

# Urinococcus 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

*Urinococcus massiliensis* strain Marseille-P1992<sup>T</sup> (= CSURP1992 = DSM100581) is a species of a new genus isolated from human urine.

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**Keywords:** Culturomics, new species, taxonogenomics, urine, *Urinococcus massiliensis*

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## 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

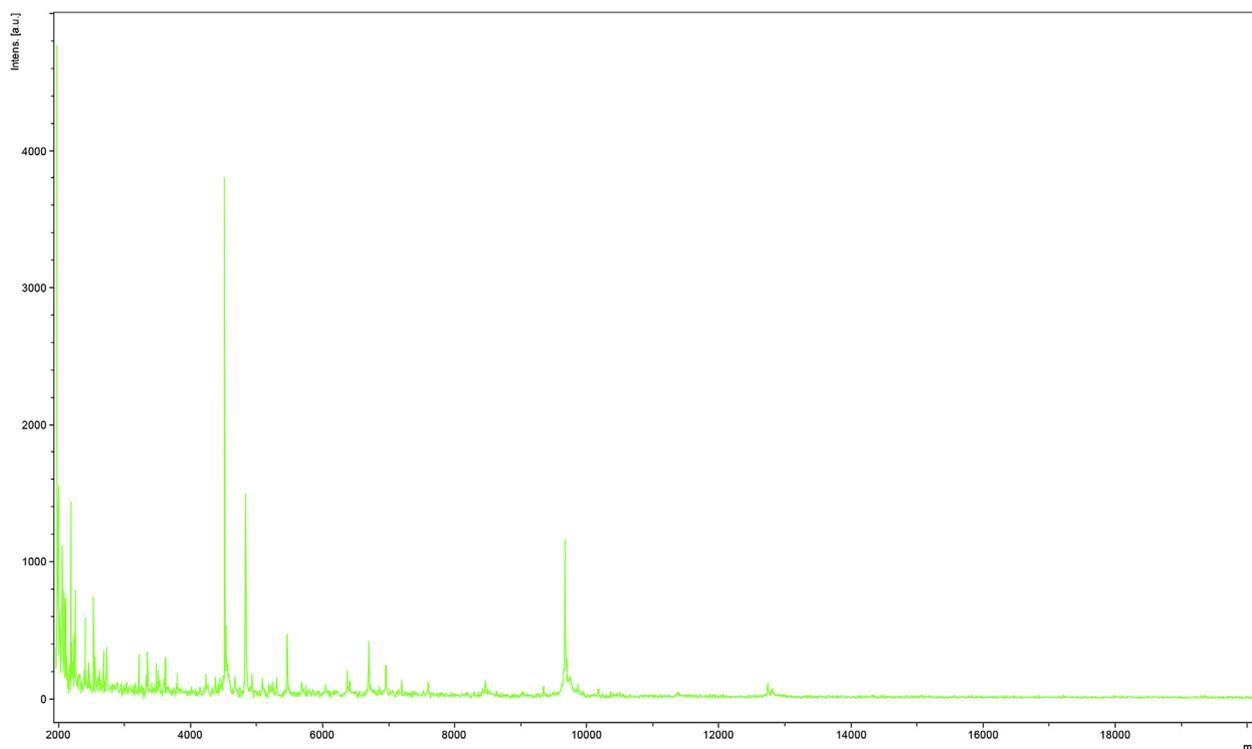
previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated <http://www.mediterraneeinfection.com/article.php?larub=280&titre=urms-database>). 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

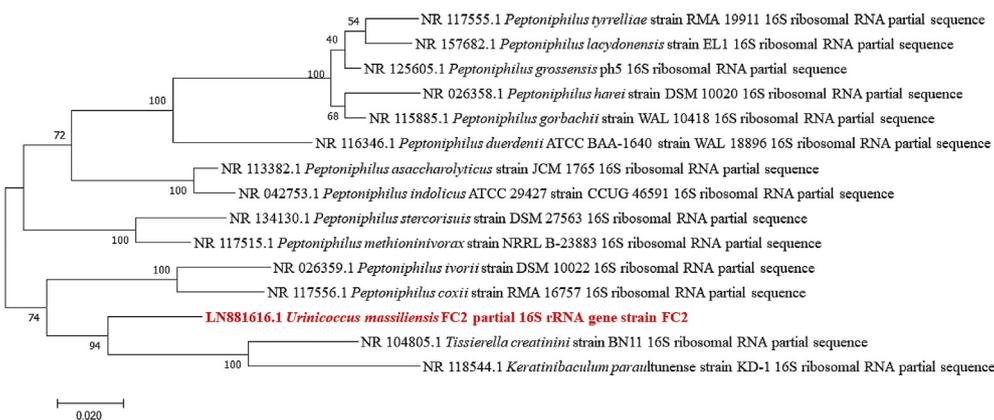
The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing was done using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (ThermoFisher, 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 *Urinococcus 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 *Urinococcus*, family *Peptoniphilaceae*, phylum *Firmicutes*.

**TABLE 1.** Description of *Urinicoccus massiliensis* according to the digitalized protologue TA00972 on the [www.imedea.uib.es/dprotologue](http://www.imedea.uib.es/dprotologue) website

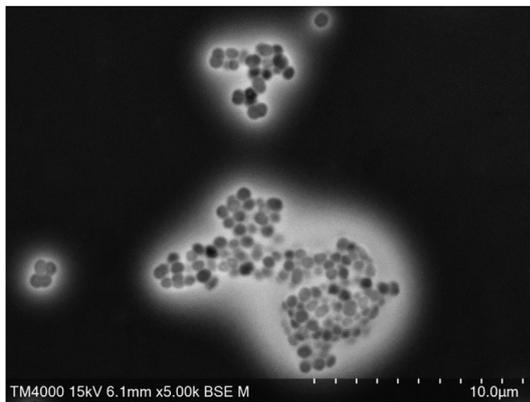
| TAXONUMBER  | TA00972   |
|---|---|
| DATE OF THE ENTRY   | 2019-05-30  |
| DRAFT NUMBER/DATE   | 001   |
| VERSION   | Submitted   |
| SPECIES NAME  | <i>Urinicoccus massiliensis</i>   |
| GENUS NAME  | Urinicoccus   |
| SPECIFIC EPITHET  | <i>Urinicoccus massiliensis</i>   |
| SPECIES STATUS  | nom. 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 |
| E-MAIL OF THE CORRESPONDING AUTHOR  | <a href="mailto:edmondkuete@yahoo.fr">edmondkuete@yahoo.fr</a>  |
| SUBMITTER   | KUETE YIMAGOU EDMOND  |
| E-MAIL OF THE SUBMITTER   | <a href="mailto:edmondkuete@yahoo.fr">edmondkuete@yahoo.fr</a>  |
| 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 *Urinicoccus massiliensis* strain Marseille-PI992<sup>T</sup> 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.



**FIG. 3.** Electron micrograph of *Urinicoccus massiliensis* strain Marseille-P1992<sup>T</sup> obtained with a Hitachi TM4000Plus tabletop scanning electron microscope.

### 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<sup>T</sup> 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.

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

| Bacteria:                        |               |
|----------------------------------|---------------|
| API ZYM                          |               |
| Test                             | Results (+/-) |
| Control                          | -             |
| Alkaline phosphatase             | +             |
| Esterase (C4)                    | +             |
| Esterase Lipase (C8)             | +             |
| Lipase (C14)                     | -             |
| Leucine arylamidase              | -             |
| Valine arylamidase               | -             |
| Cystine arylamidase              | -             |
| Trypsine                         | -             |
| α-Chymotrypsin                   | -             |
| Acid phosphatase                 | +             |
| Naphthalo-AS-BI-phosphohydrolase | +             |
| α-Galactosidase                  | -             |
| β-Galactosidase                  | -             |
| β-Glucuronidase                  | -             |
| α-Glucosidase                    | -             |
| β-Glucosidase                    | +             |
| N-Acetyl-β-glucosaminidase       | +             |
| α-Mannosidase                    | -             |
| α-Fucosidase                     | -             |

### 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 (Trimomatic [13]) or raw data. GapCloser was used to reduce

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

| Bacteria:                       |               |                           |               |
|---------------------------------|---------------|---------------------------|---------------|
| <i>Urinicoccus massiliensis</i> |               |                           |               |
| Test                            | Results (+/-) | Test                      | Results (+/-) |
| Control                         | -             | Esculine                  | -             |
| Glycerol                        | -             | Salicine                  | +             |
| Erythrol                        | -             | D-cellobiose              | -             |
| D-arabinose                     | -             | D-maltose                 | +             |
| L-arabinose                     | +             | D-lactose                 | +             |
| D-ribose                        | -             | D-melibiose               | +             |
| D-xylose                        | +             | D-saccharose              | +             |
| L-xylose                        | +             | D-trehalose               | +             |
| D-adonitol                      | +             | Inuline                   | +             |
| Methyl-βD-xylopyranoside        | +             | D-melezitose              | -             |
| D-galactose                     | -             | D-raffinose               | +             |
| D-glucose                       | +             | Amidon                    | +             |
| D-fructose                      | +             | Glycogene                 | +             |
| D-mannose                       | +             | Xylitol                   | -             |
| L-sorbose                       | +             | Gentibiose                | +             |
| L-rhamnose                      | -             | D-turanose                | +             |
| Dulcitol                        | +             | D-lyxose                  | +             |
| Inositol                        | -             | D-tagatose                | +             |
| D-mannitol                      | -             | D-fucose                  | +             |
| D-sorbitol                      | -             | L-fucose                  | +             |
| Methyl-αD-mannopyranoside       | -             | D-arabitol                | +             |
| Methyl-αD-glucopyranoside       | -             | L-arabitol                | +             |
| N-acetylglucosamine             | -             | Potassium gluconate       | +             |
| Amygdaline                      | -             | Potassium 2-cetogluconate | -             |
| Arbutine                        | -             | Potassium 5-cetogluconate | +             |

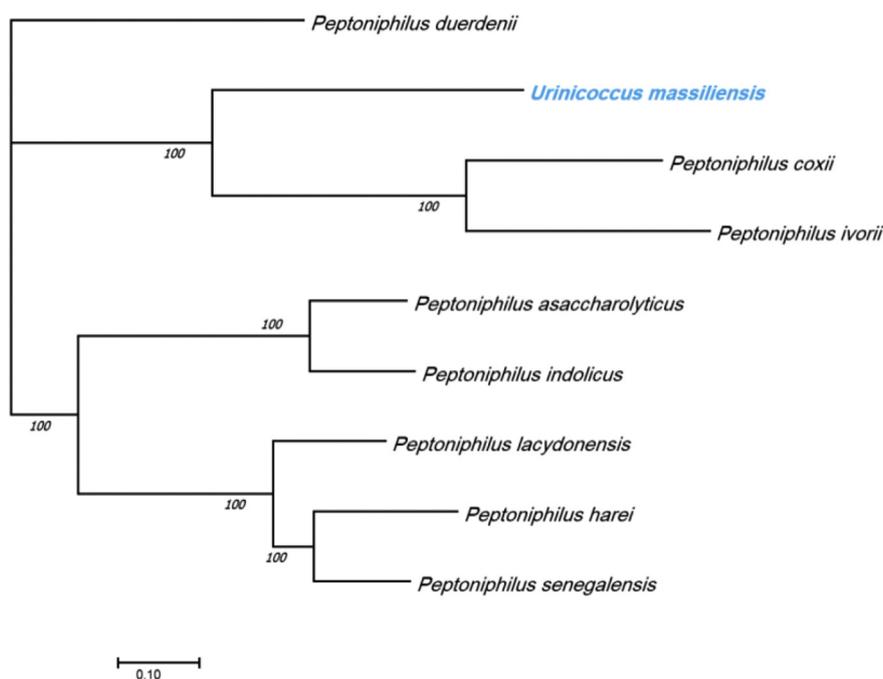
**TABLE 4. Biochemical characteristics of all studied species**

| Characteristics                | <i>Urinococcus massiliensis</i> | <i>Peptoniphilus asaccharolyticus</i> | <i>Peptoniphilus coxii</i> | <i>Peptoniphilus duerdenii</i> | <i>Peptoniphilus harei</i> | <i>Peptoniphilus indolicus</i> | <i>Peptoniphilus ivorii</i> | <i>Peptoniphilus lacydonensis</i> | <i>Peptoniphilus senegalensis</i> |
|--------------------------------|---------------------------------|---------------------------------------|----------------------------|--------------------------------|----------------------------|--------------------------------|-----------------------------|-----------------------------------|-----------------------------------|
| 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                      | -                               | -                                     | -                          | -                              | -                          | +                              | -                           | NA                                | -                                 |
| Fermentation of:               |                                 |                                       |                            |                                |                            |                                |                             |                                   |                                   |
| glucose                        | +                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| lactose                        | +                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| raffinose                      | +                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| mannose                        | +                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| Activity of:                   |                                 |                                       |                            |                                |                            |                                |                             |                                   |                                   |
| α-galactosidase                | -                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| β-galactosidase                | -                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| α-glucosidase                  | -                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| β-glucosidase                  | +                               | -                                     | -                          | -                              | -                          | -                              | -                           | -                                 | -                                 |
| arginine arylamidase           | NA                              | +                                     | -                          | -                              | +                          | +                              | -                           | NA                                | +                                 |
| proline arylamidase            | NA                              | -                                     | +                          | -                              | -                          | -                              | +                           | NA                                | -                                 |
| phenylalanine arylamidase      | NA                              | -                                     | -                          | -                              | -                          | +                              | -                           | NA                                | -                                 |
| leucine arylamidase            | -                               | SD                                    | -                          | +                              | SD                         | +                              | -                           | -                                 | WR                                |
| pyroglutamyl arylamidase       | NA                              | -                                     | -                          | -                              | -                          | -                              | -                           | NA                                | -                                 |
| histidine arylamidase          | NA                              | WR                                    | -                          | -                              | +                          | +                              | -                           | NA                                | +                                 |

SD, strain-dependent; WR: weak reaction.

**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 |
|---------------------------------------|------------------|-----------|------|--------------|
| <i>Urinicoccus massiliensis</i>       | FPLH00000000     | 2.08      | 41.7 | 2047         |
| <i>Peptoniphilus harei</i>            | AENP00000000     | 1.84      | 34.4 | 1766         |
| <i>Peptoniphilus duerdenii</i>        | AEEH00000000     | 2.08      | 34.2 | 2018         |
| <i>Peptoniphilus senegalensis</i>     | CAEL00000000     | 1.84      | 32.3 | 1726         |
| <i>Peptoniphilus coxii</i>            | LSDG00000000     | 1.84      | 44.6 | 1783         |
| <i>Peptoniphilus lacydonensis</i>     | FNWF00000000     | 1.85      | 29.9 | 1788         |
| <i>Peptoniphilus asaccharolyticus</i> | FWVW00000000     | 2.23      | 32.3 | 2268         |
| <i>Peptoniphilus ivorii</i>           | LR134523.1       | 1.59      | 53.2 | 1569         |
| <i>Peptoniphilus indolicus</i>        | AGBB00000000     | 2.24      | 31.7 | 2145         |

**FIG. 4.** Phylogenetic tree based on core genes highlighting the position of *Urinicoccus massiliensis* (blue) relative to other closely related bacterial taxa. The annotated GFF3 file of reference genomes was used as matrix in Roary version 3.10.2 on galaxy online site (<http://www.usegalaxy.org.au>) choosing a minimum percentage blastp identity of 50% as previously described [17]. Core-genome alignment was uploaded in NG-PHYLOGENY platform (<https://ngphylogeny.fr/>). Using the 7.0 version MEGA software, core genome sequences were realigned using Muscle v3.8.31 with default parameters and phylogenetic relationships inferred using the Maximum Likelihood method with 1000 bootstrap replicates. The scale bar indicates a 10% nucleotide sequence divergence.

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<sup>T</sup> 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<sup>T</sup> with closely related species was estimated using the digital DNA–DNA hybridization tool [16]. Values among closely related species (Table 6) ranged from 53.6 ± 5.4% between *Peptoniphilus asaccharolyticus* and *Peptoniphilus coxii* to 17.5 ± 4.5% between *Urinicoccus massiliensis* and *Peptoniphilus senegalensis*. When the isolate was compared to these closely related species, values ranged from 17.5 ± 4.5% with *Peptoniphilus senegalensis* to 38.6 ± 5% with *Peptoniphilus asaccharolyticus*.



Heatmap generated with OrthoANI values calculated from the OAT software. Please cite Lee et al. 2015.

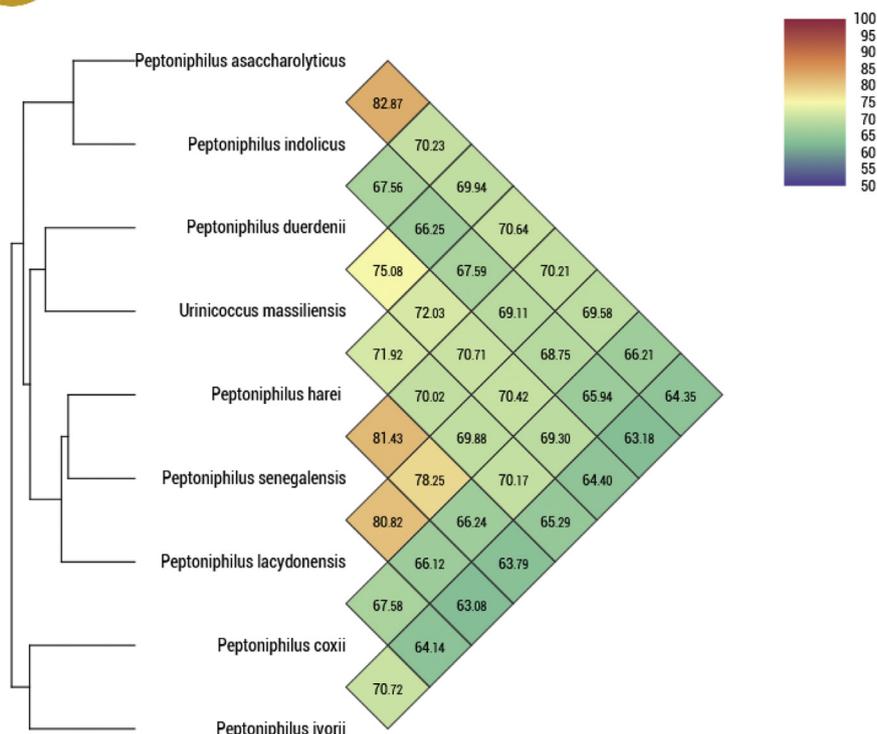


FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software between genus species and other closely related species with standing in nomenclature.

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 <i>Peptoniphilus asaccharolyticus</i> | 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 <i>Peptoniphilus coxii</i>            |     | 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 <i>Peptoniphilus duerdenii</i>        |     |            | 100        | 35.4 ± 4.9 | 34.5 ± 4.9 | 34.3 ± 5   | 33.4 ± 4.9 | 33.3 ± 4.9 | 32.9 ± 5   |
| 4 <i>Peptoniphilus harei</i>            |     |            |            | 100        | 32.2 ± 4.9 | 32 ± 4.9   | 31 ± 4.9   | 30.7 ± 4.9 | 30.2 ± 4.9 |
| 5 <i>Peptoniphilus indolicus</i>        |     |            |            |            | 100        | 27 ± 4.9   | 26.2 ± 4.9 | 24.7 ± 4.8 | 24.3 ± 4.7 |
| 6 <i>Peptoniphilus ivorii</i>           |     |            |            |            |            | 100        | 24.1 ± 4.8 | 23.8 ± 4.8 | 22.4 ± 4.7 |
| 7 <i>Peptoniphilus lacydonensis</i>     |     |            |            |            |            |            | 100        | 20.3 ± 4.6 | 20 ± 4.7   |
| 8 <i>Peptoniphilus senegalensis</i>     |     |            |            |            |            |            |            | 100        | 17.5 ± 4.5 |
| 9 <i>Urinicoccus massiliensis</i>       |     |            |            |            |            |            |            |            | 100        |

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

## 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-PI992<sup>T</sup> was deposited in two different strain collections (= CSURPI992 = DSM100581).

## 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-IAHU- 03).

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