

Corynebacterium marinum sp. nov. isolated from coastal sediment
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1 ***Corynebacterium marinum* sp. nov. isolated from coastal sediment in Qingdao,**
2 **China**

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15 **Running title:** *Corynebacterium marinum* sp. nov.

16

17 The GenBank accession number for the 16S rRNA gene sequence of *Corynebacterium marinum*

18 D7015^T is DQ219354. A representative phase-contrast micrograph of strain D7015^T and a table

19 showing the cellular fatty acid profiles of strain D7015^T and related species are available as

20 supplementary material in IJSEM Online.

21 A taxonomic study was performed on strain D7015^T, which was isolated from coastal sediment
22 close to a coal-fired power station in Qingdao, China. Strain D7015^T comprised Gram-positive,
23 non-motile diphtheroid rods, which grew in the presence of 0-8% (w/v) NaCl and at 4-37°C, with
24 optimum growth at 1% (w/v) NaCl and 30-32°C. The G+C content was 65.0 mol%. The major fatty
25 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%).
26 On the basis of the morphological, physiological and phylogenetic characteristics, strain D7015^T
27 was classified in the genus *Corynebacterium*. It exhibited a 16S rRNA gene sequence similarity of
28 95.9% and a DNA:DNA relatedness value of 20.4% with *Corynebacterium halotolerans* DSM
29 44683^T. Strain D7015^T was sufficiently different from hitherto described *Corynebacterium* species
30 to be considered as a novel species. The name *Corynebacterium marinum* sp. nov. is proposed, with
31 strain D7015^T (=CGMCC 1.6998^T =NRRL B-24779^T) as the type strain.

32
33 The genus *Corynebacterium* was proposed by Lehmann & Neumann (1896) and represents a large
34 group of Gram-positive, asporogenous, rod-shaped bacteria with high DNA G+C content.
35 Corynebacteria have been isolated from a wide range of environments, namely dairy products, soil,
36 sewage, sediments, plant materials and aquatic sources, but the majority of novel species described
37 in recent years have originated from human or animal clinical samples (e.g. Renaud *et al.*, 2007;
38 Yassin & Siering, 2008; Yassin, 2009; Funke *et al.*, 2009). However, some corynebacterial species
39 have been isolated from the marine environment, and they may occur as part of the indigenous flora
40 of marine animals. For example, *Corynebacterium phocae* and *Corynebacterium caspium* were
41 isolated from seals (Pascual *et al.*, 1998; Collins *et al.*, 2004), *Corynebacterium spheniscorum* and
42 *Corynebacterium sphenisci* were recovered from wild penguins (Goyache *et al.*, 2003a, 2003b),
43 and *Corynebacterium maris* Coryn-1^T was found in the mucus of the coral, *Fungia granulose*
44 (Ben-Dov *et al.*, 2009). Here, we report the taxonomic characteristics of a novel *Corynebacterium*
45 species that originated from coastal sediment close to a coal-fired power station in Qingdao, China.

46

47 Strain D7015^T was isolated from coastal sediment in 2000 (Du *et al.*, 2002). The isolate was grown
48 aerobically at 28°C on marine 2216E agar (MA; Difco) for 48 h. Cultures were maintained on MA
49 slants at room temperature, and stock cultures were kept in tryptone soya broth (Oxoid)
50 supplemented with 1% (w/v) NaCl (= TNB) and 20% (v/v) glycerol at -70°C. Identification of
51 strain D7015^T was performed as described by Jordan *et al.* (2007). For phenotypic tests, the strain
52 was grown on MA for 48 h at 28°C, and cells were resuspended in saline for use as an inoculum.
53 Tolerance of 1, 3, 5, 7, 8 and 10% (w/v) NaCl was assessed on appropriately modified tryptone
54 soya agar (TSA; Oxoid). Growth in the absence of NaCl was assessed on plate count agar (PCA;
55 Oxoid). Inoculated plates were incubated at 28°C for up to 5 days. The effects of different
56 temperatures on growth were assessed on TSA plates supplemented with 1.0% (w/v) NaCl (TNA)
57 with incubation at 4, 10, 15, 28, 30, 32, 37, 42 and 45°C. The reduction of nitrate was assessed in
58 nitrate broth, prepared according to the method of Cowan & Steel (1974), and incubated at room
59 temperature for 10 days. Oxidase and catalase activities were determined by using standard
60 methods. The culture was characterized biochemically using the API Coryne, API 50CH and API
61 ZYM systems according to the manufacturer's instructions (bioMérieux). Measuring turbidity was
62 used to evaluate the growth of strain D7015^T in the API 50CH and the API 20NE systems. The API
63 50 CH strips were read after 7 days incubation at 28°C.

64

65 The almost-complete 16S rRNA gene sequence (1444 nt) of strain D7015^T was obtained using the
66 universal primers 27f and 1492r (MWG Biotech; Lane, 1991). The 16S rRNA gene sequence of
67 strain D7015^T was submitted to GenBank and EMBL to search for similar sequences using the
68 BLAST algorithm. A phylogenetic dendrogram of strain D7015^T and some closely related
69 members of the genus *Corynebacterium* based on 16S rRNA gene homology was constructed using
70 the neighbor-joining method of the MEGA software version 4.1 (Tamura *et al.*, 2007). The

71 resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates.

72

73 Cellular fatty acids were determined on a 3 day old culture grown on marine 2216E agar plates after
74 incubation at 28°C. The fatty acids were extracted, methylated and analyzed using the standard
75 MIDI (Microbial Identification) system (Sasser, 1990). The G+C content of the DNA was
76 determined directly by high pressure liquid chromatography according to a method described
77 previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA:DNA hybridization between
78 strain D7015^T and *Corynebacterium halotolerans* DSM 44683^T was carried out by applying the
79 optical renaturation method (De Ley *et al.*, 1970; Huss *et al.*, 1983; Jahnke, 1992) under optimal
80 hybridization conditions.

81

82 Microscopic examination of strain D7015^T suggested that it belonged to the genus
83 *Corynebacterium*, as cells stained Gram-positive and produced short, diphtheroid rods with some
84 of the cells arranged in a V formation due to their snapping division (Collins & Cummins, 1986). A
85 representative phase-contrast micrograph of strain D7015^T is available as supplementary material
86 in IJSEM Online. Strain D7015^T was non-motile and non-endospore-forming. The isolate was
87 catalase-positive and oxidase-negative. The following results of carbon source assimilation were
88 positive in API 50CH: aesculin ferric citrate, salicin, D-maltose and glycogen. Strain D7015^T
89 displayed a numerical profile of 3200127 with the commercial API Coryne system, which
90 corresponded to a “doubtful” identification as *Corynebacterium glucuronolyticum* (with a
91 confidence level of 99.2%). However, further biochemical analyses, using API ZYM and
92 additional phenotypic tests revealed that strain D7015^T could be distinguished from *C.*
93 *glucuronolyticum* on the basis of its ability to produce α -chymotrypsin and its inability to produce
94 esterase lipase (C4). Strain D7015^T produced acid from glucose, maltose, sucrose and glycogen,
95 but not from ribose, xylose, mannitol and lactose. Strain D7015^T was also different from *C.*

96 *spheniscorum* notably in the inability of the latter to reduce nitrate, or produce β -glucuronidase, α -
97 chymotrypsin or naphthol-AS-BI-phosphohydrolase. The complete morphological and
98 biochemical characteristics for strain D7015^T are given in the species description.

99

100 Phylogenetic analyses performed with nearly complete sequences of members of closely related
101 species (Fig. 1) showed that no sequence available in the GenBank database exhibited more than
102 96% similarity. The sequence similarity observed between the new isolate and its closest relative,
103 *C. halotolerans*, was 95.9%, which is a lower value than the borderline used for defining bacterial
104 species (i.e. 97%) as proposed by Stackebrandt & Goebel (1994). *C. halotolerans* was isolated
105 from a saline soil sample in China (Chen *et al.*, 2004). For strain D7015^T, growth was not observed
106 at 10% (w/v) NaCl. However, optimum growth of *C. halotolerans* was in 10% (w/v) KCl, NaCl or
107 MgCl₂·6H₂O. These two strains were also different biochemically, with strain D7015^T able to
108 hydrolyse starch and Tween 20-80, but unable to produce esterase lipase (C4). The DNA:DNA
109 relatedness value of $20.4 \pm 0.1\%$ (experiment repeated twice) was significantly lower than 70%,
110 which is considered to be the threshold value for the delineation of genomic species (Wayne *et al.*,
111 1987). Clearly, strain D7015^T differed biochemically from all of its closest relatives, both in its
112 ability to hydrolyse casein and in its inability to produce esterase lipase C4. The phenotypic
113 features that differentiate strain D7015^T from its closest phylogenetic relatives are provided in
114 Table 1.

115

116 The cellular fatty acids in strain D7015^T were C_{18:1}ω9c (56.18%), C_{16:0} (38.02%), C_{16:1}ω7c (4.45%),
117 C_{18:0} (1.0%) and C_{14:0} (0.35%). The genomic DNA G+C content was 65.0 mol%.

118

119 Based on these molecular, chemotaxonomic and phenotypic results, it is proposed that strain
120 D7015^T should be classified as a new species of the genus *Corynebacterium*, for which the name

121 *Corynebacterium marinum* sp. nov. is proposed.

122

123 **Description of *Corynebacterium marinum* sp. nov.**

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

125

126 Cells are short Gram-positive, non-motile, diphtheroid rods; some of the cells are arranged in a V
127 formation. Colonies on marine 2216E agar medium are circular, erose, convex, yellow and of a
128 creamy consistency and are 0.5-1.5 mm in diameter after 48 h at 28°C. Facultatively anaerobic,
129 catalase-positive and oxidase-negative. Bacteria are methyl-red negative, Voges-Proskauer
130 positive, reduce nitrate, and lyse horse blood cells. Aesculin and urea are not hydrolysed, but casein
131 is digested and starch and pullulan are hydrolysed. Gelatin is not liquefied. Tween 20-80 are
132 hydrolysed. Cells grow in the presence of 0-8% (w/v) NaCl and at 4-37°C. Prolific growth occurs
133 at 30-32°C in media that contain 1% (w/v) NaCl. Using the API 50CH system, aesculin ferric
134 citrate, salicin, D-maltose, and glycogen are utilized. Acid is produced from glucose, maltose,
135 sucrose and glycogen, but not from ribose, xylose, mannitol or lactose. Activities for esterase lipase
136 (C8), leucine arylamidase, α -chymotrypsin, naphthol-as-bi-phosphohydrolase, pyrazinamidase and
137 β -glucuronidase are positive. Esterase lipase (C4), lipase (C14), valine arylamidase, cystine
138 arylamidase, trypsin, alkaline phosphatase, pyrrolidonyl arylamidase, acid phosphatase,
139 α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase,
140 α -mannosidase and α -fucosidase are negative. The type strain is resistant to nalidixic acid (30 μ g),
141 nitrofurantoin (50 μ g), sulphamethizole (200 μ g), tetracycline (100 μ g) and cotrimoxazole (25 μ g),
142 but sensitive to ampicillin (25 μ g), chloramphenicol (50 μ g), gentamycin (10 μ g), kanamycin (30
143 μ g), carbenicillin (100 μ g) and streptomycin (25 μ g) as determined by antibiotic discs. Major fatty
144 acids produced are C_{18:1 ω 9c} (56.18%), C_{16:0} (38.02%), C_{16:1 ω 7c} (4.45%), C_{18:0} (1.0%) and C_{14:0}
145 (0.35%). The percentage whole-cell fatty acid compositions of strain D7015^T and related species is

146 available as supplementary material in IJSEM Online. The genomic DNA G+C content is 65.0
147 mol% for the type strain.

148

149 The type strain is D7015^T (=CGMCC 1.6998^T = NRRL B-24779^T), which was isolated from coastal
150 sediment close to a coal-fired power station in Qingdao, China.

151

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158

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228

229 **Table 1.** Comparison of strain D7015^T with the phylogenetically related species of the genus
 230 *Corynebacterium*
 231 Species: 1, Strain D7015^T, 2, *Corynebacterium efficiens* DSM 44549^T (Fudou *et al.*, 2002); 3,
 232 *Corynebacterium halotolerans* DSM 44683^T (Chen *et al.*, 2004). +, positive; -, negative; ND, no
 233 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 produced from:			
ribose	-	+	-
maltose	+	+	-
glycogen	+	-	-
DNA G+C content (mol%)	65.0	59.0-60.2	63.0

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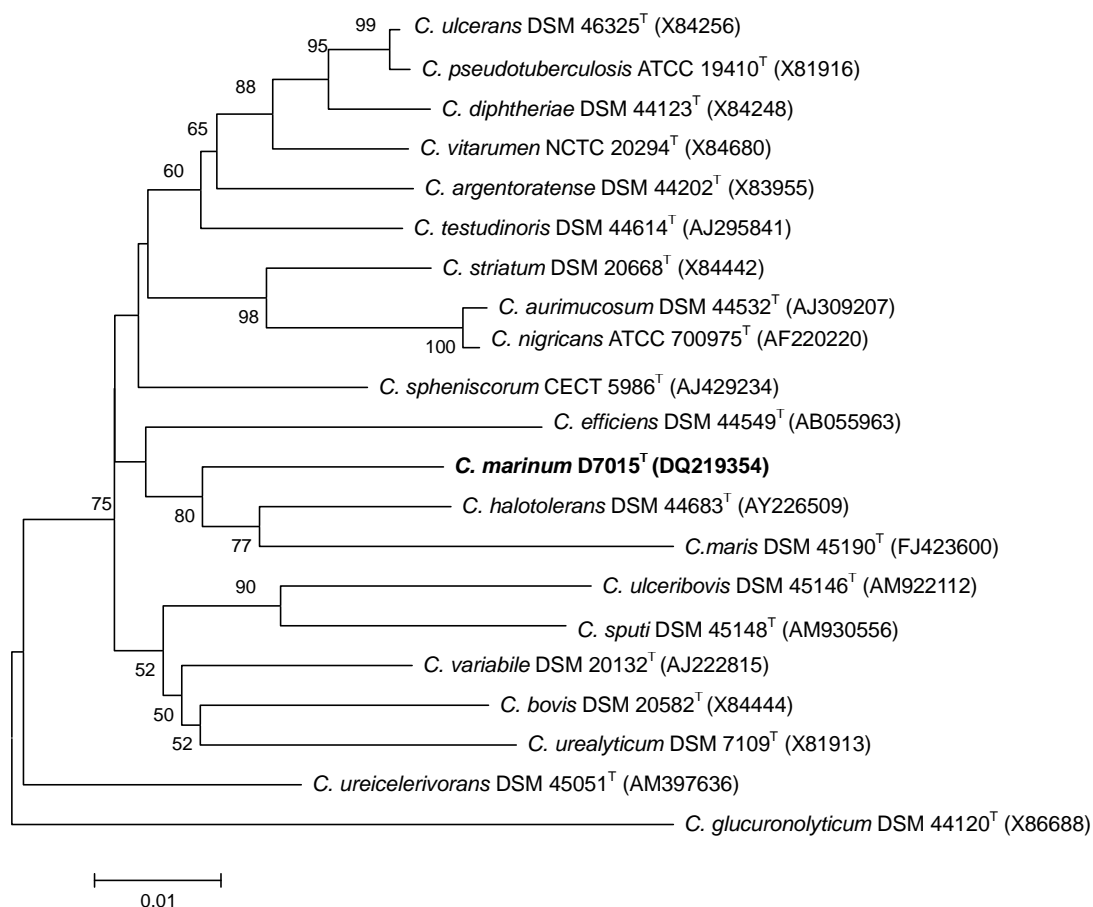
245 **Figure legend:**

246 **Fig. 1.** Neighbor-joining phylogeny of *Corynebacterium marinum* and closely related species.

247 Numbers along branches represent bootstrap values; only values greater than 50% are shown.

248 GenBank accession numbers for the 16S rRNA sequences used in the tree reconstruction are given
249 in the parentheses next to each taxon. Bar, 0.01 substitutions per nucleotide.

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