Corynebacterium timonense sp. nov. and *Corynebacterium massiliense* sp. nov., isolated from human blood and human articular hip fluid

Vicky Merhej,¹ Enevold Falsen,² Didier Raoult¹ and Véronique Roux¹

Correspondence

Véronique Roux

vroux91@hotmail.com

¹Laboratoire de Bactériologie – Virologie, Hôpital de la Timone, URMITE CNRS-IRD UMR6236, 264 rue Saint-Pierre, 13385 Marseille, Cedex 05, France

²Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Guldhedsg.10, S-413 46 Göteborg, Sweden

Gram-positive, facultatively anaerobic, rod-shaped bacteria were isolated from the blood of a patient with endocarditis (strain 5401744^T) and from the hip joint fluid of a patient with an infected orthopaedic prosthesis (strain 5402485^T). These strains were characterized by using a polyphasic taxonomic approach. Based on cellular morphology and biochemical criteria the two isolates were tentatively assigned to the genus *Corynebacterium*, although they did not correspond to any recognized species. The predominant fatty acids were a mix of C_{18:2}ω6,9c and anteiso-C_{18:0} (32.1 % of the total), C_{16:0} (26.3 %) and C_{18:1}ω9c (22.5 %) for strain 5402485^T and C_{18:1}ω9c (36.4 %), C_{17:1}ω9c (27.1 %) and C_{16:0} (10.9 %) for strain 5401744^T.

Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain 5401744^T was closely related to the type strains of *Corynebacterium auris*, *Corynebacterium capitovis*, *Corynebacterium lipophiloflavum* and *Corynebacterium mycetoides* (97.0, 96.6, 96.5 and 96.3 % similarity, respectively) and strain 5402485^T was closely related to the type strains of *Corynebacterium macginleyi*, *Corynebacterium accolens*, *Corynebacterium tuberculostearicum*, *Corynebacterium confusum*, *Corynebacterium mastitidis* and *Corynebacterium renale* (95.6, 95.3, 95.3, 94.5, 94.0 and 93.5 %, respectively). On the basis of phenotypic data and phylogenetic inference, these isolates are considered to represent two novel species of the genus *Corynebacterium*, for which the names *Corynebacterium timonense* sp. nov. (type strain, 5401744^T=CSUR P20^T=CIP 109424^T=CCUG 53856^T) and *Corynebacterium massiliense* sp. nov. (type strain, 5402485^T=CSUR P19^T=CIP 109423^T=CCUG 53857^T) are proposed.

The genus *Corynebacterium* is one of the largest genera in the coryneform group of bacteria, which consists of irregularly shaped, Gram-positive, strictly aerobic or facultatively anaerobic, asporogenous rods. Among this group, phenotypic identification at the species level on the basis of biochemical criteria remains problematic because of a lack of characteristic traits. The combination of chemotaxonomic markers (Collins *et al.*, 1982; Von Graevenitz *et al.*, 1994) and molecular approaches based on 16S rRNA and *rpoB* gene sequence analyses has

improved the identification of members of the genus *Corynebacterium* (Khamis *et al.*, 2004, 2005; Pascual *et al.*, 1995; Roux *et al.*, 2004; Ruimy *et al.*, 1995; Tang *et al.*, 2000). At the time of writing, the genus comprised 70 recognized species, most of which have been described since 2000. These *Corynebacterium* species have been isolated from human clinical sources (Feurer *et al.*, 2004; Funke *et al.*, 1995, 1997a, b, 1998; Riegel *et al.*, 2006; Yassin, 2007), animal sources (Collins *et al.*, 2001, 2004; Fernández-Garayzábal *et al.*, 2004; Vela *et al.*, 2003), and soil and vegetables (Chen *et al.*, 2004; Fudou *et al.*, 2002). In the present report, we describe two novel species of the genus *Corynebacterium* that were isolated from human blood and human articular hip fluid.

In May 2005, a 56-year-old man with a history of infective endocarditis underwent aortic and mitral valve replacement. Following surgery he developed bradycardia that required a pacemaker implant. Two months later, the patient was admitted to the emergency unit of the Timone

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains 5401744^T and 5402485^T are EF217055 and EF217056, respectively; those for the *rpoB* gene sequence of strains 5401744^T and 5402485^T are EF217058 and EF217057, respectively.

Electron micrographs of cells of strains 5401744^T and 5402485^T and neighbour-joining phylogenetic trees showing the position of strains 5401744^T and 5402485^T among representatives of the genus *Corynebacterium* inferred from 16S rRNA and *rpoB* gene sequence comparisons are available as supplementary material with the online version of this paper.

Hospital. He presented with persistent fever, elevated erythrocyte sedimentation rate (62 mm h^{-1}) and elevated C-reactive protein (200 mg l^{-1}). His white blood cell count was $10\,500 \text{ ml}^{-1}$. Gram-positive coryneform bacteria (representative strain 5401744^T) were isolated from five of six blood cultures (three samples obtained on the first day of hospitalization and three others obtained on the second day). The patient was treated with vancomycin (2 g per day) and gentamicin (200 mg per day) intravenously and recovered.

In September 2005, an 84-year-old man who had previously undergone arthroplasty of his left hip presented with left knee pain and intermittent fever at Marseilles Hopital-Nord emergency unit. Synovial fluid was sampled and sent to the laboratory for bacterial culture. It contained many neutrophils without crystals. Gram-positive bacilli (representative strain 5402485^T) were isolated and identified as representing *Corynebacterium* sp. The patient was treated with antibiotics (amoxicillin, 3 g per day; rifampicin, 900 mg per day) administered orally three times a day for 6 months.

In both cases no phenotypic identification of the bacteria was obtained. Genotypic identification based on 16S rRNA gene sequence comparisons suggested that strains 5401744^T and 5402485^T represent two novel species of the genus *Corynebacterium*.

Minimum inhibitory concentrations were determined according to the disc diffusion method, as there is, to our knowledge, no international guidelines for the antimicrobial susceptibility testing of coryneform bacteria. The two novel strains were susceptible to amoxicillin, doxycycline, rifampicin, vancomycin, trimethoprim-sulfamethoxazole, gentamicin, ofloxacin and clindamycin. Strain 5402485^T was susceptible to erythromycin whereas strain 5401744^T was resistant.

Surface colonies on sheep blood agar (bioMérieux) in 5% CO₂ were circular, 1–2 mm in diameter, smooth, shiny and yellow for strain 5401744^T after 24 h incubation, and 0.5–1 mm in diameter, circular, glistening and greyish for strain 5402485^T after 48 h incubation. After 24 h incubation the colonies were not haemolytic. Growth was tested under anaerobic and microaerophilic atmospheres, which were created by using a GENbag anaer and GENbag microaer (bioMérieux), respectively, and in presence of air or 5% CO₂. Growth temperature was tested by incubation at 25, 30, 37, 44 and 50 °C. The bacteria grew in aerobic and anaerobic atmospheres. Optimum growth was observed at 37 °C but occurred between 25 and 50 °C for strain 5401744^T and between 30 and 44 °C for strain 5402485^T. The size and ultrastructure of cells were determined by using transmission electron microscopy. Cells were grown in liquid trypticase soy broth (TSB; Becton Dickinson) medium for 48 h, collected by pipetting and stained with 1% (w/v) phosphotungstic acid. Samples were examined on a Morgagni 268D (Philips) electron microscope at an operating voltage of 60 kV. Cells of strains 5401744^T and 5402485^T were Gram-positive, non-

motile, non-spore-forming rods, 0.6–2.1 µm long and 0.4–0.6 µm wide, and 0.2–1.8 µm long and 0.3–0.7 µm wide, respectively (see Supplementary Fig. S1 in IJSEM Online).

Catalase activity was determined by using the ID colour catalase test kit (bioMérieux). Oxidase activity was assayed by applying the cells to moistened discs that were impregnated with dimethyl-*p*-phenylene diamine (bioMérieux). Strains 5401744^T and 5402485^T were catalase-positive but oxidase-negative. The novel strains were characterized biochemically by using API Coryne and API ZYM strips (bioMérieux) according to the manufacturer's instructions. Phenotypic characteristics were compared with those of *Corynebacterium auris* CIP 104632^T, *Corynebacterium lipophiloflavum* CIP 105127^T, *Corynebacterium mycetoides* CIP 55.51^T, *Corynebacterium capitovis* CIP 106739^T, *Corynebacterium casei* CIP 107182^T, *Corynebacterium ammoniagenes* CIP 101283^T, *Corynebacterium confusum* CIP 105403^T, *Corynebacterium macginleyi* CIP 104099^T, *Corynebacterium accolens* CIP 104783^T, *Corynebacterium tuberculostearicum* CIP 107291^T and *Corynebacterium renale* CIP 103421^T, which were the most closely related species based on 16S rRNA and *rpoB* gene sequence comparisons (see below). Characteristic traits are presented in Table 1. Results of other tests for the novel strains are given in the species descriptions below.

Preparation and determination of cellular fatty acids were carried out according to the procedures given for the Sherlock Microbial Identification System (MIDI) except that the database and software package used to obtain a comparison of a new profile with one from the database were developed by the Culture Collection, University of Göteborg (CCUG). The cellular fatty acid profiles of strains 5401744^T and 5402485^T are detailed in Table 2.

Bacterial DNA was extracted by using the Magna Pure LC DNA isolation kit III (Roche) with a Magna Pure LC instrument, as described by the manufacturer. PCR amplification of the 16S rRNA and *rpoB* genes was performed by using the universal primer pair fd1 and rp2 (Weisburg *et al.*, 1991) and the primer pair cory2700f and cory3130r (Khamis *et al.*, 2004), respectively. PCR products were purified by using MultiScreen PCR (Millipore) and sequencing reactions were carried out by using a DNA sequencing kit (BigDye Terminator Cycle Sequencing v1.1 Ready Reactions; PE Biosystems) according to the manufacturer's instructions. Sequencing products were purified and electrophoresis was performed with a 3100 Genetic Analyzer (Applied Biosystem). Gene sequences were aligned via the multisequence alignment program CLUSTAL_X (v. 1.8) (Thompson *et al.*, 1997). Phylogenetic relationships with closely related species were determined by using MEGA version 4 (Tamura *et al.*, 2007). Distance matrices were determined following the assumptions described by Kimura (1980) and were used to elaborate the dendrogram according to the neighbour-joining method (Saitou & Nei, 1987). The maximum-parsimony algorithm was also used to infer phylogenetic analysis. Bootstrap analysis (bootstrap values were

Table 1. Differential phenotypic characteristics between strains 5401744^T and 5402485^T and related species of the genus *Corynebacterium*

Strains: 1, *C. auris* CIP 104632^T; 2, *C. lipophiloflavum* CIP 105127^T; 3, *C. mycetoides* CIP 55.51^T; 4, *C. capitovis* CIP 106739^T; 5, 5401744^T (*C. timonense* sp. nov.); 6, *C. cystitidis* CIP 103424^T; 7, *C. confusum* CIP 105403^T; 8, *C. macginleyi* CIP 104099^T; 9, *C. accolens* CIP 104783^T; 10, *C. tuberculostearicum* CIP 107291^T; 11, *C. casei* CIP 107182^T; 12, *C. ammoniogenes* CIP 101283^T; 13, 5402485^T (*C. massiliense* sp. nov.); 14, *C. renale* CIP 103421^T; 15, *C. mastitidis* CECT 4843^T. w, Weakly positive; +, positive; –, negative; v, variable.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15*
Nitrate reduction	–	–	–	–	–	–	+	+	+	w	+	+	–	–	–
Aesculin hydrolysis	+	–	–	–	w	–	–	–	–	–	–	+	–	–	–
Acid production from:															
Glucose	–	–	+	+	+	+	+	+	+	+	+	+	–	+	–
Ribose	–	–	–	–	+	+	+	+	+	+	+	+	–	+	–
Xylose	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Maltose	–	–	+	–	–	–	–	–	+	+	–	–	–	–	–
Sucrose	–	–	+	–	–	–	–	+	–	+	–	–	–	–	–
Enzyme activities (API Coryne)															
Pyrazinamidase	+	+	+	–	+	+	+	–	–	+	–	+	w	+	+
Pyrrolidonyl arylamidase	–	–	w	–	–	–	–	–	+	–	+	–	–	–	–
Urease	–	–	–	–	–	+	–	–	–	–	–	+	–	+	v
Enzyme activities (API ZYM)															
Alkaline phosphatase	+	+	+	+	+	+	+	+	–	w	+	–	w	–	+
Esterase (C4)	+	+	+	w	+	+	+	+	w	+	+	–	w	+	+
Esterase lipase (C8)	+	+	+	+	+	+	+	+	+	+	+	+	w	+	+
Lipase (C14)	+	w	w	w	w	–	+	w	+	–	+	–	–	–	+
Leucine arylamidase	+	+	+	+	+	+	+	w	+	+	+	+	+	w	+
Valine arylamidase	w	–	+	–	+	–	–	–	–	w	–	–	–	–	+
Cystine arylamidase	+	–	+	–	–	+	w	–	–	w	+	–	–	–	+
Trypsin	+	–	+	–	–	–	–	w	w	–	–	–	–	–	–
Acid phosphatase	+	–	w	+	+	+	w	+	–	w	+	–	–	–	+

*Results from Fernandez-Garayzabal *et al.* (1997).

obtained for a consensus tree based on 1000 randomly generated trees) was performed to investigate the stability of the trees obtained. Levels of 16S rRNA gene sequence similarity were determined by using BioEdit software. Levels of similarity between strain 5401744^T and the type strains of *C. auris*, *C. capitovis*, *C. lipophiloflavum* and *C. mycetoides* were 97.0, 96.6, 96.5 and 96.3 %, respectively; those between strain 5402485^T and the type strains of *C. macginleyi*, *C. accolens*, *C. tuberculostearicum*, *C. confusum*, *C. mastitidis* and *C. renale* were 95.6, 95.3, 95.3, 94.5, 94.0 and 93.5 %, respectively. 16S rRNA gene sequence similarity between strains 5401744^T and 5402485^T was 94.2 %. All the above values are ≤ 97 %, confirming that strains 5401744^T and 5402485^T represent two novel species of the genus *Corynebacterium* (Stackebrandt *et al.*, 2002). Phylogenetic analysis inferred from 16S rRNA gene and *rpoB* gene sequence comparisons according to the neighbour-joining and maximum-parsimony methods showed that strain 5401744^T grouped with *C. auris* in a cluster also including *C. capitovis*, *C. mycetoides* and *C. lipophiloflavum* (Figs 1 and 2). Phylogenetic analysis inferred from 16S rRNA gene sequence comparisons according to the neighbour-joining method showed that strain 5402485^T grouped with *C. confusum* in a cluster also including *C.*

tuberculostearicum, *C. accolens* and *C. macginleyi* (Fig. 1). In the maximum-parsimony tree, strain 5402485^T grouped with *C. renale* and *C. mastitidis* in a cluster also including *C. tuberculostearicum*, *C. accolens*, *C. macginleyi* and *C. confusum* (data not shown). On the basis of *rpoB* gene sequence comparisons, strain 5402485^T formed a distinct subline within the genus *Corynebacterium* and clustered with *C. casei* and *C. ammoniogenes* (Fig. 2). None of the bootstrap values found were representative of stable nodes (all were < 95 %). The phylogenetic analyses based on 16S rRNA and *rpoB* gene sequence comparisons gave different clusterings for *C. massiliense*. Comparisons based on biochemical characteristics or fatty acid patterns (Table 1) did not allow us to determine the best phylogenetic approach.

Based on both phylogenetic and phenotypic evidence, strain 5401744^T isolated from human blood culture and strain 5402485^T isolated from articular hip fluid are considered to represent separate novel species of the genus *Corynebacterium*, for which the names *Corynebacterium timonense* sp. nov. and *Corynebacterium massiliense* sp. nov. are proposed, respectively.

Two other strains of *C. massiliense* sp. nov. (CCUG 49336 and CCUG 49589) and one other strain of *C. timonense* sp.

Table 2. Whole-cell fatty acid compositions (% of total) of strains 5401744^T and 5402485^T and related species of the genus *Corynebacterium*

Strains: 1, 5401744^T (*C. timonense* sp. nov.); 2, *C. auris* CIP 104632^T; 3, *C. lipophiloflavum* CIP 105127^T; 4, *C. mycetoides* CIP 55.51^T; 5, *C. capitovis* CIP 106739^T; 6, 5402485^T (*C. massiliense* sp. nov.); 7, *C. cystitidis* CIP 103424^T; 8, *C. confusum* CIP 105403^T; 9, *C. macginleyi* CIP 104099^T; 10, *C. accolens* CIP 104783^T; 11, *C. tuberculostearicum* CIP 107291^T; 12, *C. ammoniogenes* CIP 101283^T; 13, *C. renale* 103421^T; 14, *C. mastitidis* CIP 105509^T. —, Not detected.

Fatty acid	1*	2*	3*	4*	5†	6*	7*	8*	9*	10*	11*	12*	13*	14*
C _{14:0}	—	0.5	—	—	—	1.2	—	—	1.9	—	—	0.4	—	1.6
C _{15:1ω8c}	—	2.3	—	—	—	—	—	—	—	—	—	—	—	—
C _{16:1ω11c}	2.4	—	—	—	—	—	—	—	—	—	—	—	—	—
C _{16:1ω9c}	—	0.8	—	3.2	3.0	—	—	1.8	—	—	—	—	—	—
C _{16:1ω7c}	—	—	—	—	—	2.5	—	—	3.8	—	—	—	—	2.3
C _{16:0}	10.9	11.5	19.5	20.0	23.0	26.3	40.6	34.0	16.0	34.7	8.6	24.6	32.7	26.5
iso-C _{17:1ω5c}	—	0.4	—	—	—	—	—	—	—	—	—	—	—	—
anteiso-C _{17:0}	3.3	3.2	—	—	—	—	—	—	—	—	3.8	—	—	0.4
C _{17:1ω9c}	27.1	—	—	—	—	—	—	—	—	—	16.4	1.4	18.0	—
C _{17:1ω8c}	—	27.8	—	5.5	—	—	—	—	—	—	—	—	—	0.5
C _{17:0}	—	13.3	15.6	8.3	—	1.6	—	—	3.0	—	0.8	—	—	—
C _{18:2ω6,9c/anteiso-C_{18:0}}	4.4	1.7	9.2	—	—	32.1	3.8	3.0	5.6	24.5	5.9	0.6	5.5	16.6
C _{18:1ω9c}	36.4	22.1	39.4	56.1	67.8	22.5	50.8	56.3	49.5	27.7	58.7	39.7	40.2	28.1
C _{18:0}	6.1	5.2	16.5	6.8	6.1	11.0	4.9	4.9	7.3	13.2	3.7	3.9	3.8	13.5
10-Methyl C _{18:0} (TSBA)‡	—	—	—	—	—	2.7	—	—	—	—	1.3	2.3	—	—
C _{18:0} 2-OH	—	—	—	—	—	—	—	—	—	—	—	—	—	1.4
C _{20:4ω6,9,12,15c}	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1
C _{18:0} 3-OH	—	—	—	—	—	—	—	—	—	—	—	—	—	1.9
Unidentified	9.4	11.4	—	—	—	—	—	—	12.7	—	0.8	27.3	—	5.8

*Result obtained from CCUG.

†Result obtained from Collins *et al.* (2001).

‡TSBA, Tuberculostearic acid.

nov. (CCUG 47998) were deposited in the Culture Collection of the University of Göteborg. The biochemical characteristics and fatty acid profiles of strains CCUG 49336 and CCUG 49589 were similar to those of strain 5402485^T. 16S rRNA (605 bp) and *rpoB* (366 bp) gene sequence comparisons revealed 100 % similarity for all three isolates of the species. The biochemical characteristics of strain CCUG 47998 were similar to those of strain 5401744^T. The fatty acid profiles of the two strains were different (Table 3) but 16S rRNA (702 bp) and *rpoB* (406 bp) gene sequence comparisons revealed 100 % similarity.

Description of *Corynebacterium timonense* sp. nov.

Corynebacterium timonense (ti.mo.nen'se. N.L. neut. adj. *timonense* from the name Hôpital de la Timone, the name of the hospital in Marseille, France, where the type strain was isolated).

Cells are Gram-positive, non-spore-forming and non-motile. They are typically club-shaped rods that occur as single cells, in pairs or in small clusters. The rods are 0.6–2.1 µm in length and 0.4–0.6 µm in diameter after 48 h growth in TSB medium (as determined by electron

microscopy). After 24 h growth on sheep blood agar at 37 °C, surface colonies are circular, yellow, shiny and 1–2 mm in diameter. Capable of aerobic and anaerobic growth. Temperature range for growth is 25–50 °C (optimum, 37 °C). Catalase-positive. With the API Coryne system, acid is produced from D-glucose and D-ribose, but not from D-xylose, D-mannitol, maltose, D-lactose, sucrose or glycogen. Nitrate is not reduced. Aesculin is weakly hydrolysed but urea and gelatin are not hydrolysed. With API Coryne and API ZYM strips, positive for pyrazinamidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14) (weakly), leucine arylamidase, valine arylamidase and acid phosphatase, but negative for pyrrolidonyl arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The fatty acid profile is characterized by the predominance of C_{18:1ω9c}, C_{17:1ω9c}, C_{16:0} and C_{18:0}. Tuberculostearic acid is not detected.

The type strain, 5401744^T (=CSUR P20^T=CIP 109424^T=CCUG 53856^T), was isolated from human blood. CCUG 47998 is a second strain of the species.

Fig. 1. Phylogenetic tree showing the positions of strains 5401744^T and 5402485^T among representatives of the genus *Corynebacterium* inferred from 16S rRNA gene sequence comparisons (1274 nt fragment) according to the neighbour-joining method. Numbers at nodes are bootstrap percentages based on 1000 resamplings. The sequence of the type strain of *Mycobacterium smegmatis* was used as the outgroup (AB305022; not shown). Bar, 0.005 changes per nucleotide position. An extended tree containing a wider selection of reference sequences is available in Supplementary Fig. S2 in IJSEM Online. Asterisks indicate nodes that were conserved when using the maximum-parsimony method.

Fig. 2. Phylogenetic trees showing the positions of strains 5401744^T and 5402485^T among representatives of the genus *Corynebacterium* inferred from *rpoB* gene sequence comparisons (406 nt fragment) according to the neighbour-joining method. Numbers at nodes are bootstrap percentages based on 1000 resamplings that support the topology shown. The sequence of the type strain of *Mycobacterium smegmatis* was used as the outgroup (CP000480; not shown). Bar, 0.02 changes per nucleotide position. An extended tree containing a wider selection of reference sequences is available in Supplementary Fig. S3 in IJSEM Online. Asterisks indicate nodes that were conserved when using the maximum-parsimony method.

Table 3. Whole-cell fatty acid compositions (% of the total) of strains 5401744^T and CCUG 47998

–, Not detected.

Fatty acid	5401744 ^T	CCUG 47998
C _{14:0}	–	0.7
Unknown	4.0	–
C _{15:0}	–	8.0
C _{16:1} ω11c	2.4	0.5
C _{16:0}	10.9	21.4
anteiso-C _{17:0}	3.3	2.8
C _{17:1} ω9c	27.1	4.1
Unknown	5.4	–
C _{17:0}	–	4.0
C _{18:2} ω6,9c/anteiso-C _{18:0}	4.4	10.3
C _{18:1} ω9c	36.4	36.2
C _{18:0}	6.1	12.1

Description of *Corynebacterium massiliense* sp. nov.

Corynebacterium massiliense (mas.si.li.en'se. L. neut. adj. *massiliense* of Massilia, the old Roman name for Marseille, from where the type strain was isolated).

Cells are Gram-positive, non-spore-forming and non-motile. They are typically club-shaped rods that occur as single cells, in pairs or in small clusters. The rods are 0.2–1.8 µm in length and 0.3–0.7 µm in diameter after 48 h growth in TSB medium (as determined by electron microscopy). After 48 h growth on sheep blood agar, surface colonies are circular, greyish, glistening and 0.5–1 mm in diameter. Capable of aerobic growth but only weak anaerobic and microaerophilic growth. Temperature range for growth is 30–44 °C (optimum, 37 °C). Catalase-positive. With the API Coryne system, acid is not produced from D-glucose, D-ribose, D-xylose, D-mannitol, maltose, D-lactose, sucrose or glycogen. Nitrate is not reduced. Urea, gelatin and aesculin are not hydrolysed. With API Coryne and API ZYM strips, positive for leucine arylamidase, weakly positive for pyrazinamidase, alkaline phosphatase, esterase (C4) and esterase lipase (C8), but negative for pyrrolidonyl arylamidase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The fatty acid profile is characterized by the predominance of a mixture of C_{18:2}ω6,9c and anteiso-C_{18:0}, C_{16:0}, C_{18:1}ω9c and C_{18:0}. Tuberculostearic acid is detected for strains 5402485^T and CCUG 49589).

The type strain, 5402485^T (=CSUR P19^T=CIP 109423^T=CCUG 53857^T), was isolated from human hip joint fluid. CCUG 49336 and CCUG 49589 are additional strains of the species.

Acknowledgements

We are grateful to Bernard Campagna and Nicolas Aldrovendi for their technical assistance with electron microscopy and to Kent Molin (University of Göteborg, Sweden) for his technical assistance in cellular fatty acid analysis.

References

- Chen, H.-H., Li, W.-J., Tang, S.-K., Kroppenstedt, R. M., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2004). *Corynebacterium halotolerans* sp. nov., isolated from saline soil in the west of China. *Int J Syst Evol Microbiol* **54**, 779–782.
- Collins, M. D., Goodfellow, M. & Minnikin, D. E. (1982). A survey of the structures of mycolic acids in *Corynebacterium* and related taxa. *J Gen Microbiol* **128**, 129–149.
- Collins, M. D., Hoyles, L., Foster, G., Sjoden, B. & Falsen, E. (2001). *Corynebacterium capitolis* sp. nov., from a sheep. *Int J Syst Evol Microbiol* **51**, 857–860.
- Collins, M. D., Hoyles, L., Foster, G. & Falsen, E. (2004). *Corynebacterium caspium* sp. nov., from a Caspian seal (*Phoca caspica*). *Int J Syst Evol Microbiol* **54**, 925–928.
- Fernandez-Garayzabal, J. F., Collins, M. D., Hutson, R. A., Fernandez, E., Monasterio, R., Marco, J. & Dominguez, L. (1997). *Corynebacterium mastitidis* sp. nov., isolated from milk of sheep with subclinical mastitis. *Int J Syst Bacteriol* **47**, 1082–1085.
- Fernández-Garayzábal, J. F., Vela, A. I., Egido, R., Hutson, R. A., Lanzarot, M. P., Fernández-García, M. & Collins, M. D. (2004). *Corynebacterium ciconiae* sp. nov., isolated from the trachea of black storks (*Ciconia nigra*). *Int J Syst Evol Microbiol* **54**, 2191–2195.
- Feurer, C., Clermont, D., Bimet, F., Candrea, A., Jackson, M., Glaser, P., Bizet, C. & Dauga, C. (2004). Taxonomic characterization of nine strains isolated from clinical and environmental specimens, and proposal of *Corynebacterium tuberculostearicum* sp. nov. *Int J Syst Evol Microbiol* **54**, 1055–1061.
- Fudou, R., Jojima, Y., Seto, A., Yamada, K., Kimura, E., Nakamatsu, T., Hiraishi, A. & Yamanaka, S. (2002). *Corynebacterium efficiens* sp. nov., a glutamic-acid-producing species from soil and vegetables. *Int J Syst Evol Microbiol* **52**, 1127–1131.
- Funke, G., Lawson, P. A. & Collins, M. D. (1995). Heterogeneity within human-derived centers for disease control and prevention (CDC) coryneform group ANF-1-like bacteria and description of *Corynebacterium auris* sp. nov. *Int J Syst Bacteriol* **45**, 735–739.
- Funke, G., Hutson, R. A., Hillergermann, M., Heizmann, W. R. & Collins, M. D. (1997a). *Corynebacterium lipophiloflavum* sp. nov. isolated from a patient with bacterial vaginosis. *FEMS Microbiol Lett* **150**, 219–224.
- Funke, G., Lawson, P. A. & Collins, M. D. (1997b). *Corynebacterium mucifaciens* sp. nov., an unusual species from human clinical material. *Int J Syst Bacteriol* **47**, 952–957.
- Funke, G., Osorio, C. R., Frei, R., Riegel, P. & Collins, M. D. (1998). *Corynebacterium confusum* sp. nov., isolated from human clinical specimens. *Int J Syst Bacteriol* **48**, 1291–1296.
- Khamis, A., Raoult, D. & La Scola, B. (2004). *rpoB* gene sequencing for identification of *Corynebacterium* species. *J Clin Microbiol* **42**, 3925–3931.
- Khamis, A., Raoult, D. & La Scola, B. (2005). Comparison between *rpoB* and 16S rRNA gene sequencing for molecular identification of 168 clinical isolates of *Corynebacterium*. *J Clin Microbiol* **43**, 1934–1936.

- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Pascual, C., Lawson, P. A., Farrow, J. A., Gimenez, M. N. & Collins, M. D. (1995).** Phylogenetic analysis of the genus *Corynebacterium* based on 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 724–728.
- Riegel, P., Creti, R., Mattei, R., Nieri, A. & von Hunolstein, C. (2006).** Isolation of *Corynebacterium tuscaniae* sp. nov. from blood cultures of a patient with endocarditis. *J Clin Microbiol* **44**, 307–312.
- Roux, V., Drancourt, M., Stein, A., Riegel, P., Raoult, D. & La Scola, B. (2004).** *Corynebacterium* species isolated from bone and joint infections identified by 16S rRNA gene sequence analysis. *J Clin Microbiol* **42**, 2231–2233.
- Ruimy, R., Riegel, P., Boiron, P., Monteil, H. & Christen, R. (1995).** Phylogeny of the genus *Corynebacterium* deduced from analyses of small-subunit ribosomal DNA sequences. *Int J Syst Bacteriol* **45**, 740–746.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rossello-Mora, R., Swings, J. & other authors (2002).** Report of the *ad hoc* committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Tang, Y. W., Von Graevenitz, A., Waddington, M. G., Hopkins, M. K., Smith, D. H., Li, H., Kolbert, C. P., Montgomery, S. O. & Persing, D. H. (2000).** Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. *J Clin Microbiol* **38**, 1676–1678.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Vela, A. I., Mateos, A., Collins, M. D., Briones, V., Hutson, R. A., Dominguez, L. & Fernandez-Garayzabal, J. F. (2003).** *Corynebacterium suicordis* sp. nov., from pigs. *Int J Syst Evol Microbiol* **53**, 2027–2031.
- Von Graevenitz, A., Pünter, V., Gruner, E., Pfyffer, G. E. & Funke, G. (1994).** Identification of coryneform and other gram-positive rods with several methods. *APMIS* **102**, 381–389.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Yassin, A. F. (2007).** *Corynebacterium ureicelerivorans* sp. nov., a lipophilic bacterium isolated from blood culture. *Int J Syst Evol Microbiol* **57**, 1200–1203.