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^T 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^T 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 Bruker database, which is continually incremented with the MEPHI database. The strain was isolated on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Étoile, France) at 37°C and pH 7.5 in an

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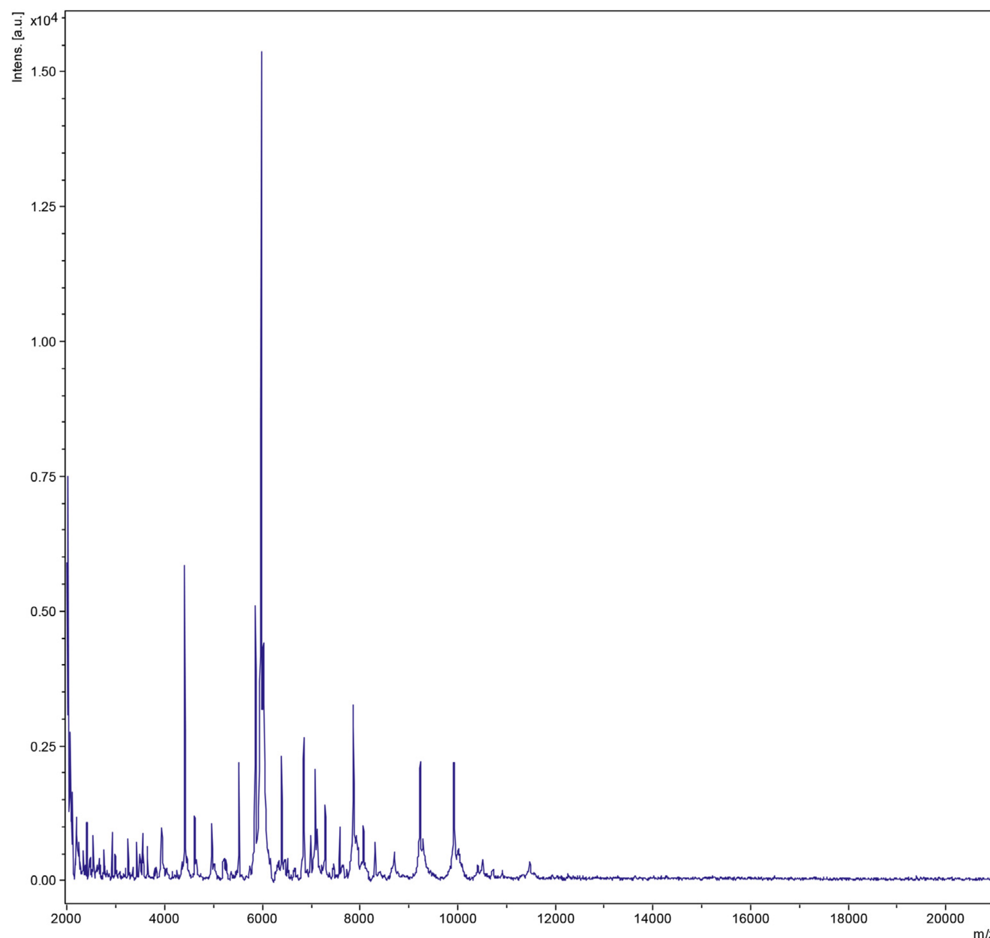

FIG. 1. MALDI-TOF MS reference spectrum of *Urinicoccus timonensis* gen. nov., sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

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-P3926^T 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-P3926^T exhibited a 90.80% 16S rRNA similarity with *Peptoniphilus ivorii* DSM 10022 (GenBank accession number NR_026359). We consequently proposed to classify Strain Marseille-P3926^T as a new species within the genus *Urinicoccus* in the phylum *Firmicutes* (Fig. 3).

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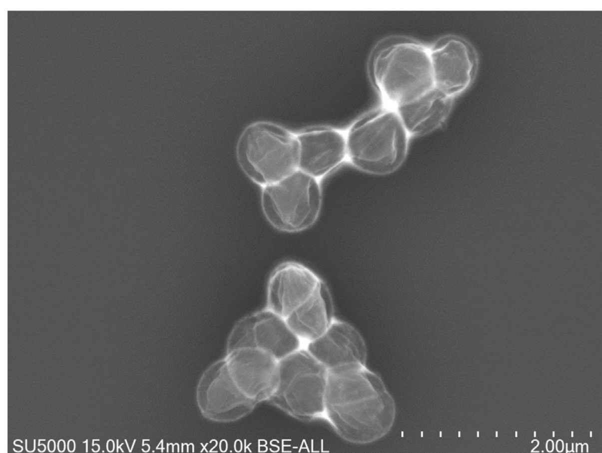

FIG. 2. Scanning electron microscopy (SEM) of stained *Urinicoccus timonensis* gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes 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 SU5000) approximately 60 cm in height and 33 cm in width to evaluate bacteria structure. Scales and acquisition settings are shown in the figure.

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^T 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,

TABLE 1. Phenotypic characterization of *Urinicoccus timonensis* based on the biochemical tests. Profile Index (A) API 50 CH, (B) API ZYM

Bacteria: *Urinicoccus timonensis*

(A) API 50 CH

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-β-D-xylopyranoside	—	d-melezitose	—
D-galactose	+	d-raffinose	—
D-glucose	+	Amidon	—
D-fructose	—	Glycogene	—
D-mannose	—	Xylitol	—
L-sorbose	—	Gentibiose	—
L-rhammose	—	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 5-cetogluconate	+

Bacteria: *Urinicoccus timonensis*

API ZYM

Test	Results (+/−)
Control	—
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	—

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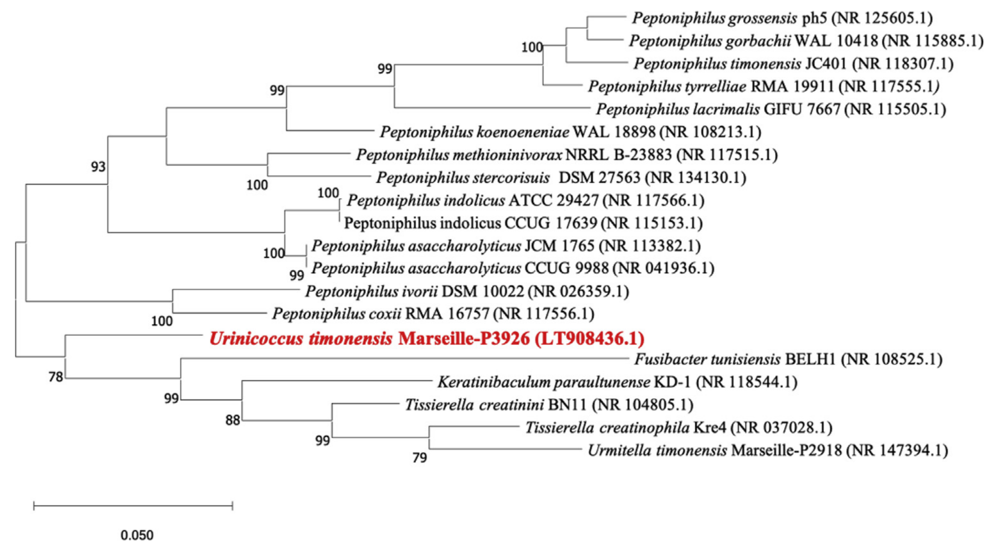

FIG. 3. Phylogenetic tree highlighting the position of *Urinicoccus timonensis* gen. nov., sp. nov. with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inferences were 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.

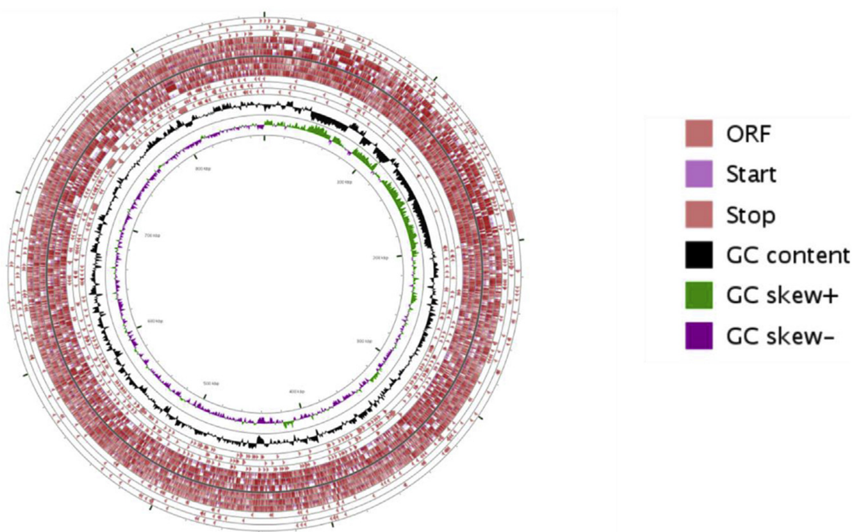

FIG. 4. A circular map generated using the CGView [15] Server showing a complete view of the genome of *Urinicoccus timonensis* gen. nov., sp. nov.

TABLE 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_HE978566.1	2 101 866	29	1983	2012	32.7
Peptoniphilus coxii	NZ_KQ960154.1	1 837 050	39	1713	1752	44.5
Peptoniphilus asaccharolyticus	NZ_FWWR0100017.1	2 232 586	32	2283	2315	32.2
Keratinibaculum paraultunense	SWAE01000001.1	2 248 613	55	2156	2211	29.4
Peptoniphilus ivorii	NZ_LR134523.1	1 587 771	64	1488	1574	53.2

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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#), 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%	30.10% (27.7%–32.6%)	17.80% (15.7%–20.2%)	17.50% (15.4%–19.9%)	33.10% (30.7%–35.6%)	18.40% (16.2%–20.8%)	31.90% (29.5%–34.4%)
Peptoniphilus_indolicus			100%	23.70% (21.4%–26.2%)	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_grossensis				100%	41.70% (39.2%–44.3%)	26.70% (24.3%–29.1%)	28.50% (26.1%–31%)	39.70% (37.2%–42.2%)
Peptoniphilus_coxii					100%	35.40% (33%–37.9%)	33.20% (30.8%–35.7%)	17.50% (15.4%–19.9%)
Peptoniphilus_asaccharolyticus						100%	30.20% (27.9%–32.7%)	35.40% (33%–38%)
Keratinibaculum_paraultunense							100%	29.10% (26.7%–31.6%)
Peptoniphilus ivorii								100%

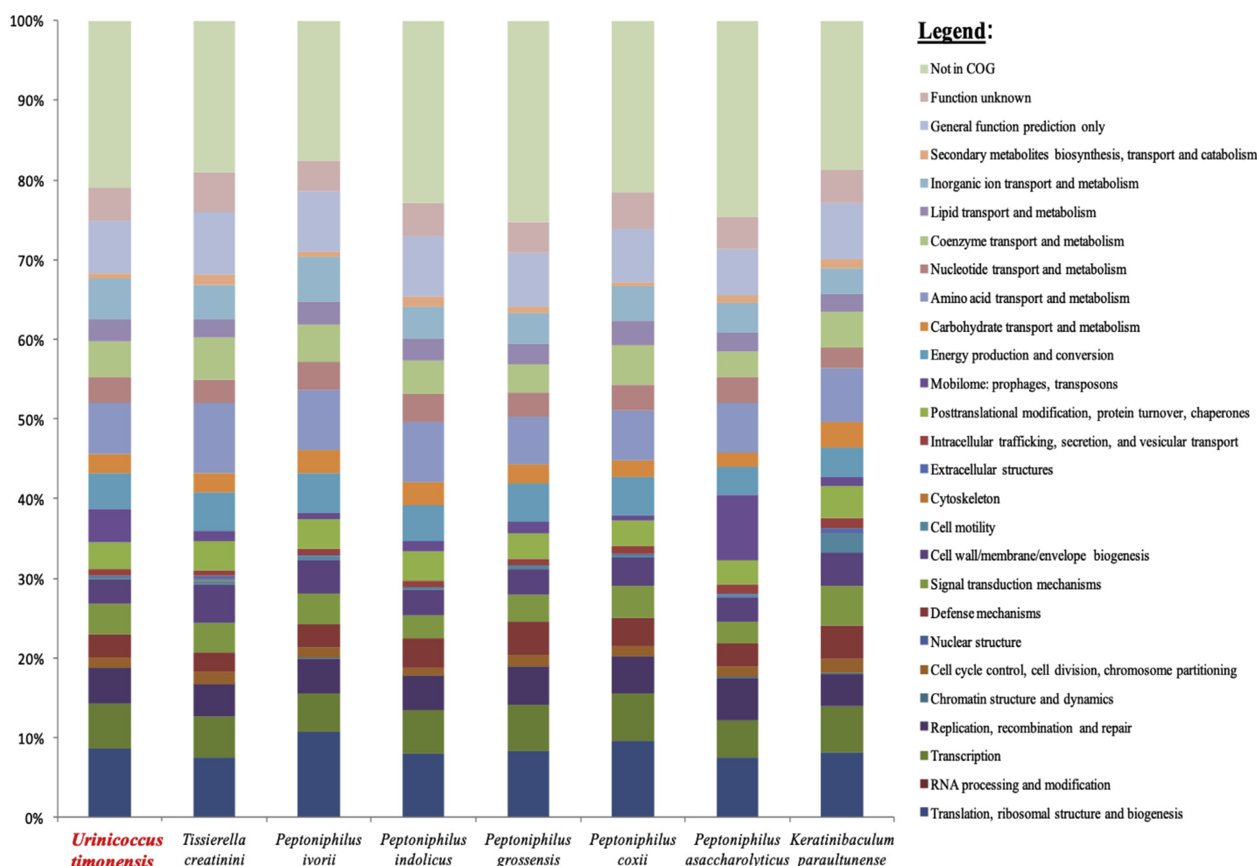

FIG. 5. Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Urinicoccus timonensis* gen. nov., sp. nov., among other closely related species.

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TABLE 4. Number of genes associated with the 25 general clusters of orthologous group functional categories

	Urinicoccus timonensis	Peptoniphilus ivorii	Tissierella creatinini	Peptoniphilus indolicus	Peptoniphilus grossensis	Peptoniphilus coxii	Peptoniphilus asaccharolyticus	Keratinibaculum paraultunense	Description
[J]	178	178	209	186	179	182	188	196	Translation, ribosomal structure and biogenesis
[A]	0	0	0	0	0	0	0	0	RNA processing and modification
[K]	115	79	141	130	126	111	116	140	Transcription
[L]	90	74	110	99	104	88	133	99	Replication, recombination and repair
[B]	1	1	1	1	1	1	1	2	Chromatin structure and dynamics
[D]	27	22	43	24	30	23	32	44	Cell cycle control, cell division, chromosome partitioning
[Y]	0	0	0	0	0	0	0	0	Nuclear structure
[V]	59	48	66	85	89	68	73	100	Defence mechanisms
[T]	79	63	104	69	74	73	70	120	Signal transduction mechanisms
[M]	63	69	132	74	71	67	73	100	Cell wall/ membrane/ envelope biogenesis
[N]	7	9	14	6	8	8	10	60	Cell motility
[Z]	0	0	2	0	0	0	0	1	Cytoskeleton
[W]	3	3	13	1	2	3	2	15	Extracellular structures
[U]	15	13	20	21	18	16	29	31	Intracellular trafficking, secretion, and vesicular transport
[O]	71	62	99	86	69	62	77	97	Post-translational modification, protein turnover, chaperones
[X]	83	13	38	30	30	13	205	27	Mobilome: prophages, transposons
[C]	93	82	130	105	106	89	86	88	Energy production and conversion
[G]	48	49	67	66	52	40	43	80	Carbohydrate transport and metabolism
[E]	132	123	245	177	127	118	159	162	Amino acid transport and metabolism
[F]	68	59	80	83	67	61	78	62	Nucleotide transport and metabolism
[H]	90	79	144	98	77	93	81	107	Coenzyme transport and metabolism
[I]	57	47	62	62	55	57	58	57	Lipid transport and metabolism
[P]	104	92	121	97	82	82	94	78	Inorganic ion transport and metabolism
[Q]	15	12	34	30	19	11	24	24	Secondary metabolites biosynthesis, transport and catabolism
[R]	135	125	219	175	145	125	145	171	General function prediction only
[S]	86	65	138	96	84	85	98	103	Function unknown
–	427	290	523	536	546	407	614	450	Not in cluster of orthologous group

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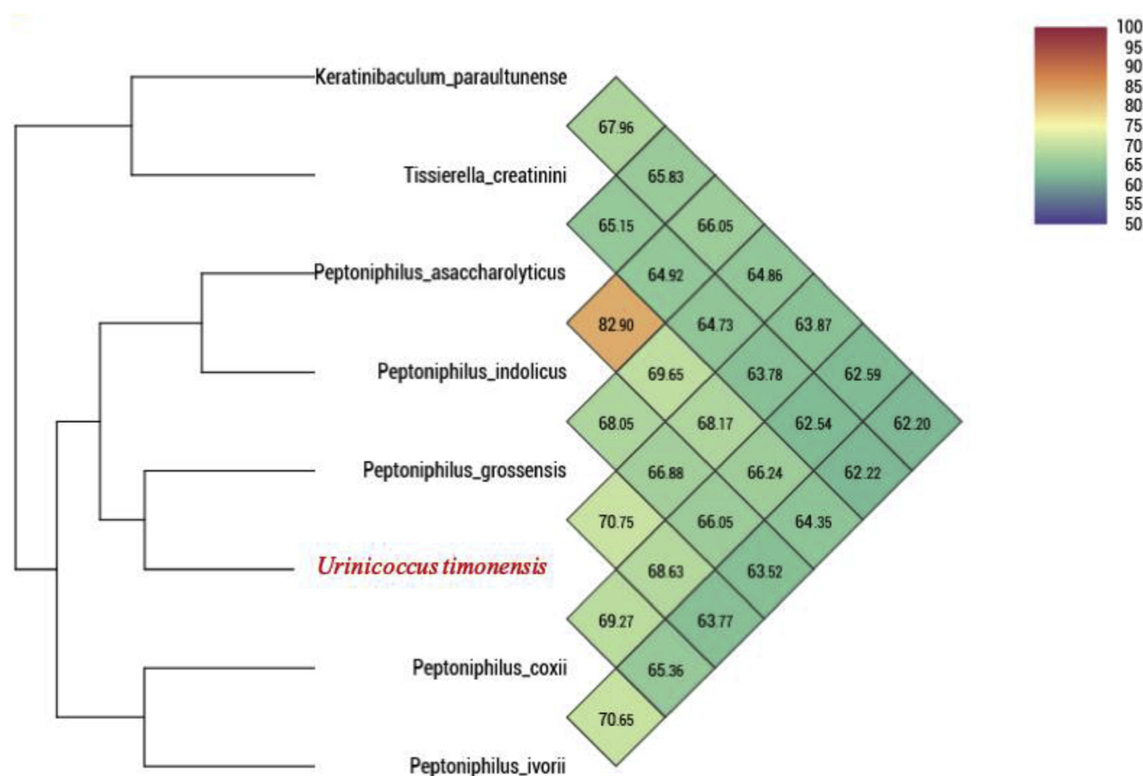

FIG. 6. Heatmap generated with OrthoANI values calculated using the OAT software between *Urinicoccus timonensis* gen. nov., sp. nov. and other closely related species with standing in nomenclature.

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-P3926^{T} 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.

U.ri.ni.coc.cus N.L. fem. n. *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-P3926^{T} 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-P3926^{T} was cultivated). is classified as a member of the family *Peptoniphilaceae* in the phylum *Firmicutes*. Strain Marseille-P3926^{T} 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-P3926^{T} were white and smooth with a mean diameter of 1–4 mm. This bacterial strain does not present any catalase and oxidase ac-

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tivities. The genome size of *Urinicoccus timonensis* strain Marseille-P3926[T] 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[T] 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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