Corynebacterium urinapleomorphum sp. nov., a new bacterial species isolated from human urine sample

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Abstract

Corynebacterium urinapleomorphum sp. nov. stain Marseille-P2799^T (= CSURP2799; = DSM103272) is a new species from the order *Corynebacteriales* that was isolated from urine of a 2-month-old child with gastroenteritis.

Keywords: *Corynebacteriales*, *Corynebacterium urinapleomorphum*sp. nov., culturomics, human microbiota, urinary microbiota
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Introduction

Currently, the implication of bacterial diversity for normal physiological functions and disease must be understood [1]. To explore the diversity of human intestinal bacteria, the culturomics approach, based on diversified culture conditions, was designed to isolate species never cultivated before and also to complete the metagenomics of 16S rRNAs [2–4]. This culturomics approach has also been extended for the characterization of other human bacterial systems such as those of the vaginal and urinary tract [5,6]. Recently, a new taxonomic method called taxonogenomics has been developed for a description associating the analysis of complete sequences of the genome and the phenotypic characteristics of new bacterial species [7]. Herein, we give a short description, based on taxonogenomics, of a new species within the genus *Corynebacterium*, isolated from a young boy's urine.

Isolation and growth conditions

In 2016, the strain Marseille-P2799^T was isolated from a urine sample from a 2-month-old child with rotavirus gastroenteritis. Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), the strain has not been identified. The screening was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), as previously reported [8]. Spectra obtained (Fig. 1) were imported and analysed using the BIOTYPER 3.0 software against the Bruker database, which was constantly updating from the MEPHI database http://www.mediterranee-infection.com/article.php?larub=280&titre=umrs-database [1]. The strain Marseille-P2799^T was isolated after 72 hours of incubation at 37°C on 5% sheep's blood–antioxidant agar homemade R-medium (Hôpital de la Timone, Marseille, France) in anaerobic atmosphere generated using the GENbag anaer system (bioMérieux, Marcy-l'Étoile, France) [9].

Phenotypic characteristics

Colonies of the Strain Marseille-P2799^T were pale grey and had a mean diameter of 0.5 mm. Bacterial cells were non-

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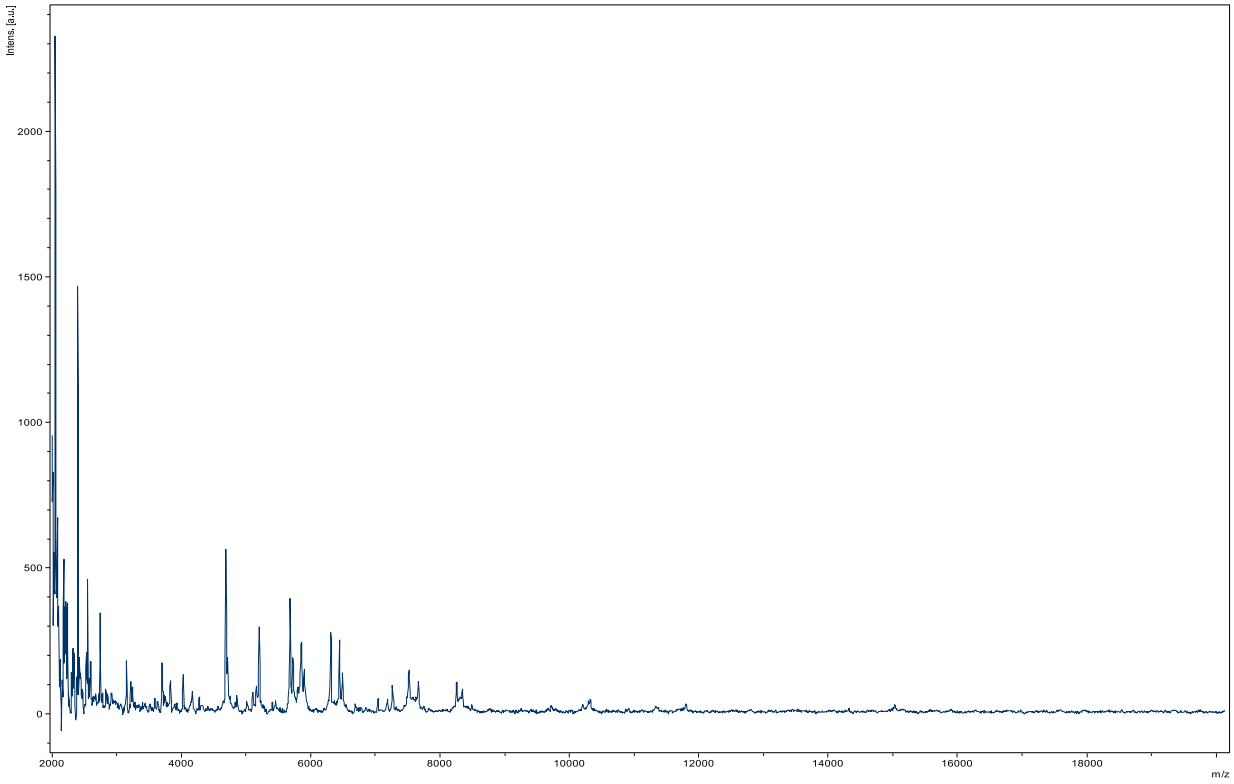

FIG. 1. MALDI-TOF MS reference spectrum of *Corynebacterium urinapleomorphum* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

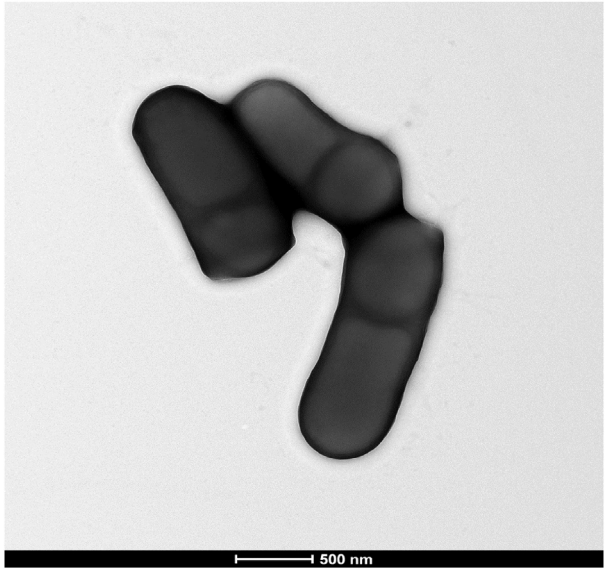

FIG. 2. Scanning electron microscopy (SEM) of stained *Corynebacterium urinapleomorphum* sp. nov. A colony was collected from agar and immersed in a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000) approximately 60 cm in height and 33 cm in width to evaluate bacteria structure. The scale is shown on the figure.

TABLE 1. Phenotypic characterization of *Corynebacterium urinapleomorphum* sp. nov., based on analytical profile index (API) tests

Biochemical characteristics	*Corynebacterium urinapleomorphum* sp. nov.
Alkaline phosphatase	+
Esterase (C-4)	+
Esterase lipase (C-8)	+
Lipase (C-14)	−
Leucine arylamidase	+
Valine arylamidase	−
Cystine arylamidase	−
Trypsine	−
α-chymotrypsine	−
Acid phosphatase	+
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	−
β-galactosidase	−
β-glucuronidase	−
α-glucosidase	−
β-glucosidase	−
N-acetyl-β-glucosaminidase	−
α-mannosidase	−
α-fucosidase	−
Nitrates to nitrites	−
Indole	−
Glucose fermentation	−
Arginine dihydrolase	+
Urease	+
Protease	+
Glucose assimilation	−
Arabinose	−
Mannose	−
Mannitol	−
N-acetyl-glucosamine	−
Maltose	−
Potassium gluconate	−
Capric acid	−
Adipic acid	−
Malate	−
Trisodium citrate	−
Phenylacetic acid	−

motile, Gram-positive, pleomorphic bacilli with a length ranging from 0.7 to 2 µm and width ranging from 0.4 to 0.6 µm (Fig. 2). Strain Marseille-P2799^T was catalase positive and oxidase negative. API ZYM and API 20NE tests were performed at 37°C under aerobic conditions (Table 1). Table 2 compares the main biochemical characteristics of the closest *Corynebacterium* species with standing in nomenclature.

Strain identification

In order to classify this bacterium, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [10]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com).

Strain Marseille-P2799^T exhibited 98% sequence similarities to *Corynebacterium appendicis* strain IMMIB R-3491^T (GenBank Accession no. AJ314919), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed classifying strain Marseille-P2799^T as a new species within the genus *Corynebacterium* belonging to the phylum *Actinobacteria*.

TABLE 2. Differential phenotypic characteristics of *Corynebacterium urinapleomorphum* sp. nov. (1), *Corynebacterium phoceense* (2), *Corynebacterium freiburgense* (3), *Corynebacterium aurimucosum* (4) and *Corynebacterium appendicis* (5)

Properties	1	2	3	4	5
Cell diameter (µm)	0.2	0.5	0.5	0.5	0.3
Oxygen requirement	+	+	+	+	−
Gram stain	+	+	+	+	+
Salt requirement	−	−	−	−	−
Motility	−	−	−	−	−
Endospore formation	−	+	−	−	−
Alkaline phosphatase	+	+	−	+	+
Catalase	+	+	+	+	+
Oxidase	−	−	na	na	na
Nitrate reductase		+	+	−	−
Urease	+	−	−	−	+
β-galactosidase	−	−	+	−	−
N-acetyl-glucosamine	−	−	−	−	−
Arabinose	−	na	−	+	−
Lipase (C8)	+	+	+	−	−
Pyrrolidonyl arylamidase	−	+	−	−	−
Mannose	−	+	+	−	+
Mannitol	−	−	+	−	−
Sucrose		−	+	+	−
D-glucose	−	+	+	+	+
D-fructose	−	+	+	+	na
D-maltose	−	+	+	+	+
Habitat	Human	Human	Human	Human	Human

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

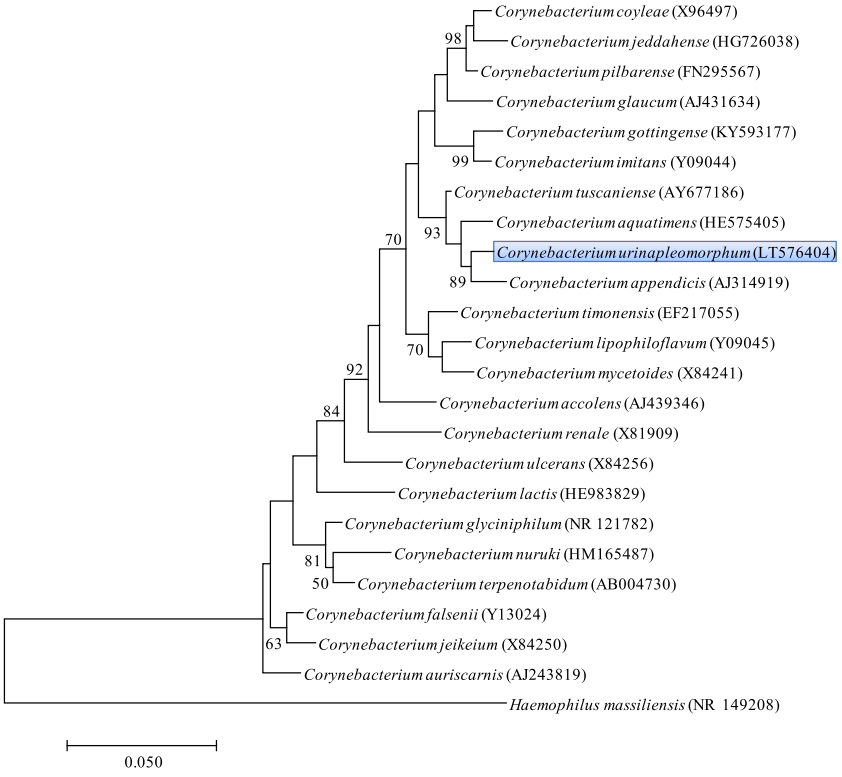

FIG. 3. Phylogenetic tree highlighting the position of *Corynebacterium urinapleomorphum* sp. nov., relative to the most closely related type strains within the genus *Corynebacterium*. GenBank Accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference was obtained using the maximum likelihood method and MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 5% nucleotide sequence divergence. *Haemophilus massiliensis* was used as an outgroup.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired End (Illumina), as previously described [11]. The assembly was performed using a pipeline containing several softwares (Velvet [12], Spades [13] and Soap Denovo [14]), and trimmed (MiSeq and Trimmomatic [15] softwares) or untrimmed (only MiSeq software) data. GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of Strain Marseille-P2799^T was 2.26 Mb with 63.4% G + C content. The degree of genomic similarity of the strain with closely related species was calculated using OrthoANI software [16]. OrthoANI values among closely related species (Fig. 4) ranged from 60.04% between *Corynebacterium gottingense* and *Corynebacterium lipophiloflavum* to 84.98% between *Corynebacterium imitans* and *Corynebacterium gottingense*. When *Corynebacterium urinapleomorphum* was compared with these closely related species, values ranged from 62.43% with *Corynebacterium gottingense* to 74.85% with *Corynebacterium mycetoides*.

Conclusion

On the basis of unique phenotypic features, including MALDI-TOF spectrum, a 16S rRNA sequence divergence >1.3% and an OrthoANI value < 95% with the phylogenetically closest species with standing in nomenclature, we formally proposed strain Marseille-P2799^T as the type strain of *Corynebacterium urinapleomorphum* sp. nov, which is a new species in the genus *Corynebacterium*.

Description of *Corynebacterium urinapleomorphum* strain Marseille-P2799^T sp. nov.

Corynebacterium urinapleomorphum (u.ri.na.pleo.morph.um composed of u.ri.na L.N. gen. fem. *urina*, the Latin word for 'urine', as strain Marseille-P2799 was first found in a paediatric urine sample, and pleo. morph.um. L. neutral. adj. *pleomorphum* of pleo, 'several' or 'different', and morph, 'shape', as cells were bacilli with cytoplasmic inclusions that could make us think that the bacterium was a catenary Gram-positive coccus). The strain grows at temperatures ranging between 37°C and 45°C in anaerobic conditions (at an optimum temperature of 37°C). This is a facultative aero-

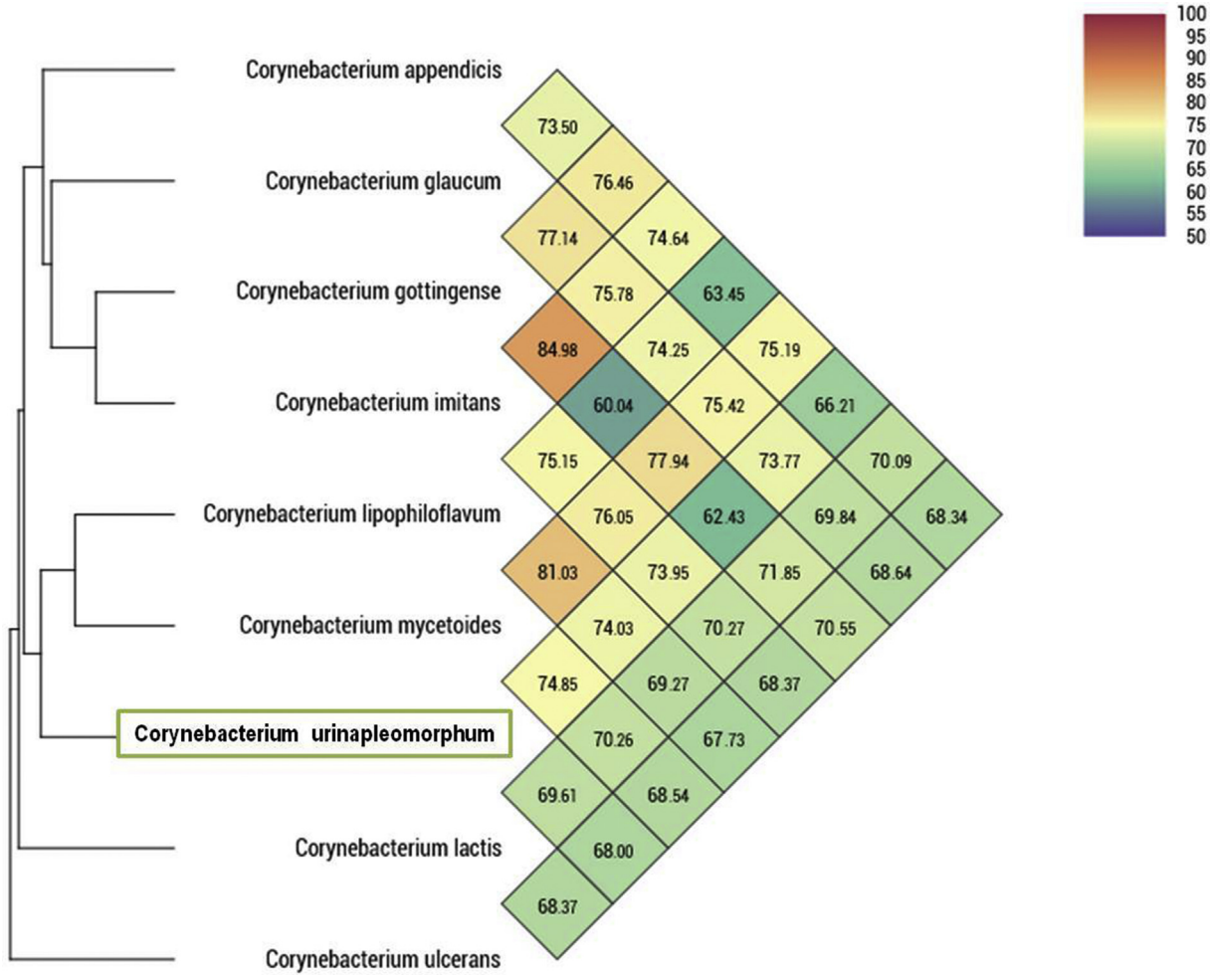

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Corynebacterium urinapleomorphum* sp. nov. and other closely related species with standing in nomenclature.

anaerobic bacterium. Salinity range growth was tested between 10% and 20% (no growth was observed), and pH growth occurred between pH 5 and 8 (with an optimum of pH 7). The potential pathogenicity of the type strain Marseille-P2799^T (= CSURP2799; = DSM103272) is unknown. However, this bacterium, as well as *Staphylococcus saprophyticus* and *Helicobacter pylori*, has recently been isolated from the gallbladder of a patient with acute cholecystitis [17]. It was isolated from the urine sample of a 2-month-old child who came into our hospital with gastroenteritis. This strain exhibited a G + C content of 63.4%.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under Accession number LT576404 and FTLL00000000, respectively.

Deposit in culture collections

Strain Marseille-P2799^T was deposited in two different strain collections under following numbers (= CSURP2799; = DSM103272).

Ethics and consent

The study was **approved** by the ethics committee of the Institut Federatif de Recherche 48 under reference 2016-010. The patient's guardian gave an approved and signed consent for participating in this study.

Conflicts of interest

None to declare.

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