C_{18:0} (0.51 %), C_{14:0} (0.73 %), 10-methyl C_{18:0} (0.45 %) and C_{16:0} 3-OH (0.36 %), and monounsaturated fatty acids, namely C_{18:1}ω9c (51.96 %) and C_{16:1}ω9c (0.48 %). The strain was found to contain mycolic acids. Analysis of cell membrane polar lipids revealed the presence of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and several glycolipids. The DNA G+C content of strain GIMN1.010^T was 61.7 mol%. These characteristics matched well with the description of the genus *Corynebacterium*.

The 16S rRNA gene sequence of strain GIMN1.010^T determined in this study was a continuous stretch of 1478 bp. A phylogenetic tree of *Corynebacterium* 16S rRNA gene sequences was reconstructed by using the neighbour-joining method of Saitou & Nei (1987) with CLUSTAL W (version 1.81) and MEGA (version 3.1, Kumar *et al.*, 2004). For the neighbour-joining analysis, a distance matrix was calculated according to Kimura's two-parameter correction model. The minimum-evolution and maximum-parsimony methods were also used for tree reconstruction. Fig. 1 shows the close phylogenetic association of strain GIMN1.010^T with the type strains of some recognized species of the genus *Corynebacterium*.

Fig. 1. Unrooted neighbour-joining tree reconstructed from *Corynebacterium* 16S rRNA gene sequences (>1343 bp), showing the phylogenetic relationship between strain GIMN1.010^T and the type strains of recognized species of the genus *Corynebacterium*. *Gordonia amarae* DSM 43392^T was used as the outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. GenBank sequence accession numbers are given in parentheses. Branches marked with an asterisk were also conserved in the trees generated with the minimum-evolution and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position.

Strain GIMN1.010^T shared highest 16S rRNA gene sequence similarity (98.4 %) with *Corynebacterium glutamicum* ATCC 13032^T, but the mean ($\pm$ SD) level of DNA–DNA relatedness between the two was 22.4 ± 1.72 %. The latter value is well below the 70 % cut-off point for species classification, as recommended by Wayne *et al.* (1987), thus confirming that the isolated strain represents a novel species of the genus *Corynebacterium*. The chemotaxonomic characteristics of strain GIMN1.010^T were in agreement with the results of the phylogenetic inference, confirming that the novel bacterium belongs to the genus *Corynebacterium*. However, strain GIMN1.010^T clearly differed from related species in terms of physiological and biochemical characteristics (Table 1). These results strongly suggest that strain GIMN1.010^T represents a genetically distinct species, for which we propose the name *Corynebacterium deserti* sp. nov.

Description of *Corynebacterium deserti* sp. nov.

Corynebacterium deserti (des.er'ti. L. gen. n. *deserti* referring to the isolation of the type strain from desert).

Cells are aerobic, Gram-stain-positive, non-spore-forming, irregular rods. Colonies on TGY agar medium are moderately lemon yellow, circular, entire, opaque, convex with regular edges and approximately 0.5 mm in diameter after 24 h at 30 °C. Optimum growth temperature is 30 °C. Optimum growth occurs at pH 7.0. Positive for catalase and urease, but negative for oxidase. Nitrate reduction, gelatin liquefaction, casein hydrolysis and the Voges–Proskauer test are negative. Utilizes glucose, sucrose, xylose, trehalose and salicin as sole carbon sources, but not lactose, raffinose,

D-arabinose, fructose, mannose, maltose or L-tyrosine. The predominant menaquinones are MK9 (H₂) and MK8 (H₂). Contains straight-chain saturated fatty acids (C_{16:0}, C_{18:0}, C_{14:0}, 10-methyl C_{18:0} and C_{16:0} 3-OH) and monounsaturated fatty acids (C_{18:1}ω9c and C_{16:1}ω9c). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl-inositol and several glycolipids. The DNA G + C content of the type strain is 61.7 mol%. Mycolic acids are present.

The type strain, GIMN1.010^T (=CCTCC AB 2010341^T =NRRL B-59552), was isolated from a sand sample from the desert in the west of China.

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Table 1. Characteristics that differentiate strain GIMN1.010^T from its closest phylogenetic relatives in the genus *Corynebacterium*

Strains: 1, GIMN1.010^T; 2, *C. glutamicum* ATCC 13032^T; 3, *C. callunae* NCFB 10338^T. w, Weakly positive. All three strains used sucrose and L-arabinose as sole carbon sources. All data are from the present study and were taken under the same conditions except where indicated.

Characteristic	1	2	3
Nitrate reduction	–	+	–
Urease	+	+	–
Carbon utilization			
Fructose	–	+	+
Mannose	–	+	+
Maltose	–	+	+
Trehalose	w	+	+
Xylose	+	–	–
Salicin	+	–	+
Raffinose	–	+	+
DNA G + C content (mol%)*	61.7	54.6	53.6

*DNA G + C content of the reference strains taken from Liebl *et al.* (1991).

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