**New Species**

**Genome sequence and description of *Urinicoccus timonensis* gen. nov., sp. nov., a new bacterium isolated from a human stool sample**

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**Abstract**

*Urinicoccus timonensis* gen. nov., sp. nov. strain Marseille-P3926<sup>T</sup> is a new species from the phylum Firmicutes and the family Peptoniphilaceae that was isolated from a human faeces sample. Genome was 1,978,908 bp long with a 41.1 G + C content. The closest species based on 16S ribosomal RNA was *Peptoniphilus ivorii* DSM 10022 with 90.8% sequence similarity. Considering phenotypic features, 16S rRNA sequence and comparative genome studies, we proposed Marseille-P3926<sup>T</sup> as the strain type of *Urinicoccus timonensis* gen. nov., sp. nov.

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**Introduction**

Deciphering the bacterial diversity linked to normal and pathogenic functions appears to be fundamental [1]. The culturomic approach, complementary to the metagenomic method, based on the increase of culture conditions, has considerably broadened our knowledge of the human gut microbiota [2–4]. The isolation, culture and characterization of microorganisms are essential to understand the overall physiology of the microbiota of the human gastrointestinal (GI) tract [5]. In this report, we report the isolation of a novel genus and species, *Urinicoccus timonensis*, which was isolated from a sample of human faeces.

**Isolation and growth conditions**

In February 2017, we isolated from a human faeces sample, a bacterial strain that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The screening was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously reported [6]. The spectra obtained (Fig. 1) were imported and analysed using the Biotyper 3.0 software against the MEPHI database. The strain was isolated on 5% sheep blood-enriched Columbia agar (bio-Mérieux, Marcy l’Étoile, France) at 37°C and pH 7.5 in an...
anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France) after a 15-day pre-incubation in an anaerobic bottle containing blood. The culture vial (Becton Dickson, Le Pont-de-Claix, France) was supplemented with 5 mL of 0.2-μm filtered rumen fluid.

Phenotypic characteristics

Colonies were white and smooth with a mean diameter of 1 to 4 mm. Bacterial cells were Gram-positive cocci with a mean diameter of 0.67 μm (Fig. 2). Strain Marseille-P3926T exhibited neither catalase nor oxidase activities. API 50 CH and API ZYM test were performed at 37°C under anaerobic conditions and the results are summarized in Table 1.

Strain identification

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 [7]. The 16S rRNA nucleotide sequence was assembled and corrected using CodonCode Aligner software (http://www.codoncode.com).

Strain Marseille-P3926T exhibited a 90.80% 16S rRNA similarity with Peptoniphilus ivorii DSM 10022 (GenBank accession number NR_026359). We consequently proposed to classify Strain Marseille-P3926T as a new species within the genus Urinococcus in the phylum Firmicutes (Fig. 3).
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 preparation kit and Nextera XT Paired End (Illumina), as previously described [8]. The assembly was performed using a pipeline containing several software (Velvet [9], Spades [10] and Soap Denovo [11]), on trimmed data (MiSeq and Trimmomatic [12] software) or untrimmed data (only MiSeq software). 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 using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P3926 was 1 978 908 bp long with a 41.1 mol% G + C content (Fig. 4; Table 2). The digital DNA–DNA hybridization (dDDH) values obtained from U. timonensis strain Marseille-P3926 by comparison with other close strains are detailed in Table 3. These dDDH values were <70% of the recommended threshold for species demarcation [13], confirming that the strain studied is representative of a new species. Of the 1931 predicted genes,
FIG. 3. Phylogenetic tree highlighting the position of *Urinicoccus timonensis* gen. nov., sp. nov. with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inferences were obtained using the maximum likelihood method and the MEGA 7 software. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 5% nucleotide sequence divergence.

TABLE 2. Genomic comparison of *Urinicoccus timonensis* gen. nov., sp. nov., and other closely related species with standing in nomenclature

| GenBank accession numbers | Size | Number of RNAs | Number of protein-coding genes | Number of genes | G + C content (%) |
|---------------------------|------|----------------|--------------------------------|-----------------|------------------|
| *Urinicoccus timonensis* | 0CTU01000001.1 | 1 978 908 | 60 | 1871 | 1931 | 41.1 |
| *Tissierella creatinini* | SUSS01000001.1 | 2 611 442 | 58 | 2471 | 2533 | 37.7 |
| *Peptoniphilus indolicus* | NZ_JH165061.1 | 2 237 864 | 33 | 2133 | 2166 | 29.7 |
| *Peptoniphilus grossensis* | NZ_HE971666.1 | 2 101 866 | 29 | 1983 | 2032 | 32.7 |
| *Peptoniphilus cauli* | NZ_KQ960154.1 | 1 837 050 | 39 | 1713 | 1752 | 44.5 |
| *Peptoniphilus asaccharolyticus* | NZ_FYWR0100017.1 | 2 232 586 | 32 | 2283 | 2315 | 32.2 |
| *Keraatinibaculum paraultunense* | SWAE01000001.1 | 2 248 613 | 55 | 2156 | 2211 | 29.4 |
| *Peptoniphilus ivori* | NZ_LR134523.1 | 1 587 771 | 64 | 1488 | 1574 | 53.2 |

FIG. 4. A circular map generated using the CGView [15] Server showing a complete view of the genome of *Urinicoccus timonensis* gen. nov., sp. nov.
### TABLE 3. Numerical DNA–DNA hybridization (DDH) values (%) obtained by comparison between *Urinicoccus timonensis* gen. nov., sp. nov., and other closely related species using GGDC formula 2 software (DDH estimates based on HSP identities/length) ([https://ggdc.dsmz.de/ggdc.php#](https://ggdc.dsmz.de/ggdc.php#)), top right

|                      | *Urinicoccus timonensis* | *Tissierella creatinini* | *Peptoniphilus indolicus* | *Peptoniphilus grossensis* | *Peptoniphilus coxii* | *Peptoniphilus asaccharolyticus* | *Keratinibaculum paraultunense* | *Peptoniphilus ivorii* |
|----------------------|--------------------------|--------------------------|---------------------------|---------------------------|------------------------|---------------------------------|-------------------------------|-----------------------|
| *Urinicoccus timonensis* | 100%                     | 33.00% (30.6%–35.5%)     | 38.60% (36.1%–41.1%)     | 42.50% (39.9%–45%)        | 39.00% (36.5%–41.5%)  | 33.20% (30.8%–35.7%)           | 28.50% (26.1%–31%)            | 32.70% (30.3%–35.2%)         |
| *Tissierella creatinini* | 100%                     | 100%                     | 30.10% (27.7%–32.6%)     | 17.80% (15.7%–20.2%)      | 45.10% (42.6%–47.7%)  | 26.90% (24.6%–29.4%)           | 21.80% (19.6%–24.3%)          | 30.70% (28.3%–33.2%)         |
| *Peptoniphilus indolicus* | 100%                     | 100%                     | 41.70% (39.2%–44.3%)     | 37.50% (35.1%–40.5%)      | 41.70% (39.2%–44.3%)  | 28.50% (26.1%–31%)             | 30.70% (28.3%–33.2%)          | 30.70% (28.3%–33.2%)         |
| *Peptoniphilus grossensis* | 100%                     | 100%                     | 26.70% (24.3%–29.1%)     | 35.40% (33%–37.9%)        | 35.40% (33%–37.9%)     | 35.40% (33%–37.9%)             | 35.40% (33%–37.9%)            | 35.40% (33%–37.9%)         |
| *Peptoniphilus coxii* | 100%                     | 100%                     | 26.70% (24.3%–29.1%)     | 35.40% (33%–37.9%)        | 35.40% (33%–37.9%)     | 35.40% (33%–37.9%)             | 35.40% (33%–37.9%)            | 35.40% (33%–37.9%)         |
| *Peptoniphilus asaccharolyticus* | 100%                     | 100%                     | 26.70% (24.3%–29.1%)     | 35.40% (33%–37.9%)        | 35.40% (33%–37.9%)     | 35.40% (33%–37.9%)             | 35.40% (33%–37.9%)            | 35.40% (33%–37.9%)         |
| *Keratinibaculum paraultunense* | 100%                     | 100%                     | 26.70% (24.3%–29.1%)     | 35.40% (33%–37.9%)        | 35.40% (33%–37.9%)     | 35.40% (33%–37.9%)             | 35.40% (33%–37.9%)            | 35.40% (33%–37.9%)         |
| *Peptoniphilus ivorii* | 100%                     | 100%                     | 26.70% (24.3%–29.1%)     | 35.40% (33%–37.9%)        | 35.40% (33%–37.9%)     | 35.40% (33%–37.9%)             | 35.40% (33%–37.9%)            | 35.40% (33%–37.9%)         |

**Legend:**
- NC: Not in COG
- FA: Function unknown
- GF: General function prediction only
- M: Inorganic ion transport and metabolism
- L: Lipid transport and metabolism
- C: Coenzyme transport and metabolism
- N: Nucleotide transport and metabolism
- A: Amino acid transport and metabolism
- C: Carbohydrate transport and metabolism
- E: Energy production and conversion
- M: Methylamine, propanol, toluene, xenobiotics
- P: Posttranslational modification, protein turnover, chaperones
- I: Intracellular trafficking, secretion, and vesicular transport
- EX: Extracellular structures
- B: Cytoskeleton
- C: Cell motility
- W: Cell wall/membrane/envelope biogenesis
- S: Signal transduction mechanisms
- D: Defense mechanisms
- K: Nuclear structure
- C: Cell cycle control, cell division, chromosome partitioning
- C: Chromatin structure and dynamics
- R: Replication, recombination and repair
- T: Translation
- P: RNA processing and modification
- B: Translation, ribosomal structure and biogenesis

**FIG. 5.** Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Urinicoccus timonensis* gen. nov., sp. nov., among other closely related species.
### TABLE 4. Number of genes associated with the 25 general clusters of orthologous group functional categories

| Cluster | Urinicoccus | Peptoniphilus | Peptoniphilus | Peptoniphilus | Peptoniphilus | Peptoniphilus | Keratinibaculum | Description |
|---------|--------------|---------------|---------------|---------------|---------------|---------------|----------------|-------------|
| [A]     | 0            | 0             | 0             | 0             | 0             | 0             | 0              |             |
| [B]     | 1            | 1             | 1             | 1             | 1             | 1             | 2              |             |
| [C]     | 178          | 178           | 209           | 186           | 179           | 182           | 188            | 196         |
| [D]     | 90           | 74            | 110           | 99            | 104           | 88            | 133            | 99          |
| [E]     | 63           | 69            | 132           | 74            | 71            | 67            | 73             | 100         |
| [F]     | 79           | 63            | 104           | 69            | 74            | 73            | 70             | 120         |
| [G]     | 59           | 48            | 66            | 85            | 89            | 68            | 73             | 100         |
| [H]     | 27           | 22            | 43            | 24            | 30            | 23            | 32             | 44          |
| [I]     | 115          | 79            | 141           | 130           | 126           | 111           | 116            | 140         |
| [J]     | 1            | 1             | 1             | 1             | 1             | 1             | 1              |             |
| [K]     | 0            | 0             | 0             | 0             | 0             | 0             | 0              |             |
| [L]     | 0            | 0             | 0             | 0             | 0             | 0             | 0              |             |
| [M]     | 1            | 1             | 1             | 1             | 1             | 1             | 1              |             |
| [N]     | 9            | 6             | 14            | 6             | 8             | 8             | 10             | 60          |
| [O]     | 7            | 9             | 14            | 6             | 8             | 8             | 10             | 60          |
| [P]     | 0            | 0             | 2             | 0             | 0             | 0             | 0              |             |
| [Q]     | 0            | 0             | 2             | 0             | 0             | 0             | 0              |             |
| [R]     | 15           | 13            | 20            | 21            | 18            | 16            | 29             | 31          |
| [S]     | 79           | 63            | 104           | 69            | 74            | 73            | 70             | 120         |
| [T]     | 79           | 63            | 104           | 69            | 74            | 73            | 70             | 120         |
| [U]     | 63           | 69            | 132           | 74            | 71            | 67            | 73             | 100         |
| [V]     | 59           | 48            | 66            | 85            | 89            | 68            | 73             | 100         |
| [W]     | 27           | 22            | 43            | 24            | 30            | 23            | 32             | 44          |
| [X]     | 15           | 13            | 20            | 21            | 18            | 16            | 29             | 31          |
| [Y]     | 0            | 0             | 0             | 0             | 0             | 0             | 0              |             |
| [Z]     | 0            | 0             | 0             | 0             | 0             | 0             | 0              |             |

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1871 were protein-coding genes and 60 were RNAs. A total of 1619 genes were assigned as putative function and 427 genes were annotated as hypothetical proteins (Fig. 5; Table 4). The degree of genomic similarity of strain Marseille-P3926T with closely related species was estimated using OAT software [14]. OrthoANI values among closely related species (Fig. 6) ranged from 62.22% between Tissierella creatinini and Peptoniphilus ivorii to 82.9% between Peptoniphilus asaccharolyticus and Peptoniphilus indolicus. When Urinicoccus timonensis was compared with these closely related species, values ranged from 63.78% with T. creatinini to 70.75% with Peptoniphilus grossensis.

Conclusion

On the basis of 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 the creation of Urinicoccus timonensis gen. nov., sp. nov., whose type strain is strain Marseille-P3926.

Description of Urinicoccus gen. nov.

Urinicoccus, refers to urina, which is the latin name of urine and coccus, which is the name of bacteria with a round shape. Colonies were white and smooth with a mean diameter of 1–4 mm. Bacterial cells were Gram-positive cocci with a mean diameter of 0.67 μm (Fig. 2). Strain Marseille-P3926T exhibited neither catalase nor oxidase activities. The type species of the genus is Urinicoccus timonensis.

Description of Urinicoccus timonensis sp. nov.

Urinicoccus timonensis sp. nov. (ti.mo.nen’sis. L. gen. masc. timonensis, of Timone, the name of the hospital where strain Marseille-P3926T was cultivated). is classified as a member of the family Peptoniphilaceae in the phylum Firmicutes. Strain Marseille-P3926T is the type strain of the new species ‘Urinicoccus timonensis’ gen. nov., sp. nov. It is a strictly anaerobic, Gram-positive coccus. Colonies of Strain Marseille-P3926T were white and smooth with a mean diameter of 1–4 mm. This bacterial strain does not present any catalase and oxidase ac-
activities. The genome size of *Urinicoccus timonensis* strain Marseille-P3926<sup>T</sup> is 1,978,908 bp with 41.1 mol% G + C content. The GenBank accession number for the 16S rRNA gene sequence of strain Marseille-P3926<sup>T</sup> is LT908436 and for the whole genome shotgun project is NZ_OCTU00000000.1. It was isolated from a human faeces sample.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT908436 and NZ_OCTU00000000.1, respectively.

**Conflicts of interest**

None to declare.

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**Ethics and consent**

The study was approved by the ethics committee of the Institut Hospitalo-Universitaire Méditerranée Infection (IHU-MI) under reference 2016-010.

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