Urinicoccus massiliensis gen. nov., sp. nov., a new bacterium isolated from a human urine sample from a 7-year-old boy hospitalized for dental care

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Abstract

Urinicoccus massiliensis strain Marseille-P1992T (= CSURP1992 = DSM100581) is a species of a new genus isolated from human urine. © 2019 The Authors. Published by Elsevier Ltd.

Keywords: Culturomics, new species, taxonogenomics, urine, Urinicoccus massiliensis

Original Submission: 16 July 2019; Revised Submission: 10 October 2019; Accepted: 17 October 2019
Article published online: 30 October 2019

Introduction

Culturomics is a concept involving the development of different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once the bacterium was isolated, we used a taxonogenomics approach—including matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description (Table 1) and genome sequencing—to describe it [5,6].

Isolation and growth conditions

In 2015 we isolated from human urine an unidentified bacterial strain. The study was validated by the ethics committee of the IHU Méditerranée Infection under number N° 2016-011. A screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated http://www.mediterraneeinfection.com/article.php?larub=280&titre=urms-database). The initial growth was obtained 10 days after culture on a blood culture vial (Becton Dickinson, Le Pont-de-Claix, France) supplemented with 5 mL of 0.2-μm-filtered rumen fluid in anaerobic conditions at 37°C and pH 7.5.

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing was done using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com).

Strain Urinicoccus massiliensis exhibited a 90.74% sequence identity with Peptoniphilus asaccharolyticus strain JCM 1765 (Genbank accession number NR_113382.1, the phylogenetically closest species with standing in nomenclature (Fig. 2)). We consequently classify this strain as a member of a new species within the genus Urinicoccus, family Peptoniphilaceae, phylum Firmicutes.
# TABLE 1. Description of *Urinicoccus massiliensis* according to the digitalized protologue TA00972 on the www.imedea.uib.es/dprotologue website

| TAXONUMBER | TA00972 |
|------------|---------|
| DATE OF THE ENTRY | 2019-05-30 |
| DRAFT NUMBER/DATE VERSION | Submitted |
| SPECIES NAME | *Urinicoccus*
| GENUS NAME | *Urinicoccus*
| SPECIFIC EPITHET | *Urinicoccus massiliensis*
| SPECIES STATUS | nom. rev. |
| SPECIES ETYMOLOGY | mas.sil.ien.sis. L. Adj. gen. fem. massiliensis, of massilia, the Latin name of Marseille because strain FC2 was first found in the city of Marseille edmondkuete@yahoo.fr |
| E-MAIL OF THE CORRESPONDING AUTHOR | edmondkuete@yahoo.fr |
| SUBMITTER | KUETE Yimagou EDMOND |
| DESIGNATION OF THE TYPE STRAIN | Marseille-P1992 |
| STRAIN COLLECTION NUMBERS | CSURP1992 = DSM100581 |
| 16S rRNA GENE ACCESSION NUMBER | LN881616 |
| GENOME ACCESSION NUMBER (EMBL) | FPLH01000000 |
| GENOME SIZE | 2.08716 |
| GC mol % | 41.7 |
| DATA ON THE ORIGIN OF THE SAMPLE FROM WHICH THE STRAIN HAD BEEN ISOLATED | Country of Origin: France Region of Origin: Bouches du Rhône Date of Isolation: 2015-02-13 Source of Isolation: Urine Sampling Date: 2015-02-03 |
| SALINITY OF THE SAMPLE (%) | 7.5 |
| GROWTH MEDIUM, INCUBATION CONDITIONS (Temperature, pH, and further information) USED FOR STANDARD CULTIVATION | Blood culture vial (Becton Dickinson, Le Pont-de-Claix, France) supplemented with 5 mL of 0.2-μm filtered rumen fluid |
| GRAM STAIN | positive |
| CELL SHAPE | coccus |
| CELL SIZE (length or diameter) | 2.08716 |
| MOTILITY | Non-motile |
| SPORULATION (resting cells) | none |
| LOWEST TEMPERATURE FOR GROWTH | 25°C |
| HIGHEST TEMPERATURE FOR GROWTH | 45°C |
| TEMPERATURE OPTIMUM | 37°C |
| OXIDASE | Negative |
| CATALASE | Negative |
FIG. 1. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

FIG. 2. Phylogenetic tree showing the position of Urinococcus massiliensis strain Marseille-P1992\textsuperscript{T} relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. The scale bar indicates a 2% nucleotide sequence divergence.
**Phenotypic characteristics**

Colonies were translucent with a mean diameter of 1 μm. Bacterial cells were gram-positive, rod-shaped, ranging in length from 0.3 μm to 0.5 μm (Fig. 3). Strain Marseille-P1992^T^ showed catalase-negative and oxidase-negative activities (Table 1). API 50CH and API ZYM tests were performed at 37°C under anaerobic conditions. Results are summarized in Tables 2 and 3. Table 4 compares the main biochemical characteristics of *Urinicoccus massiliensis* and the closest related taxa with standing in nomenclature.

**Genome sequencing**

DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced with the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different softwares (Velvet [10], Spades [11] and Soap Denovo [12]) on trimmed (Trimmomatic [13]) or raw data. GapCloser was used to reduce

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**TABLE 2. Phenotypic characterization of *Urinicoccus massiliensis* based on the biochemical tests API 50 CH**

| Bacteria: *Urinicoccus massiliensis* | Test | Results (+/−) | Test | Results (+/−) |
|-------------------------------------|------|---------------|------|---------------|
| Control                             | −    | Esculine       | −    | −             |
| Glycerol                            | −    | Salicine      | +    | +             |
| Erythrol                            | −    | D-cellobiose  | −    | −             |
| D-arabinose                         | +    | D-maltose     | +    | +             |
| L-arabinose                         | −    | D-lactose     | −    | −             |
| D-ribose                           | −    | D-melibiose   | −    | −             |
| D-xylene                           | −    | D-saccharose  | +    | +             |
| L-xylene                           | +    | D-trahalose   | +    | +             |
| D-ribose                           | +    | Inuline       | +    | +             |
| Methyl-β-D-xylopyranoside           | +    | D-melezitose  | −    | −             |
| D-glucosamine                      | −    | D-raffinose   | +    | +             |
| D-glucose                          | +    | Amidon        | +    | +             |
| D-fructose                         | +    | Glycogen      | +    | +             |
| D-mannose                          | +    | Xylool        | −    | −             |
| L-sorbose                          | +    | Galactosio    | +    | +             |
| L-rhamnose                         | −    | D-turanose    | +    | +             |
| Dulcitol                           | +    | D-lyxose      | +    | +             |
| Inositol                           | −    | D-tagatose    | +    | +             |
| D-mannitol                         | +    | D-fucose      | +    | +             |
| D-sorbitol                         | +    | L-fucose      | +    | +             |
| Methyl-α-D-mannopyranoside         | −    | D-arabitol    | +    | +             |
| Methyl-α-D-glucopyranoside         | −    | L-arabitol    | +    | +             |
| N-acetylglucosamine                | −    | Potassium gluconate | − | − |
| Amygdaline                         | −    | Potassium 2-cetogluconate | − | − |
| Arbutine                           | −    | Potassium 3-cetogluconate | + | + |

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**TABLE 3. Phenotypic characterization of *Urinicoccus massiliensis* based on the biochemical tests API ZYM**

| Bacteria: *Urinicoccus massiliensis* | API ZYM | Test | Results (+/−) | Test | Results (+/−) |
|-------------------------------------|---------|------|---------------|------|---------------|
| Control                             | −       | Alkaline phosphatase | +    | −             |
| Glycerol                            | −       | Esterase (C4)       | +    | −             |
| D-arabinose                         | +       | Esterase Lipase (C8) | +    | −             |
| L-arabinose                         | −       | Lipase (C14)        | −    | −             |
| D-ribose                           | −       | Leucine amygdalase  | −    | −             |
| D-xylene                           | +       | Valine amygdalase   | −    | −             |
| L-xylene                           | +       | Cystine amygdalase  | −    | −             |
| D-ribose                           | +       | Trypsine            | −    | −             |
| Methyl-β-D-xylopyranoside           | +       | α-Chymotrypsin      | −    | −             |
| D-glucosamine                      | +       | Acid phosphatase    | +    | +             |
| L-rhamnose                          | −       | Naphtho-AS-Bi-phosphohydrolase | − | − |
| D-melibiose                         | −       | α-Galactosidase     | −    | −             |
| D-lyxose                           | +       | β-Galactosidase     | −    | −             |
| L-sorbose                           | +       | β-Glucosidase       | −    | −             |
| D-glucosamine                      | +       | N-Acetyl-β-glucosaminidase | + | + |
| D-glucosamine                      | +       | α-Mannosidase       | −    | −             |
| D-glucosamine                      | +       | α-Fucosidase        | −    | −             |
| Characteristics                        | Urinicoccus massiliensis | Peptoniphilus asaccharolyticus | Peptoniphilus coxii | Peptoniphilus duodenii | Peptoniphilus harei | Peptoniphilus indolicus | Peptoniphilus ivorii | Peptoniphilus lacydonensis | Peptoniphilus senegalensis |
|---------------------------------------|--------------------------|-------------------------------|--------------------|------------------------|--------------------|------------------------|------------------------|---------------------------|------------------------|
| Major cellular fatty acid             | NA                       | Butyrate                      | Butyrate           | Butyrate               | Butyrate           | Butyrate               | Butyrate               | Butyrate                  | Butyrate               |
| Peptone as major energy source        | NA                       | +                             | +                   | +                      | +                   | +                      | +                      | +                         | +                      |
| Production of:                        |                          |                               |                    |                        |                    |                        |                        |                           |                        |
| indole                                | NA                       | SD                            | −                   | +                      | SD                 | +                      | −                      | +                         | +                      |
| urease                                | NA                       | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| catalase                              | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| alkaline phosphatase                  | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| coagulase                             | −                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| Fermentation of:                      |                          |                               |                    |                        |                    |                        |                        |                           |                        |
| glucose                               | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| lactose                               | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| raffinose                             | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| mannose                               | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| Activity of:                          |                          |                               |                    |                        |                    |                        |                        |                           |                        |
| α-galactosidase                       | −                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| β-galactosidase                       | −                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| α-glucosidase                         | −                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| β-glucosidase                         | +                        | −                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| arginine arylamidase                  | NA                       | +                             | −                   | −                      | −                   | −                      | −                      | −                         | −                      |
| proline arylamidase                   | NA                       | −                             | +                   | −                      | −                   | −                      | −                      | −                         | −                      |
| phenylalanine arylamidase             | NA                       | −                             | +                   | +                      | −                   | −                      | −                      | −                         | −                      |
| leucine arylamidase                   | NA                       | −                             | +                   | +                      | −                   | −                      | −                      | −                         | −                      |
| histidine arylamidase                 | NA                       | −                             | +                   | +                      | −                   | −                      | −                      | −                         | −                      |
| pyroglutamyl arylamidase              | NA                       | −                             | +                   | +                      | −                   | −                      | −                      | −                         | −                      |

SD, strain-dependent; WR: weak reaction.
assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed [14]. The best assembly was selected by using different criteria (17 scaffolds, 19 contigs). Core-genome-based phylogenetic relationships of strain Marseille-P1992 and the closest species (Table 5) are presented in Fig. 4. The degree of genomic similarity between strain Marseille-P1992 T and closely related species was estimated using the OrthoANI software [15]. Values among closely related species (Fig. 5) ranged from 63.08% between Peptoniphilus senegalensis and Peptoniphilus ivorii to 82.87% between Peptoniphilus asaccharolyticus and Peptoniphilus indolicus. When the isolate was compared to these closely related species, values ranged from 65.29% with Peptoniphilus ivorii to 75.08% with Peptoniphilus duerdenii.

The degree of genomic similarity of strain Marseille-P1992 T with closely related species was estimated using the digital DNA–DNA hybridization tool [16]. Values among closely related species (Table 6) ranged from 17.5 ± 4.5% between Peptoniphilus asaccharolyticus and Peptoniphilus coxii to 38.6 ± 5% with Peptoniphilus asaccharolyticus.

### Table 5. Genomic characteristics of *Urinicoccus massiliensis* gen. nov., sp. nov. and the eight most closely related bacterial taxa for which genome sequences are available

| Type strains                        | Accession number | Size (Mb) | GC % | Gene content |
|------------------------------------|------------------|-----------|------|--------------|
| *Urinicoccus massiliensis*         | FPLH0000000000   | 2.08      | 41.7 | 2047         |
| Peptoniphilus harei                | AENP0000000000   | 1.84      | 34.4 | 1766         |
| Peptoniphilus duerdeni             | AEEH0000000000   | 2.08      | 34.2 | 2018         |
| Peptoniphilus senegalensis         | CAEL0000000000   | 1.84      | 32.3 | 1726         |
| Peptoniphilus coxii                | LSDG0000000000   | 1.84      | 46.6 | 1783         |
| Peptoniphilus laeydonensis         | FNVR0000000000   | 1.85      | 29.9 | 1788         |
| Peptoniphilus asaccharolyticus     | FWWR0000000000   | 2.23      | 32.3 | 2268         |
| Peptoniphilus ivorii               | LR134523.1       | 1.59      | 53.2 | 1569         |
| Peptoniphilus indolicus            | AGBB0000000000   | 2.24      | 31.7 | 2145         |

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Conclusion

Strain *Urinicoccus massiliensis* exhibited a 16S rRNA sequence identity <95%, an OrthoANI value < 95% and an dDDH value < 70% with the phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. It is consequently proposed as the type strain of a new genus: *Urinicoccus massiliensis* gen. nov., sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in Genbank under accession number LN881616 and FPLH01000000 respectively.

Deposit in culture collections

Strain Marseille-P1992T was deposited in two different strain collections (= CSURP1992 = DSM100581).

TABLE 6. Digital DNA–DNA hybridization (dDDH) values obtained by comparison of all studied genomes

|    | 1     | 2      | 3    | 4      | 5       | 6       | 7       | 8       | 9       |
|----|-------|--------|------|--------|---------|---------|---------|---------|---------|
| 1  | Peptoniphilus asaccharolyticus | 100    | 53.6 ± 5.4 | 50.1 ± 5.3 | 50 ± 5.3 | 45.1 ± 5.1 | 43.2 ± 5 | 40.4 ± 5 | 39.2 ± 5 | 38.6 ± 5 |
| 2  | Peptoniphilus coxii | 100    | 38.3 ± 5 | 38.3 ± 5 | 37.6 ± 5 | 37.2 ± 4.9 | 37.2 ± 5 | 35.8 ± 4.9 | 35.4 ± 5 |
| 3  | Peptoniphilus duerdenii | 100    | 35.4 ± 4.9 | 34.5 ± 4.9 | 34.3 ± 4.9 | 32.2 ± 4.9 | 32 ± 4.9 | 31 ± 4.9 | 30.7 ± 4.9 | 30.2 ± 4.9 |
| 4  | Peptoniphilus harei | 100    | 100    | 27 ± 4.9 | 26.2 ± 4.9 | 24.1 ± 4.8 | 24 ± 4.8 | 24.2 ± 4.7 |
| 5  | Peptoniphilus indolicus | 100    | 100    | 100    | 100    | 100    | 100    | 100    | 100    |
| 6  | Peptoniphilus ivorii | 100    | 24.1 ± 4.8 | 23.8 ± 4.8 | 23.4 ± 4.7 | 20.3 ± 4.6 | 20 ± 4.7 |
| 7  | Peptoniphilus lapidysenensis | 100    | 100    | 100    | 100    | 100    | 100    | 100    | 100 |
| 8  | Peptoniphilus senegalensis | 100    | 100    | 100    | 100    | 100    | 100    | 100    | 100    |
| 9  | *Urinicoccus massiliensis* | 100    | 100    | 100    | 100    | 100    | 100    | 100    | 100 |

The words in bold represent the studied bacteria in this manuscript. Numbers (100) represent the percentage of similarity between each strain with itself.

FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software between genus species and other closely related species with standing in nomenclature.
Conflict of Interest

The authors declare no conflicts of interest. This work was funded by the IHU Méditerranée Infection (Marseille, France) and by the French Government under the Investissements d’Avenir (Investments for the Future) programme managed by the Agence Nationale de la Recherche (ANR, fr: National Agency for Research) (reference: Méditerranée Infection 10-IHU-03).

Acknowledgements

The authors thank Hitachi Corporation for providing the TM4000Plus Tabletop microscope. They also thank Aurelia Caputo from IHU-Méditerranée Infection, Marseille, France for submitting the genomic sequences to GenBank.

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