# *Corynebacterium phoceense* sp. nov., strain MC1$^T$ a new bacterial species isolated from human urine

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## Abstract

*Corynebacterium phoceense* strain MC1 (= CSUR P1905 = DSM 100570) is a novel *Corynebacterium* species isolated from the urine of a kidney transplant recipient as a part of a culturomics study. *Corynebacterium phoceense* is a Gram-positive, sporogenous, strictly aerobic, and nonmotile coccobacillus. Here we describe strain MC1 and provide its complete annotated genome sequence according to the taxonogenomics concept. Its genome is 2 793 568 bp long and contains 2575 protein-coding genes and 67 RNA genes, including eight rRNA genes.



**Keywords:** *Corynebacterium phoceense*, culturomics, human urine, kidney transplant recipient, taxonogenomics
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## Introduction

The genus *Corynebacterium* was described in 1896 by Lehmann and Neumann and belongs to the *Actinobacteria* class [1]. Currently it consists of a heterogeneous group of Gram-positive, non-spore-forming, rod-shaped bacteria with a high DNA G+C content [2]. In the genus *Corynebacterium* many species are involved in human and animal diseases, whereas many others are normal flora on skin and mucous membranes [3–5]. Corynebacteria are found in different environments such as water, soil, plants and human samples [6–10]. Among corynebacteria, the most significant human pathogen is *Corynebacterium diphtheriae,* which causes diphtheria worldwide [11]. However, most corynebacteria are opportunistic pathogens [6]. Bacteria found in urine are occasionally associated with urinary infection [12,13].

Currently bacterial classification is focused on a polyphasic approach with phenotypic and genotypic characteristics such as DNA-DNA hybridization, G+C content and 16S rRNA sequence similarity [14–16]. This classification system has its limitations, such as the high cost of the DNA-DNA hybridization technique and its low reproducibility [14,17]. A new bacterial description concept was developed in our laboratory [18–22] with the recent development of genome sequencing technology [23]. This concept, taxonogenomics [24], is a combination of proteomic description and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profile [25] associated with a phenotypic description and the sequencing, annotation and comparison of the complete genome of the new bacteria species [26].

Here we describe *Corynebacterium phoceense* sp. nov., strain MC1 (= CSUR P1905 = DSM 100570), according the taxonogenomics concept.

## Material and methods

### Organism information

*Corynebacterium phoceense* was isolated from culture of a midstream urine specimen from a 25-year-old kidney transplant



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**TABLE 1. Classification and general features of *Corynebacterium phoceense* strain MC1$^T$**

| Property | Term |
|---|---|
| Current classification | Domain: *Bacteria*<br>Phylum: *Actinobacteria*<br>Class: *Actinobacteria*<br>Order: *Actinomycetales*<br>Family: *Corynebacteriaceae*<br>Genus: *Corynebacterium*<br>Species: *Corynebacterium phoceense*<br>Type strain: MC1 |
| Gram stain | Positive |
| Cell shape | Rod |
| Motility | Nonmotile |
| Sporulation | Non–spore forming |
| Temperature range | Mesophilic |
| Optimum temperature | 37°C |

recipient from Comoros. He underwent transplantation to treat faecal and segmental glomerulosclerosis in Marseille, France. A urine sample was collected 2 years after his kidney transplantation without clinical signs of urinary tract infection. The patient did not receive antibiotics at the time of urine collection. Informed consent was obtained from the patient, and the study was approved by the Institut Fédératif de Recherche 48, Faculty of Medicine, Marseille, France, under agreement 09-022.

### Strain identification by MALDI-TOF MS and 16S rRNA sequencing

Among the 18 culture conditions previously selected by culturomics [27], strain MC1 grew on sheep's blood–enriched Colombia agar (bioMérieux, Marcy l'Etoile, France). The colonies were obtained by spreading samples on a solid medium. They were then purified by subculture and identified by MALDI-TOF MS [28,29]. Colonies were deposited in duplicate on a MTP 96 MALDI-TOF MS target plate (Bruker Daltonics, Leipzig, Germany), which was analysed with a Microflex spectrometer (Bruker). The 12 spectra obtained were matched against the references of the 7567 bacteria contained in the database by standard pattern matching (with default parameter settings), with MALDI BioTyper database software 2.0 (Bruker). An identification score over 1.9 with a validated species allows identification at the species level, and a score under 1.7 does not enable any identification. When identification by MALDI-TOF MS failed, the 16S rRNA was sequenced [30]. Stackebrandt and Ebers [31] suggest similarity levels of 98.7% with the 16S rRNA sequence as a threshold to define a new species without performing DNA-DNA hybridization.

### Growth conditions

To establish our strain's optimal growth conditions, different temperatures (25, 28, 37, 45 and 56°C) and atmospheres (aerobic, microaerophilic and anaerobic) were tested. GENbag anaer and GENbag microaer systems (bioMérieux) were used respectively to test anaerobic and microaerophilic growth. Aerobic growth was carried out with and without 5% $CO_2$.

### Morphologic, biochemical and antibiotic susceptibility testing

Gram staining, motility, catalase, oxidase and sporulation were tested as previously described [27]. To perform a biochemical description, according to the manufacturer's instructions, we use API Coryne (bioMérieux) to identify coryneiforms, API ZYM (bioMérieux) to search enzymatic activities and API 50CH (bioMérieux) to estimate capacity to ferment different carbohydrates. Cellular fatty acid methyl ester (FAME) analysis was performed by gas chromatography/mass spectrometry (GC/MS). Two samples were prepared with approximately 60 mg of bacterial biomass per tube collected from several culture plates. FAMEs were prepared as previously described (http://www.midi-inc.com/pdf/MIS_Technote_101.pdf), and GC/MS analyses were carried out as previously described [32]. Briefly, FAMEs were separated using an Elite 5-MS column and monitored by mass spectrometry (Clarus 500-SQ 8 S; Perkin Elmer, Courtaboeuf, France). A spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (NIST, Gaithersburg, MD, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK).

Antibiotic susceptibility testing was performed using the disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2015 recommendations (http://www.eucast.org/). *Corynebacterium phoceense* resistance and susceptibility are estimated with 22 antibiotic treatments (vancomycin 30 µg, rifampicin 30 µg, doxycycline 30 IU, erythromycin 15 IU, amoxicillin 25 µg, nitrofurantoin 300 µg, gentamicin 15 µg, ciprofloxacin 5 µg, ceftriaxone 30 µg, amoxicillin 20 µg + clavulanic acid 10 µg, penicillin G 10 µg, gentamicin 500 µg, trimethoprim 1.25 µg + sulfamethoxazole 23.75 µg, oxacillin 5 µg, imipenem 10 µg, tobramycin 10 g, metronidazole 4 µg, amikacin 30 µg, linezolid 30 µg, clindamycin 15 µg, daptomycin in stripe 0.016–256 µg (bioMérieux) and chloramphenicol 5 mg, (Sigma-Aldrich, St. Louis, MO, USA)). The bacterial suspension (0.5 McFarland standard) is made in 2 mL NaCl 0.85% medium. Petri dishes with Mueller-Hinton + 5% sheep's blood (Becton Dickinson, San Diego, CA, USA) are seeded by swabbing with bacteria suspension. Different antibiotic dishes (SirScan) are dispensed on petri dishes. Electron microscopy figure was obtained by performing a negative staining of strain MC1. Detection Formvar-coated grids were deposited on a 40 µL bacterial suspension drop, then incubated at 37°C for 30 minutes and on ammonium molybdate 1% for 10 seconds. The dried grids on

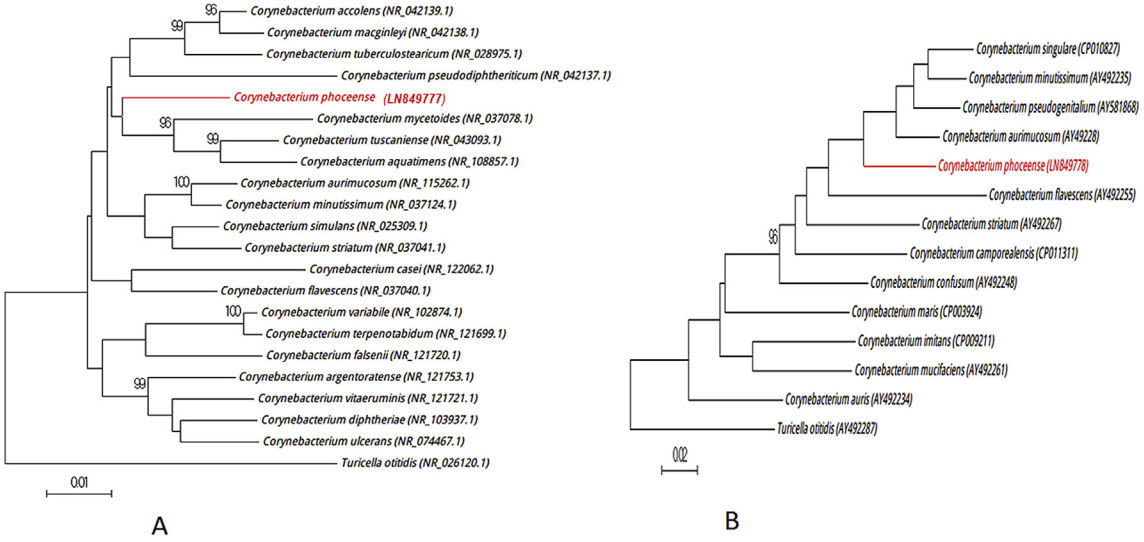


FIG. 1. Phylogenetic trees highlighting position of *Corynebacterium phoceense* strain MC1 (=CSUR P1905 = DSM 100570) relative to other strains within genus *Corynebacterium*. Sequences of 16S rRNA gene (A) and *rpoB* genes (B) were aligned by CLUSTALW. Scale bar represents 1 and 2% nucleotide sequence divergence for (A) and (B) respectively.

blotted paper were observed with a Tecnai G20 transmission electron microscope (FEI Company, Limeil-Brevannes, France).

### Growth conditions and genomic DNA preparation

*Corynebacterium phoceense* strain MC1 (= CSUR P1905 = DSM 100570) was grown on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C in aerobic atmosphere. Bacteria grown on three petri dishes were collected and resuspended in 4 × 100 µL of Tris-EDTA (TE) buffer. Then 200 µL of this suspension was diluted in 1 mL TE buffer for lysis treatment that included a 30-minute incubation with 2.5 µg/µL lysozyme at 37°C, followed by an overnight incubation with 20 µg/µL

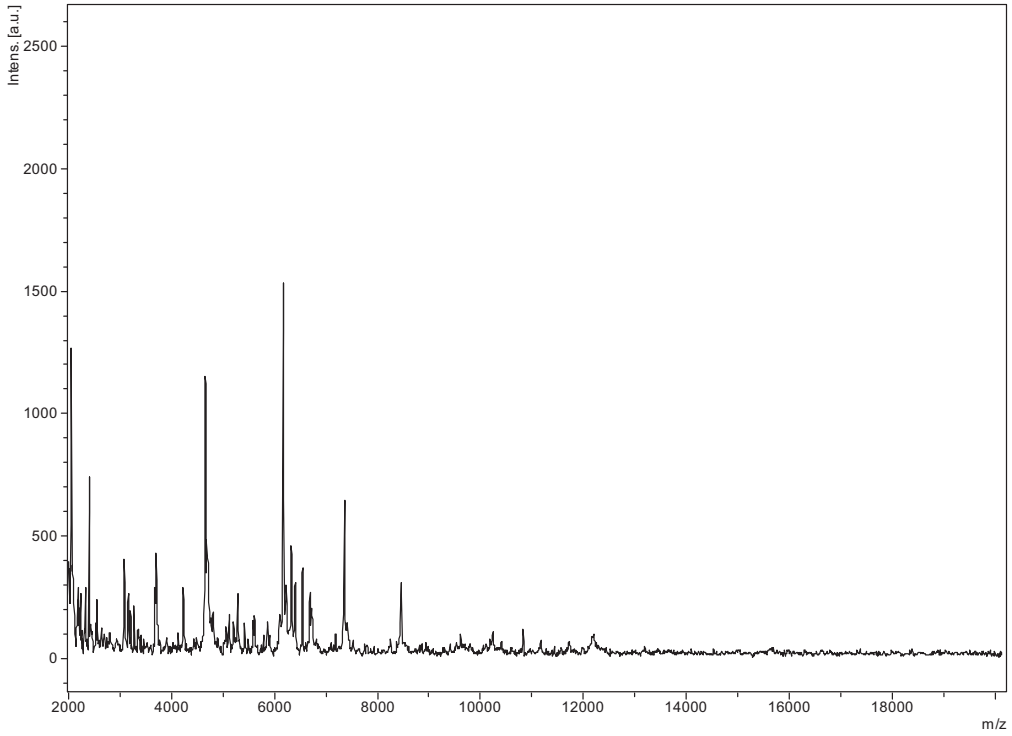


FIG. 2. Reference mass spectrum from *Corynebacterium phoceense* strain MC1. Spectra from 12 individual colonies were compared and reference spectrum was generated.

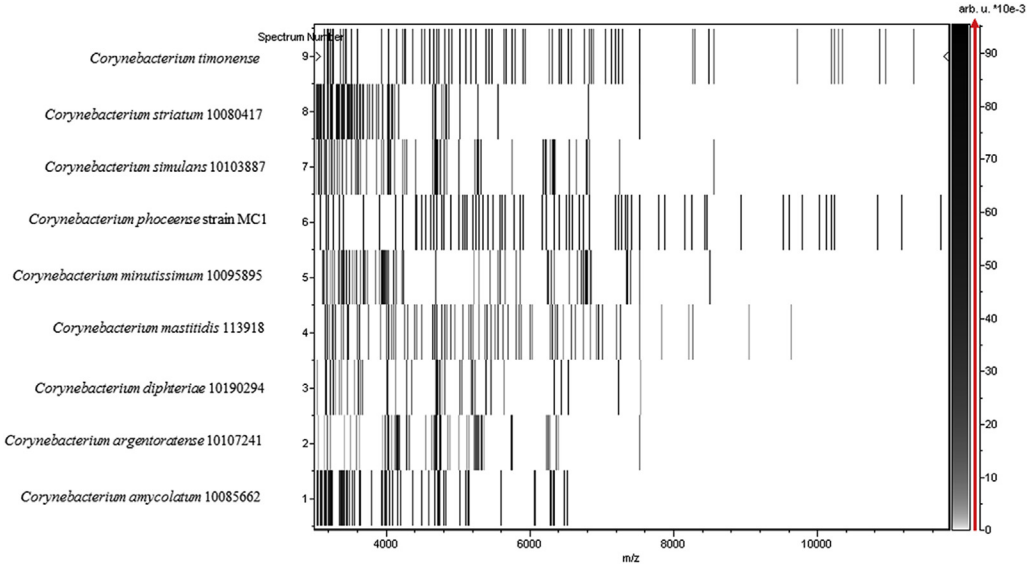


**FIG. 3.** Gel view comparing *Corynebacterium phoceense* strain MC1 (= CSUR P1905 = DSM 100570) to other species within *Corynebacteriaceae* family. Gel view displays raw spectra of loaded spectrum files arranged in pseudo-gel-like look. *x*-axis records m/z value. Left *y*-axis displays running spectrum number originating from subsequent spectra loading. Peak intensity is expressed by greyscale scheme code. Colour bar and right y-axis indicate relation between colour peak displayed and peak intensity in arbitrary units. Displayed species are indicated at left.

proteinase K at 37°C. Extracted DNA was then purified using three successive phenol–chloroform extractions and ethanol precipitations at −20°C overnight. After centrifugation, the DNA was resuspended in 160 µL TE buffer.

### Genome sequencing and assembly

Genomic DNA (gDNA) of *Corynebacterium phoceense* was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the mate pair strategy. The gDNA was barcoded in order to be mixed with nine other projects with the Nextera Mate Pair sample prep kit (Illumina).

gDNA was quantified by a Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA) to 59.1 ng/µL. The mate pair library was prepared with 1.5 µg of genomic DNA using the Nextera mate pair Illumina guide. The genomic DNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The fragmentation pattern was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 lab chip. The DNA fragments ranged in size from 1.5 to 11 kb, with an

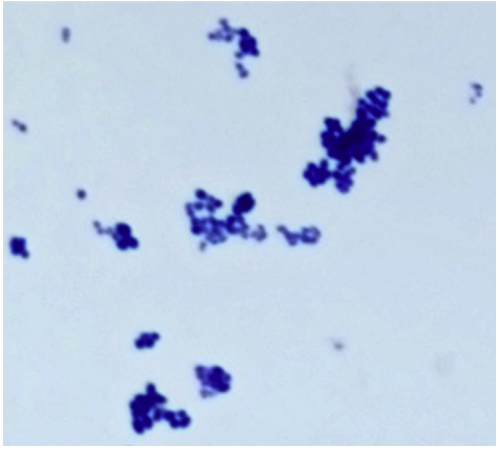

**FIG. 4.** Gram staining of *Corynebacterium phoceense* strain MC1.

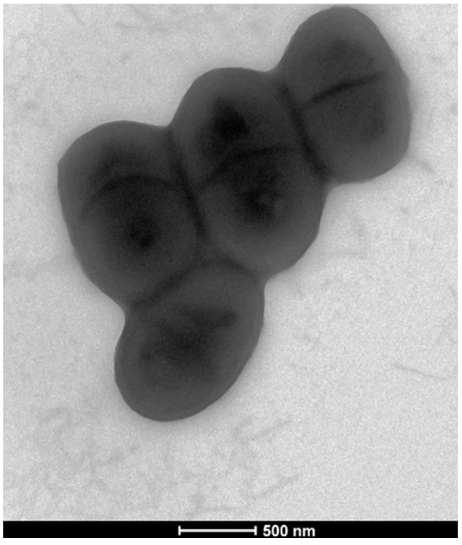


**FIG. 5.** Transmission electron microscopy of *Corynebacterium phoceense* strain MC1$^T$ with Morgani 268D (Philips, Amsterdam, the Netherlands) at operating voltage of 60 kV. Scale bar = 500 nm.

**TABLE 2. Cellular fatty acid composition (%)**

| Fatty acid | Name | Mean relative %[a] |
|---|---|---|
| 18:1n9 | 9-Octadecenoic acid | 51.1 ± 0.5 |
| 16:0 | Hexadecanoic acid | 46.0 ± 0.3 |
| 18:0 | Octadecanoic acid | 2.1 ± 0.2 |
| 15:0 | Pentadecanoic acid | TR |
| 14:0 | Tetradecanoic acid | TR |
| 18:2n6 | 9,12-Octadecadienoic acid | TR |
| 16:1n7 | 9-Hexadecenoic acid | TR |
| 17:0 | Heptadecanoic acid | TR |
| 16:1n5 | 11-Hexadecenoic acid | TR |

[a]Mean peak area percentage; TR = trace amounts <1%.

optimal size at 7.364 kb. No size selection was performed, and 600 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared to small fragments with an optimal at 589 bp on the Covaris S2 device in T6 tubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies), and the final concentration library was measured at 25.29 nmol/L.

The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and sequencing runs were performed in a single 39-hour run in a 2 × 151 bp read length.

Total information of 0.7 Gb was obtained from a 129K/mm$^2$ cluster density with a cluster passing quality control filters of 80.3% (2 435 000 passing filter paired reads). Within this run, the index representation for *Corynebacterium phoceense* was determined to 11.16%. The 271 698 paired reads were trimmed, then assembled in 14 scaffolds.

## Genome annotation and comparison

Genome annotation and comparison open reading frames (ORFs) were predicted using Prodigal [33] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region (contains N). The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups (COGs) database using BLASTP (*E* value 1e-03, coverage 70%, identity percentage 30%). If no hit was found, it searched against the NR database using BLASTP with an *E* value of 1e-03 coverage 70% and identity percentage of 30%. If sequence lengths were smaller than 80 amino acids, we used an *E* value of 1e-05. The tRNAScanSE tool [34] was used to find tRNA genes, whereas rRNAs were found using RNAmmer [35]. Lipoprotein signal peptides and the number of transmembrane helices were predicted using Phobius [36]. ORFans were identified if all the performed BLASTP procedures did not give positive results (*E* value smaller than 1e-03 for ORFs with sequence size higher than 80 aa or *E* value smaller than 1e-05 for ORFs with a sequence length smaller than 80 aa). Such parameter thresholds have already been used in previous work to define ORFans.

Genomes were automatically retrieved from the 16s RNA tree using Xegen's PhyloPattern software [37]. For each selected genome, complete genome sequence, proteome and

**TABLE 3. Differential characteristics of *Corynebacterium phoceense* strain MC1$^T$, *Corynebacterium freiburgense* strain 1045$^T$, *Corynebacterium mastitidis* strain CECT 4843$^T$, *Corynebacterium terpenotabidum* strain Y-1 1$^T$, *Corynebacterium lactis* strain RW2-5$^T$, *Corynebacterium aurimucosum* strain IMMIB D-1488$^T$ and *Corynebacterium mustelae* strain 3105$^T$ [41–46]**

| Property | *C. phoceense* | *C. freiburgense* | *C. mastitidis* | *C. terpenotabidum* | *C. lactis* | *C. aurimucosum* | *C. mustelae* |
|---|---|---|---|---|---|---|---|
| Cell diameter (µm) | 0.5 | 0.5 | 0.5 | 0.7 | 0.6 | 0.5 | 0.5 |
| Oxygen requirement | + | + | + | + | + | + | + |
| Gram stain | + | + | + | + | + | + | + |
| Salt requirement | − | − | − | − | − | − | − |
| Motility | − | − | − | − | − | − | − |
| Endospore formation | + | − | − | − | − | − | − |
| Production of: Alkaline phosphatase | + | − | + | NA | + | + | − |
| Catalase | + | + | + | + | + | + | + |
| Oxidase | − | NA | − | − | − | NA | − |
| Nitrate reductase | + | + | − | − | NA | − | − |
| Urease | − | − | v | + | − | − | − |
| β-Galactosidase | − | + | − | NA | − | − | − |
| N-acetyl-glucosamine | − | − | − | + | − | − | + |
| Ribose | + | + | − | − | + | − | + |
| Pyrazinamidase | + | − | + | NA | + | + | + |
| Pyrrolidonyl arylamidase | + | − | − | NA | − | − | − |
| Mannose | + | + | NA | + | + | − | + |
| Mannitol | − | + | − | − | − | − | − |
| Sucrose | − | + | − | − | NA | + | + |
| D-Glucose | + | + | − | − | + | + | + |
| D-Fructose | + | + | NA | − | + | + | + |
| D-Maltose | + | + | − | − | − | + | + |
| D-Lactose | + | + | − | − | − | − | − |
| Habitat | Human | Human | Sheep's milk | Soil | Cow's milk | Human | Human |

+, positive result; −, negative result; NA, data not available.

**TABLE 4. Nucleotide content and gene count levels of genome**

| | Genome (total) | |
|---|---|---|
| **Attribute** | **Value** | **% of total[a]** |
| Size (bp) | 2 793 868 | 100.0 |
| G+C content (bp) | 1 753 790 | 63.2 |
| Coding region (bp) | 2 265 533 | 81.1 |
| Total of genes | 2575 | 100.0 |
| RNA genes | 67 | 2.6 |
| Protein-coding genes | 2508 | 100.0 |
| Protein with function prediction | 1804 | 71.9 |
| Protein assigned to COGs | 1511 | 60.2 |
| Genes with peptid signals | 313 | 12.4 |
| Genes with transmembrane helices | 612 | 24.4 |

COGs, Clusters of Orthologous Groups database.
[a]Total is based on either size of genome in base pairs or total number of protein coding genes in annotated genome.

ORFeome genome sequence were retrieved from the National Center for Biotechnology Information FTP site. All proteomes were analysed with proteinOrtho [38]. Then for each couple of genomes a similarity score was computed. This score is the mean value of nucleotide similarity between all orthologue couples between the two genomes studied (AGIOS) [26].

The entire proteome was annotated to define the distribution of functional classes of predicted genes according to the clusters of orthologous protein groups (using the same method as for the genome annotation). To evaluate the genomic similarity among the compared strains, we determined two parameters: digital DNA-DNA hybridization, which exhibits a high correlation with DNA-DNA hybridization (DDH) [39,40], and AGIOS [26], which was designed to be independent from DDH.

## Results

### Strain identification and phylogenetic analyses

Strain MC1 (Table 1) was first isolated in February 2015 by spreading a urine sample on 5% sheep's blood–enriched Colombia agar (bioMérieux) in an aerobic atmosphere at 37°C after 48 hours of incubation.

Using MALDI-TOF MS for identification, no significant score was obtained for strain MC1, suggesting that this isolate's spectrum did not match any spectra in our MALDI-TOF MS database. The nucleotide sequences of the 16S rRNA and the *rpoB* genes of strain MC1 (GenBank accession nos. LN849777 and LN849778 respectively) showed 96.3% similarity with *Corynebacterium simulans,* the phylogenetically closest species with a validly published name (Fig. 1), therefore defining it as a new species within the

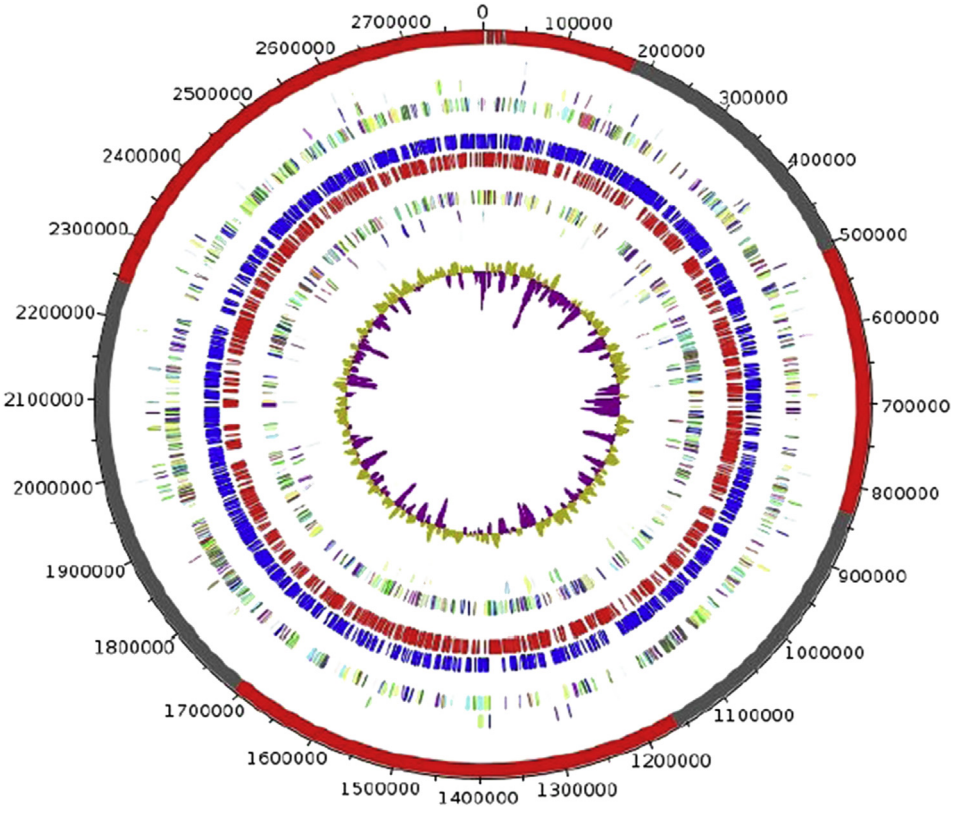


**FIG. 6.** Graphical circular map of chromosome. From outside to centre: genes on forward strain coloured by COGs categories (only gene assigned to COGs), RNA genes (tRNAs green, rRNAs red), GC content and GC skew.

**TABLE 5. Number of genes associated with 25 general COGs functional categories**

| Code | Value | % of total[a] | Description |
|---|---|---|---|
| J | 153 | 6.10 | Translation |
| A | 1 | 0.04 | RNA processing and modification |
| K | 106 | 4.22 | Transcription |
| L | 68 | 2.71 | Replication, recombination and repair |
| B | 0 | 0 | Chromatin structure and dynamics |
| D | 18 | 0.71 | Cell cycle control, mitosis and meiosis |
| Y | 0 | 0 | Nuclear structure |
| V | 56 | 2.23 | Defense mechanisms |
| T | 55 | 2.19 | Signal transduction mechanisms |
| M | 57 | 2.27 | Cell wall/membrane biogenesis |
| N | 3 | 0.11 | Cell motility |
| Z | 0 | 0 | Cytoskeleton |
| W | 3 | 0.11 | Extracellular structures |
| U | 17 | 0.67 | Intracellular trafficking and secretion |
| O | 66 | 2.63 | Posttranslational modification, protein turnover, chaperones |
| X | 53 | 2.11 | Mobilome: prophages, transposons |
| C | 94 | 3.74 | Energy production and conversion |
| G | 129 | 5.14 | Carbohydrate transport and metabolism |
| E | 172 | 6.85 | Amino acid transport and metabolism |
| F | 74 | 2.95 | Nucleotide transport and metabolism |
| H | 105 | 4.18 | Coenzyme transport and metabolism |
| I | 73 | 2.91 | Lipid transport and metabolism |
| P | 123 | 4.90 | Inorganic ion transport and metabolism |
| Q | 44 | 1.75 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 131 | 5.22 | General function prediction only |
| S | 78 | 3.11 | Function unknown |
| — | 997 | 39.75 | Not in COGs |

COGs, Clusters of Orthologous Groups database.
[a]Total is based on total number of protein-coding genes in annotated genome.

genus *Corynebacterium*. *C. phoceense* spectra (Fig. 2) were incremented in our database. The reference spectrum for *C. phoceense* was then compared to the spectra of phylogenetically close species within the genus *Corynebacterium,* and the differences were exhibited in a gel view photo (Fig. 3).

## Phenotypic description

Growth was observed from 25 to 56°C on 5% sheep's blood–enriched Columbia agar (bioMérieux), with optimal growth at 37°C in aerobic conditions after 48 hours of incubation.

Weak cell growth was observed under microaerophilic and anaerobic conditions. The motility test was negative, and after thermal shock, we observed bacterial growth, which means *Corynebacterium phoceense* is sporogenous. Cells were Gram-positive coccobacillus (Fig. 4). On Columbian blood agar, colonies are circular with entire margin, up to 1.0 mm in diameter after 48 hours' growth at 37°C. Under electron microscopy, cells had a mean diameter of 0.5 µm and a length of 3 µm (Fig. 5).

The major fatty acids were 9-octadecenoic acid (51%) and hexadecanoic acid (46%). The other fatty acids described were below 3% (Table 2).

The presence of catalase was tested using 3% (v/v) $H_2O_2$ and gave a positive result. The oxidase test was also negative for strain MC1. *Corynebacterium phoceense* strain MC1 was capable of fermenting glucose, ribose, maltose, saccharose, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, arbutin, ferric citrate esculin, D-maltose and D-saccharose. Nitrates were reduced into nitrites. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase and naphthol-AS-BI-phosphohydrolase activities were highlighted.

*Corynebacterium phoceense* strain MC1 was susceptible to all antibiotic treatments tested except nitrofurantoin and metronidazole, to which it was resistant.

The biochemical and phenotypic features of strain MC1 were compared to the corresponding features of other close representatives of the genus *Corynebacterium* (Table 3). We particularly observed the absence of endospores forming in the closest *Corynebacterium* spp.

**TABLE 6. Genome comparison of closely related species to *Corynebacterium phoceense* strain MC1$^T$**

| Species | Size (Mb) | G+C (%) | Total genes |
|---|---|---|---|
| *Corynebacterium lactis* strain RW2-5 | 2.76 | 60.5 | 2364 |
| *Corynebacterium ulcerans* strain NCTC_7910 | 2.61 | 53.3 | 2296 |
| *Corynebacterium mustelae* strain 3105 | 3.47 | 52.5 | 3146 |
| *Corynebacterium freiburgense* strain 1045 | 2.91 | 49.8 | 2667 |
| *Corynebacterium mastitidis* strain S-8 CECT_4843 | 2.37 | 68.9 | 2241 |
| *Corynebacterium aurimucosum* strain IMMIB D-1488 | 2.90 | 59.2 | 2769 |
| *Corynebacterium phoceense* strain MC1 | 2.79 | 63.2 | 2508 |
| *Corynebacterium terpenotabidum* strain Y-11 IFO 14764 | 2.75 | 67 | 2369 |

## Genome properties

The genome is 2 793 868 bp long with 63.23% GC content (Table 4, Fig. 6). It is composed of 14 scaffolds (composed of 71 contigs). Of the 2575 predicted genes, 2508 were protein-coding genes and 67 were RNAs (four genes are 5S rRNA, three genes are 16S rRNA, three genes are 23S rRNA and 57 genes are tRNA genes). A total of 1804 genes (71.93%) were assigned as putative function (by COGs or by NR BLAST). One hundred fifty-one genes were identified as ORFans (6.02%). The remaining 475 genes were annotated as hypothetical proteins (18.94%). The National Center for Biotechnology Information ID is PRJEB14666, and the genome is deposited under accession number FLTI01000001. The distribution of genes into COGs functional categories is presented in Table 5.

## Genome comparison

Table 6 compares *Corynebacterium phoceense*'s genomic characteristics to other close species. The draft genome sequence of *C. phoceense* is smaller than those of *Corynebacterium mustelae, Corynebacterium freiburgense* and *Corynebacterium aurimucosum* (2.794, 3.474, 2.91 and 2.905 Mb respectively), but larger than those of *Corynebacterium lactis, Corynebacterium ulcerans,*

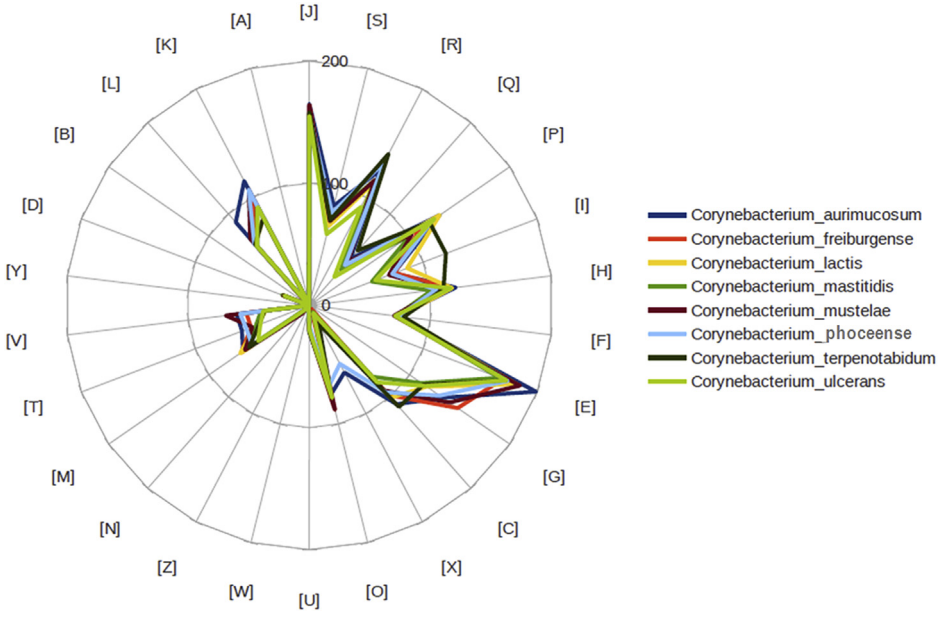


**FIG. 7.** Distribution of functional classes of predicted genes according to COGs of proteins.

*Corynebacterium mastitidis* and *Corynebacterium terpenotabidum* (2.77, 2.61, 2.37 and 2.75 Mb respectively).

The G+C content of *Corynebacterium phoceense* is smaller than those of *Corynebacterium mastitidis* and *Corynebacterium terpenotabidum* (63.23, 68.9 and 67.02% respectively), but larger than those of *Corynebacterium lactis, Corynebacterium ulcerans, Corynebacterium mustelae, Corynebacterium freiburgense* and *Corynebacterium aurimucosum* (60.53, 53.39, 52.57, 49.82 and 59.21% respectively).

The gene content of *Corynebacterium phoceense* is smaller than those of *Corynebacterium mustelae, Corynebacterium freiburgense* and *Corynebacterium aurimucosum* (2508, 3146, 2667 and 2769 respectively), but larger than those of *Corynebacterium lactis, Corynebacterium ulcerans, Corynebacterium mastitidis* and *Corynebacterium terpenotabidum* (2364, 2296, 2241 and 2369 respectively).

Finally, the distribution of genes into COGs categories was similar in all compared genomes except for those corresponding to the cell cycle control, mitosis and meiosis category, which were only present in *C. mastitidis, C. mustelae* and *C. ulcerans* (Fig. 7). *C. phoceense* strain MC1 shared 2667, 1358, 1332, 1248, 1128, 1211 and 1442 orthologous genes with *C. freiburgense, C. aurimucosum, C. ulcerans, C. lactis, C. terpenotabidum, C. mastitidis* and *C. mustelae* respectively (Table 7). Among species with standing in nomenclature, AGIOS values ranged from 54.12% between *C. terpenotabidum* and *C. freiburgense* to 74.86% between *C. aurimucosum* and *C. phoceense*. When *C. phoceense* was compared to the other

**TABLE 7. Numbers of orthologous protein shared between genomes (upper right)** [a]

| | *C. freiburgense* | *C. aurimucosum* | *C. ulcerans* | *C. lactis* | *C. phoceense* | *C. terpenotabidum* | *C. mastitidis* | *C. mustelae* |
|---|---|---|---|---|---|---|---|---|
| *Corynebacterium freiburgense* strain 1045 | **2667** | 1358 | 1332 | 1248 | 1232 | 1128 | 1211 | 1442 |
| *Corynebacterium aurimucosum* strain IMMIB | 63.97 | **2769** | 1336 | 1252 | 1407 | 1189 | 1315 | 1392 |
| *Corynebacterium ulcerans* strain NCTC 7910 | 57.66 | 58.26 | **2296** | 1197 | 1221 | 1091 | 1213 | 1354 |
| *Corynebacterium lactis* strain RW2-5 | 57.63 | 60.50 | 58.47 | **2364** | 1116 | 1105 | 1149 | 1249 |
| *Corynebacterium phoceense* strain MC1 | 63.56 | 74.86 | 58.13 | 61.48 | **2508** | 1079 | 1194 | 1264 |
| *Corynebacterium terpenotabidum* strain Y-11 IFO 14764 | 54.12 | 58.32 | 54.92 | 56.16 | 59.86 | **2369** | 1123 | 1146 |
| *Corynebacterium mastitidis* strain S-8 CECT 4843 | 63.90 | 69.01 | 57.33 | 61.39 | 70.81 | 60.55 | **2241** | 1252 |
| *Corynebacterium mustelae* strain 3105 | 65.46 | 64.85 | 58.55 | 58.25 | 64.48 | 55.39 | 64.16 | **3146** |

[a]Average percentage similarity of nucleotides corresponding to orthologous protein shared between genomes (lower left) and numbers of proteins per genome (bold).

species, AGIOS values ranged from 58.13% with *C. ulcerans* to 74.86% with *C. aurimucosum*.

## Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of *Corynebacterium phoceense* which contains the type strain MC1$^T$. This bacterial strain has been isolated from the urine of a kidney transplant recipient.

## Description of *Corynebacterium phoceense* type strain MC1$^T$ sp. nov.

*Corynebacterium phoceense* (pho.ce'en'se, L. gen. masc., phoceense, "of Phoceen," the old Latin name of the city of Marseille, where strain MC1$^T$ was isolated). Cells have mean diameter of 0.5 µm and a mean length of 3 µm. Colonies are round and 1.0 mm in diameter on 5% sheep's blood–enriched Columbia agar (bioMérieux). Positive reactions are observed for glucose, ribose, maltose, saccharose, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase and naphthol-AS-BI-phosphohydrolase. D-Ribose, D-galactose, D-glucose, D-fructose, D-mannose, arbutin, D-maltose and D-saccharose were metabolized. Cells were susceptible to vancomycin, rifampicin, doxycycline, erythromycin, amoxicillin, nitrofurantoin, gentamicin, ciprofloxacin, ceftriaxone, amoxicillin + clavulanic acid, penicillin G, gentamicin, trimethoprim + sulfamethoxazole, oxacillin, imipenem, tobramycin, metronidazole, amikacin, linezolid, clindamycin, daptomycin and chloramphenicol.

The G+C content of the genome is 63.23%. The 16S rRNA gene sequence and whole-genome shotgun sequence of *C. phoceense* strain MC1$^T$ are deposited in GenBank under accession numbers LN849777 and FLTI01000001, respectively. The type strain MC1$^T$ (= CSUR P1905 = DSM 100570) was isolated from the urine of a kidney transplant recipient.

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## Conflict of Interest

None declared.

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