Corynebacterium marinum sp. nov. isolated from coastal sediment

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A taxonomic study was performed on strain D7015^T, which was isolated from coastal sediment close to a coal-fired power station in Qingdao, China. Cells of strain D7015^T were Gram-positive, non-motile, diphtheroid rods that grew in the presence of 0–8% (w/v) NaCl and at 4–37 °C, with optimum growth at 1% (w/v) NaCl and 30–32 °C. The DNA G + C content was 65.0 mol%. The major fatty acids were C_{18:1ω9c} (56.18%), C_{16:0} (38.02%), C_{16:1ω7c} (4.45%), C_{18:0} (1.0%) and C_{14:0} (0.35%). On the basis of morphological, physiological and phylogenetic characteristics, strain D7015^T was classified in the genus *Corynebacterium*. It exhibited a 16S rRNA gene sequence similarity of 95.9% and a DNA–DNA relatedness value of 20.4% with *Corynebacterium halotolerans* DSM 44683^T. Strain D7015^T was sufficiently different from recognized species of the genus *Corynebacterium* to be considered to represent a novel species. The name *Corynebacterium marinum* sp. nov. is proposed, with strain D7015^T (=CGMCC 1.6998^T=NRRL B-24779^T) as the type strain.

The genus *Corynebacterium* was proposed by Lehmann & Neumann (1896) and represents a large group of Gram-positive, asporogenous, rod-shaped bacteria with high DNA G + C contents. Corynebacteria have been isolated from a wide range of environments, namely dairy products, soil, sewage, sediments, plant materials and aquatic sources, but the majority of novel species described in recent years have originated from human or animal clinical samples (e.g. Renaud *et al.*, 2007; Yassin & Siering, 2008; Yassin, 2009; Funke *et al.*, 2009). However, some corynebacterial species have been isolated from the marine environment and they may occur as part of the indigenous flora of marine animals. For example, *Corynebacterium phocae* and *Corynebacterium caspium* were isolated from seals (Pascual *et al.*, 1998; Collins *et al.*, 2004), *Corynebacterium spheniscorum* and *Corynebacterium sphenisci* were recovered from wild penguins (Goyache *et al.*, 2003a, 2003b), and *Corynebacterium maris* Coryn-1^T was found in the mucus of the coral *Fungia granulosa* (Ben-Dov *et al.*, 2009). Here, the taxonomic characteristics of a novel *Corynebacterium* isolate that

originated from coastal sediment close to a coal-fired power station in Qingdao, China, are reported.

Strain D7015^T was isolated from coastal sediment in 2000 (Du *et al.*, 2002). The isolate was grown aerobically at 28 °C on marine agar 2216E (MA; Difco) for 48 h. Cultures were maintained on MA slants at room temperature and stock cultures were kept in tryptone soya broth (Oxoid) supplemented with 1% (w/v) NaCl and 20% (v/v) glycerol at –70 °C. Identification of strain D7015^T was performed as described by Jordan *et al.* (2007). For phenotypic tests, the strain was grown on MA for 48 h at 28 °C and cells were resuspended in saline for use as an inoculum. Tolerance to 1, 3, 5, 7, 8 and 10% (w/v) NaCl was assessed on appropriately modified tryptone soya agar (TSA; Oxoid). Growth in the absence of NaCl was assessed on plate count agar (Oxoid). Inoculated plates were incubated at 28 °C for up to 5 days. The effects of different temperatures on growth were assessed on TSA plates supplemented with 1% (w/v) NaCl incubated at 4, 10, 15, 28, 30, 32, 37, 42 and 45 °C. Nitrate reduction was assessed in nitrate broth, prepared according to the method of Cowan & Steel (1974), incubated at room temperature for 10 days. Oxidase and catalase activities were determined by using standard methods. The culture was characterized biochemically using the API Coryne, API 50CH and API

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Corynebacterium marinum* D7015^T is DQ219354.

A representative phase-contrast micrograph of cells of strain D7015^T (Fig. S1) and cellular fatty acid profiles of strain D7015^T and related species (Table S1) are available with the online version of this paper.

ZYM systems according to the manufacturer's instructions (bioMérieux). Turbidity measurements were used to evaluate growth of strain D7015^T in the API 50CH and the API 20NE systems. API 50CH strips were read after 7 days incubation at 28 °C.

The almost-complete 16S rRNA gene sequence (1444 nt) of strain D7015^T was obtained using the universal primers 27f and 1492r (MWG Biotech; Lane, 1991). The 16S rRNA gene sequence of strain D7015^T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. A phylogenetic dendrogram of strain D7015^T and some closely related members of the genus *Corynebacterium* based on 16S rRNA gene similarities was constructed using the neighbour-joining method of the MEGA software version 4.1 (Tamura *et al.*, 2007). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates.

Cellular fatty acids were determined on a 3-day-old culture grown on MA plates after incubation at 28 °C. Fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990). The DNA G+C content was determined directly by HPLC as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA–DNA hybridization between strain D7015^T and *Corynebacterium halotolerans* DSM 44683^T was performed by applying the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) under optimal hybridization conditions.

Microscopic examination of strain D7015^T suggested that it belonged to the genus *Corynebacterium* as cells stained Gram-positive and produced short, diphtheroid rods with some of the cells arranged in a V formation due to their snapping division (Collins & Cummins, 1986). A representative phase-contrast micrograph of cells of strain D7015^T is available as Supplementary Fig. S1 (in IJSEM

Online). Strain D7015^T was non-motile and non-endospore-forming. The isolate was catalase-positive and oxidase-negative. The following results of carbon source assimilation were positive in API 50CH: aesculin ferric citrate, salicin, maltose and glycogen. Strain D7015^T displayed a numerical profile of 3200127 with the commercial API Coryne system, which corresponded to a 'doubtful' identification as *Corynebacterium glucuronolyticum* (with a confidence level of 99.2 %). However, further biochemical analyses, using API ZYM and additional phenotypic tests, revealed that strain D7015^T could be distinguished from *C. glucuronolyticum* on the basis of its ability to produce α -chymotrypsin and its inability to produce esterase lipase (C4). Strain D7015^T produced acid from glucose, maltose, sucrose and glycogen, but not from ribose, xylose, mannitol or lactose. Strain D7015^T also differed from *C. spheniscorum* notably in the inability of the latter to reduce nitrate, or produce β -glucuronidase, α -chymotrypsin or naphthol-AS-BI-phosphohydrolase. The complete morphological and biochemical characteristics of strain D7015^T are given in the species description.

Phylogenetic analyses performed with nearly complete sequences of members of closely related species (Fig. 1) showed that no sequence available in the GenBank database exhibited more than 96 % similarity. The sequence similarity observed between the isolate and its closest relative, *C. halotolerans* DSM 44683^T, was 95.9 %, which is a lower value than the borderline used for defining bacterial species (i.e. 97 %) as proposed by Stackebrandt & Goebel (1994). *C. halotolerans* was isolated from a saline soil sample in China (Chen *et al.*, 2004). Growth of strain D7015^T was not observed in 10 % (w/v) NaCl; however, optimum growth of *C. halotolerans* was in 10 % (w/v) KCl, NaCl or MgCl₂ · 6H₂O. These two strains were also different biochemically, with strain D7015^T able to hydrolyse starch and Tweens 20–80, but unable to produce esterase lipase (C4). The DNA–DNA

Fig. 1. Neighbour-joining phylogeny of *Corynebacterium marinum* D7015^T and closely related strains. Numbers along branches represent bootstrap values; only values >50 % are shown. GenBank accession numbers for the 16S rRNA sequences used in the tree reconstruction are given in parentheses. Bar, 0.01 substitutions per nucleotide.

Table 1. Characteristics of strain D7015^T and phylogenetically related species of the genus *Corynebacterium*

Strains: 1, D7015^T, 2, *Corynebacterium efficiens* DSM 44549^T (data from Fudou *et al.*, 2002); 3, *Corynebacterium halotolerans* DSM 44683^T (Chen *et al.*, 2004). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3
Starch hydrolysis	+	ND	–
Esterase lipase (C4)	–	ND	+
Growth at 45 °C	–	+	ND
Growth in 10 % (w/v) NaCl	–	+	+
Acid production from:			
Ribose	–	+	–
Maltose	+	+	–
Glycogen	+	–	–
DNA G + C content (mol%)	65.0	59.0–60.2	63.0

relatedness value of 20.4 ± 0.1 % (experiment repeated twice) between strain D7015^T and *C. halotolerans* DSM 44683^T was significantly lower than 70 %, which is considered to be the threshold value for the delineation of genomic species (Wayne *et al.*, 1987). Clearly, strain D7015^T differed biochemically from all of its closest relatives, both in its ability to hydrolyse casein and in its inability to produce esterase lipase (C4). The phenotypic features that differentiate strain D7015^T from its closest phylogenetic relatives are provided in Table 1.

The cellular fatty acids of strain D7015^T were C_{18:1}ω9c (56.18 %), C_{16:0} (38.02 %), C_{16:1}ω7c (4.45 %), C_{18:0} (1.0 %) and C_{14:0} (0.35 %); whole-cell fatty acid compositions of strain D7015^T and related species are available as Supplementary Table S1 in IJSEM Online. The genomic DNA G + C content was 65.0 mol%.

Based on these molecular, chemotaxonomic and phenotypic results, it is proposed that strain D7015^T should be classified as a representative of a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium marinum* sp. nov. is proposed.

Description of *Corynebacterium marinum* sp. nov.

Corynebacterium marinum (ma.ri'num. L. neut. adj. *marinum* of the sea, marine).

Cells are short, Gram-positive, non-motile, diphtheroid rods; some cells are arranged in a V formation. Colonies on MA medium are circular, erose, convex, yellow, of a creamy consistency and 0.5–1.5 mm in diameter after 48 h at 28 °C. Facultatively anaerobic, catalase-positive and oxidase-negative. Negative for the methyl red test and positive for the Voges–Proskauer test. Reduces nitrate and lyses horse blood cells. Aesculin and urea are not hydrolysed, but casein is digested and starch and pullulan are hydrolysed. Gelatin is not liquefied. Tweens 20–80 are hydrolysed. Cells grow in the presence of 0–8 % (w/v) NaCl

and at 4–37 °C. Prolific growth occurs at 30–32 °C in media that contain 1 % (w/v) NaCl. Using the API 50CH system, aesculin ferric citrate, salicin, maltose and glycogen are utilized. Acid is produced from glucose, maltose, sucrose and glycogen, but not from ribose, xylose, mannitol or lactose. Positive for esterase lipase (C8), leucine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, pyrazinamidase and β-glucuronidase activities. Negative for esterase lipase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, alkaline phosphatase, pyrrolidonyl arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Using antibiotic discs, resistant to (μg per disc) nalidixic acid (30), nitrofurantoin (50), sulphamethizole (200), tetracycline (100) and cotrimoxazole (25), but sensitive to ampicillin (25), chloramphenicol (50), gentamycin (10), kanamycin (30), carbenicillin (100) and streptomycin (25). Major fatty acids are C_{18:1}ω9c, C_{16:0}, C_{16:1}ω7c, C_{18:0} and C_{14:0}.

The type strain is D7015^T (=CGMCC 1.6998^T=NRRL B-24779^T), isolated from coastal sediment close to a coal-fired power station in Qingdao, China. The genomic DNA G + C content of the type strain is 65.0 mol%.

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