Corynebacterium halotolerans sp. nov., isolated from saline soil in the west of China

Hua-Hong Chen,1,2 Wen-Jun Li,1 Shu-Kun Tang,1 Reiner M. Kroppenstedt,3 Erko Stackebrandt,3 Li-Hua Xu1 and Cheng-Lin Jiang1

1The Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China
2Department of Chemistry, Chuxiong Normal College, Chuxiong, Yunnan 675000, PR China
3DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

A halotolerant, non-spore-forming actinobacterium was isolated from a soil sample from the west of China. The strain, designated YIM 70093T (=CCTCC AA 001024T = DSM 44683T), comprised Gram-positive, non-motile, diphtheroid and irregular rods. It grew in 0–25 % KCl (KCl could be substituted by NaCl or MgCl2.6H2O), with optimum growth at 10 % KCl, and its optimal pH and cultivation temperature were 7·2 and 28 °C, respectively. On the basis of its morphological, physiological and phylogenetic characteristics, strain YIM 70093T should be classified in the genus Corynebacterium. However, it is sufficiently different from hitherto described Corynebacterium species to be considered as a novel species, for which the name Corynebacterium halotolerans sp. nov. is proposed.

The genus Corynebacterium was created by Lehmann & Neumann (1896) and represents a large group of Gram-positive, asporogenous, rod-shaped bacteria with a high DNA G+C content (Collins & Cummins, 1986; Liebl, 1992). In recent years, many novel Corynebacterium species have been described, the majority of which were isolated from clinical samples or animals. Some non-clinical species of the genus Corynebacterium originated from soil or plant materials. Here, we report the taxonomic characteristics of a novel Corynebacterium species that originated from saline soil in the west of China.

Strain YIM 70093T was isolated from a saline soil sample that was collected in Xinjiang Province, China. Modified glycerol/asparagine agar (ISP 5), which contained [(1 distilled water)−1] 1·0 g L-asparagine, 10 g glycerol, 5 g yeast extract, 5 g KNO3, 1·0 g K2HPO4, 150 g KCl and 1 ml trace element solution, was used for enrichment and isolation. The strain was cultivated aerobically at 28 °C for 2–3 days. Cells for biochemical and molecular systematic analyses were grown in shaken flasks (about 150 r.p.m.) of modified ISP 5 medium broth at 28 °C for 1 week. Stock cultures were maintained at 4 °C, using modified ISP 5 agar slants that contained 10 % KCl, and as glycerol suspensions (20 %, v/v) at −20 °C.

Amino acid and sugar analyses of whole-cell hydrolysates followed procedures described by Staneck & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin et al., 1984). Menaquinoines were isolated by using the methods of Minnikin et al. (1984) and separated by HPLC (Kroppenstedt et al., 1981; Kroppenstedt, 1982). Fatty acid methyl esters and mycolic acid trimethylsilyl

---

Published online ahead of print on 7 November 2003 as DOI 10.1099/ijs.0.02919-0
esters were prepared and analysed as described previously (Klatte et al., 1994), using the standard Microbial Identification system (MIDI Inc.) for automated GC analyses (Sasser, 1990).

Genomic DNA was isolated and purified by the method of Marmur (1961). The DNA G+C content of strain YIM 70093T was measured by the thermal denaturation method (Marmur & Doty, 1962).

16S rRNA genes of the isolates were amplified by PCR using conserved primers close to the 3’ and 5’ ends of the gene, as described previously (Cui et al., 2001). Multiple alignments with sequences of a broad selection of actinobacteria and calculations of sequence similarity levels were carried out by using CLUSTAL X (Thompson et al., 1997). A phylogenetic tree was reconstructed from K$_{sub}$ values (Kimura, 1980, 1983) by using the neighbour-joining method of Saitou & Nei (1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Cells of strain YIM 70093T were aerobic, Gram-positive, non-motile, non-spore-forming, diphtheroid and irregular rods. Colonies on modified ISP 5 medium were moderately yellow, circular, entire, somewhat convex, opaque and approximately 0·5 mm in diameter after 24 h at 28°C, while those on trypticase/soy agar medium and Mueller–Hinton agar medium differed from the former only in their diameter. Strain YIM 70093T grew in modified ISP 5 medium with 0–25 % KCl, NaCl or MgCl$_2$.6H$_2$O. The isolate was catalase-positive and oxidase-negative. Urease, tyrosinase and Tween esterase activities were negative; nitrate reduction was positive, whilst nitrite reduction was negative. The carbon utilization range was wide, as the isolate could utilize most carbon sources that were tested.

The enzymic profile, obtained after 3 days incubation with API ZYM strips, was as follows: lipase and $\beta$-glucuronidase activities were positive and ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, $\alpha$- and $\beta$-galactosidase, N-acetyl-$\beta$-glucosaminidase and $\beta$-glucosidase activities were negative.

The optimum pH, cultivation temperature and NaCl, KCl and MgCl$_2$.6H$_2$O concentrations for growth were 7·2, 28°C and 10%, respectively.

Cell walls of strain YIM 70093T contained meso-diaminopimelic acid. Whole-cell hydrolysates contained mainly galactose and arabinose. Menaquinones were MK-8(H$_2$) (35·5 %) and MK-9(H$_2$) (64·5 %). Polar lipid extract contained diphasphatidylglycerol, phosphatidylglycerol, phosphatidylglycositol, glycolipid and phosphatidylinositol mannosides. Predominant cellular fatty acids were C$_{14:0}$ (7·3 %), cis-9-C$_{16:1}$ (9·8 %), C$_{16:0}$ (42·1%), cis-9-C$_{18:1}$ (28·9 %), C$_{18:1}$ (4·5 %) and 10-methyl C$_{18:0}$ (7·4 %). Short-chain mycolic acids (C$_{32}$–C$_{34}$) were present; predominant mycolic acids were C$_{32:0}$ (36·0 %), C$_{34:0}$ (20·8 %), C$_{34:1}$ (25·1 %), C$_{36:0}$ (3·6 %), C$_{36:1}$ (8·4 %) and C$_{36:2}$ (5·1 %). The DNA G+C content of strain YIM 70093T was 63 mol%.

To determine the phylogenetic position of the unknown bacterium, the 16S rRNA gene was amplified by PCR. An almost-complete 16S rDNA sequence (1492 bp) was obtained and subjected to comparative analysis. Members of the genus Corynebacterium were closest phylogenetic neighbours (Fig. 1). Binary similarity values ranged between 93·5 % (Corynebacterium callunae CCUG 28793T) and 95·8 % (Corynebacterium xerosis DSM 20743T); no sequence similarity of >97 % was obtained with any member of the genus Corynebacterium.

The genus Corynebacterium embraces a very diverse range of organisms; over 50 different species are currently assigned to the genus. It is, however, recognized that the genus is not monophyletic and actually comprises several distinct rDNA lineages. It is evident from the present 16S rDNA study that strain YIM 70093T forms a distinct subclade with C. xerosis DSM 20743T and Corynebacterium freneyi CIP 106767T within the phylogenetic tree. High sequence divergence values (>4·2 %) with other members of this genus clearly indicate that the isolate represents a novel

![Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain YIM 70093T among its phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of Rhodococcus equi DSM 20307T (GenBank no. X80614) was used as root. Bar, 1 % sequence divergence.](image-url)
species. Although there is no precise correlation between 16S rDNA divergence values and species delineation, it is generally recognized that organisms that display sequence divergence values of $\geq 3\%$ do not belong to the same species (Stackebrandt & Goebel, 1994). The sequence divergence of $>3\%$ that was observed between the unknown actinobacterium and Corynebacterium species with validly published names is therefore consistent with separate species status. Support for the distinctiveness of the unknown actinobacterium also comes from phenotypic evidence, when compared to the species C. xerosis (DSM 20743$^T$) and C. freneyi (CIP 106767$^T$) of the genus Corynebacterium (Table 1). The isolate originated from saline soil and has high tolerance to KCl, NaCl and MgCl$_2$.6H$_2$O (0–25 %, w/v) in ISP 5 medium. $\beta$-Glucuronidase activity of strain YIM 70093$^T$ is positive and $\alpha$-glucosidase activity is negative, whereas the two most closely related species show the opposite characteristics (Table 1). In addition, the unknown bacterium has different types and amounts of fatty and mycolic acids from these two species (data not shown). Therefore, based on phenotypic and molecular genetic results, it is evident that the unknown Corynebacterium strain described above should be classified as a member of a novel species of the genus Corynebacterium, for which we propose the name Corynebacterium halotolerans sp. nov.

### Description of Corynebacterium halotolerans sp. nov.

Corynebacterium halotolerans (ha.lo.to’le.rans. Gr. n. halos salt; L. part. adj. tolerans tolerating; N.L. pres. part. halotolerans referring to the ability to tolerate high salt concentrations).

Cells are aerobic, Gram-positive, non-motile, non-spore-forming, diphtheroid and irregular rods. Colonies on modified ISP 5 medium, trypticase/soy agar medium and Mueller–Hinton agar medium are moderately yellow, circular, entire, somewhat convex, opaque and 0.5–1.5 mm in diameter after 24 h at 28°C. Optimum growth temperature is 28°C. Optimum growth concentration of KCl, NaCl and MgCl$_2$.6H$_2$O is 10 %. Positive for nitrate reduction, but negative for milk peptonization and coagulation, gelatin liquefaction, growth in cellulose, production of H$_2$S and melain, starch hydrolysis and urease production. Activities for lipase and $\beta$-glucuronidase are positive. Ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, $\alpha$- and $\beta$-galactosidase, N-acetyl-$\beta$-glucosaminidase and $\beta$-glucosidase activities are negative. The following substrates are utilized: glucose, galactose, sucrose, arabinose, mannose, mannotol, maltose, starch, xylose, ribose, salicin and dextrin. Cellobiose, fructose, amygdalin and lactose are not utilized. Acid production occurs only from glucose. Cell wall contains meso-diaminopimelic acid. Whole-cell hydrolysates contain mainly galactose and arabinose. Menaquinones are MK-8(H$_2$) (35.5 %) and MK-9(H$_2$) (64.5 %); phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, glycolipid and phosphatidylinositol mannosides. Major cellular fatty acids are C$_{16}$ : 0 (7.3 %), cis-9-C$_{16}$ : 1 (9.8 %), C$_{16}$ : 0 (42.1 %), cis-9, C$_{18}$ : 1 (28.9 %), C$_{18}$ : 0 (4.5 %) and 10-methyl C$_{18}$ : 0 (7.4 %). Predominant mycolic acids are C$_{32}$ : 0 (36.0 %), C$_{34}$ : 0 (20.8 %), C$_{34}$ : 1 (25.1 %), C$_{36}$ : 0 (3.6 %), C$_{36}$ : 1 (8.4 %) and C$_{36}$ : 2 (5.1 %). DNA G+C content is 63 mol%.

The type strain is YIM 70093$^T$ (=CCTCC AA 001024$^T$ = DSM 44683$^T$). Isolated from saline soil collected in Xinjiang Province, west China.

### Acknowledgements

This research was supported by the National Natural Science Foundation of China (project no. 30270004), Yunnan Provincial Natural Science Foundation (project no. 20001C001Q) and Yunnan Education Commission Foundation (project nos 01111134 and 02QI 077).

### References


