

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

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1 Genome sequence and description of *Urinicoccus timonensis* gen. nov., sp. nov., a new 2 bacterium isolated from a human stool sample 3 Mamadou Lamine TALL^{1,2}, Cheikh Ibrahima LO^{1,3}, Edmond KUETE YIMAGOU^{1,2}, 4 Anthony FONTANINI², Jeremy DELERCE^{1,2}, Pierre-Edouard FOURNIER^{1,2}, Didier 5 RAOULT^{1,2}, Florence FENOLLAR^{1,3}, Anthony LEVASSEUR^{1,2,4,*} 6 7 8 ¹ Aix-Marseille Université, UMR MEPHI (Microbes, Evolution, Phylogeny and Infections), 9 IRD, APHM, Faculté de Médecine, Marseille 13005, France. 10 ² IHU-Méditerranée Infection, Marseille, France. ³ Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France 11 ⁴ Institut Universitaire de France (IUF), Paris, France 12 13 * Corresponding author: Pr. Anthony Levasseur, AMU, UMR MEPHI (Microbes, Evolution, 14 15 Phylogeny and Infection), UM63, IRD, APHM, IHU - Méditerranée Infection, 19-21 16 Boulevard Jean Moulin, 13385, Marseille cedex 05, France. 17 E-mail: anthony.levasseur@univ-amu.fr 18 Running title: *Urinicoccus timonensis* gen. nov., sp. nov. 19 20 Keywords: Urinicoccus timonensis gen. nov., sp. nov., culturomics, gut, taxonogenomics 21

Abstract

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

Introduction

- 9 The deciphering of the bacterial diversity linked to normal and pathogenic functions appears
- 10 to be fundamental [1]. The culturomic approach, complementary to the metagenomic method,
- based on the increase of culture conditions, has considerably broadened the repertoire 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
- 14 (GI) tract [5]. In this report, we report the isolation of a novel genus and species, *Urinicoccus*
- 15 *timonensis* which was isolated from the Human feces sample.

Isolation and growth conditions

- 17 In February 2017, we isolated from human feces sample, a bacterial strain that could not be
- 18 identified by Matrix Assisted Laser Desorption Ionization –Time of Flight Mass Spectrometry
- 19 (MALDI-TOF MS). The screening was performed on a Microflex LT spectrometer (Bruker,
- 20 Daltonics, Bremen, Germany) as previously reported [6]. The spectra obtained (Figure 1)
- were imported and analyzed using the Biotyper 3.0 software against the Bruker database that
- was continually incremented with MEPHI database. The strain was isolated on 5% sheep
- blood-enriched Columbia agar (COS) (bioMérieux, Marcy l'Etoile, France) at 37°C and PH
- 24 7.5 in anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France) after a 15-days pre-

- 25 incubation in an anaerobic bottle containing a blood. Culture vial (Becton Dickson, Le Pont-
- de-Claix, France) was supplemented with 5 ml of 0.2 µm filtered rumen fluid.

27 Phenotypic characteristics

- 28 Colonies were white and smooth with a mean diameter of 1 to 4 mm. Bacterial cells were
- 29 Gram-positive cocci with a mean diameter of 0.67 μm (Figure 2). Strain Marseille-P3926^T
- 30 exhibited neither catalase nor oxidase activities. API 50 CH and API ZYM test were
- performed at 37°C under anaerobic condition and the results are summarized in table 1.

32 Strain identification

- 33 In order to classify this bacterium, the 16S rRNA gene was amplified using the primer pair
- 34 fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator
- v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher,
- 36 Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was
- 37 assembled and corrected using CodonCode Aligner software (http://www.codoncode.com).
- 38 Strain Marseille-P3926^T exhibited a 90.80 % 16S rRNA similarity with *Peptoniphilus ivorii*
- 39 DSM 10022 (Genbank accession number: NR_026359). We consequently proposed to
- 40 classify Strain Marseille-P3926^T as a new species within the genus *Urinicoccus* in the phylum
- 41 *Firmicutes* (Figure 3).

42

Genome sequencing

- 43 Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen,
- 44 Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc, San Diego,
- 45 CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired End (Illumina),
- as previously described [8]. The assembly was performed using a pipeline containing several
- 47 software (Velvet [9], Spades [10] and Soap Denovo [11]), on trimmed (MiSeq and
- 48 Trimmomatic [12] software) or untrimmed data (only MiSeq software). GapCloser was used
- 49 to reduce assembly gaps. Scaffolds < 800 base pairs (bp) and scaffolds with a depth value

lower than 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 (Figure 4; Table 2). The values dDDH obtained from *U. timonensis* strain Marseille-P3926 by comparison with other close strains were detailed in Table 3. These dDDH values were below 70% of the recommended threshold for species demarcation [13], confirming that the strain studied is representative of a new species. Of the 1,931 predicted genes, 1,871 were protein-coding genes and 60 were RNAs. A total of 1,619 genes were assigned as putative function and 427 genes were annotated as hypothetical proteins (Figure 5; Table 4).The degree of genomic similarity of strain Marseille-P3926^T with closely related species was estimated using the OAT software [14]. OrthoANI values among closely related species (Figure 5) ranged from 62.22% between *Tissierella creatinini* and *Peptoniphilus ivorii* to 82.9 % between *Peptoniphilus asaccharolyticus* and *Peptoniphilus indolicus*. When *Urinicoccus timonensis* was compared to that of these closely 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
- 67 divergence greater than > 1.3 % and, an OrthoANI value lower than 95% with the
- 68 phylogenetically closest species with standing in nomenclature, we formally proposed the
- 69 creation of *Urinicoccus timonensis* gen. nov., sp. nov., whose type strain is strain Marseille-
- 70 P3926.

Description of *Urinicoccus* gen. nov.

- 72 U.ri.ni.coc.cus N.L. fem. n. *Urinicoccus*, refers to urina, which is the latino name of urine
- and coccus which is the name of bacteria with around shape. Colonies were white and smooth
- with a mean diameter of 1 to 4 mm. Bacterial cells were Gram-positive cocci with a mean

- 75 diameter of 0.67 µm (Figure 2). Strain Marseille-P3926^T exhibited neither catalase nor
- oxidase activities. The type species of the genus is *Urinicoccus timonensis*.
- 77 Description of *Urinicoccus timonensis* sp. nov.
- 78 Urinicoccus timonensis sp. nov. (ti.mo.nen'sis. L. gen. masc. timonensis, of Timone, the
- 79 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,
- 82 Gram-positive cocci. Colonies of Strain Marseille-P3926^T were white and smooth with a
- 83 mean diameter of 1 to 4 mm. This bacterial strain does not present any catalase and oxidase
- 84 activities. The genome size of *Urinicoccus timonensis* strain Marseille-P3926^T is 1,978,908
- 85 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 human feces sample.
- Nucleotide sequence accession number. The 16S rRNA gene and genome sequences were
- deposited in Genbank under accession number LT908436 and NZ_OCTU00000000.1,
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- 97 Conflicts of interest:
- 98 None to declare
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104	Ethics and consent
105	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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158 **Table 1:** Phenotypic characterization of *Urinicoccus timonensis* based on the biochemical

tests.

160 Profile Index: (A) API 50 CH, (B) API ZYM.

161 (A)

Bacteria: Urinicoccus timonens	sis			
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-βD-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-αD-mannopyranoside	-	D-arabitol	-	
Methyl-αD-glucopyranoside	-	L-arabitol	-	
N-acetylglucosamine	-	Potassium gluconate	-	
Amygdaline	-	Potassium 2-cetogluconate	-	
Arbutine	-	Potassium 5-cetogluconate	+	

162

163 (B)

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	-
Naphtalo-AS-BI-phosphohydrolase	-
α-galactosidase	-
β-galactosidase	-
β-glucuronidase	-
α-glucosidase	-
β-glucosidase	-
N-acetyl-β-glucosaminidase	-
α-mannosidase	-
α-fucosidase	-

Table2: 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	1,931	41.1
Tissierella_creatinini	SUSSS01000001.1	2,611,442	58	2471	2,533	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

Table 3: Numerical DNA-DNA hybridization 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
	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%

Table 4: Number of genes associated with the 25 general clusters of orthologous group (COG) 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	Defense 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
[0]	71	62	99	86	69	62	77	97	Posttranslational 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
[1]	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 cog

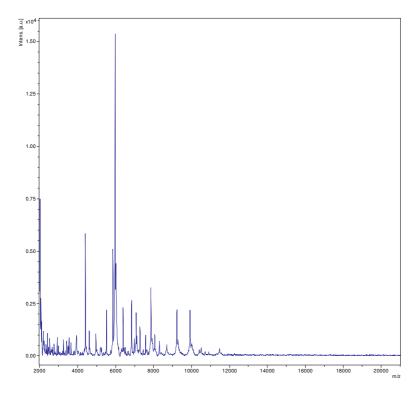


Figure 1: MALDI-TOF MS reference spectrum of *Urinicoccus timonensis* gen. nov., sp. nov.

The reference spectrum was generated by comparison of spectra from 12 individual colonies.

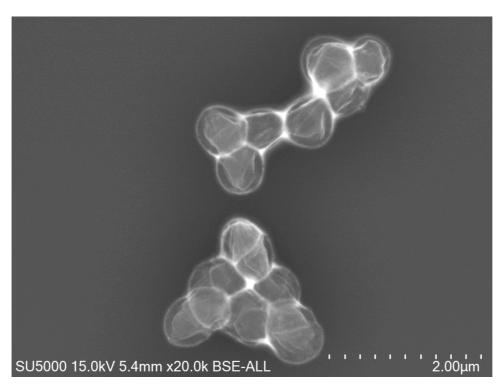


Figure 2: Scanning electron microscopy of stained *Urinicoccus timonensis* gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5 % glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine coated microscope slide for 5 minutes and treated with 1 % phosphotungstic acid (PTA) 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) with approximately 60 centimeters in height and 33 cm in width to evaluate bacteria structure. Scales and acquisition settings are shown in figure.

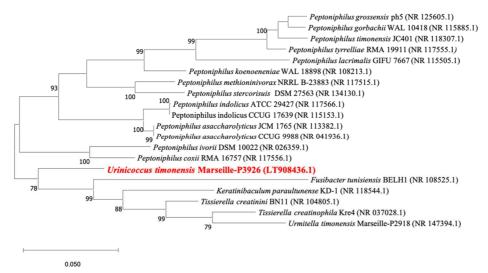


Figure 3: Phylogenetic tree highlighting the position of *Urinicoccus timonensis* gen. nov., sp. nov. with regard to other closely related species. Genbank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inferences were obtained using the Maximum Likelihood method and the MEGA 7 software. Bootstrap values obtained by repeating the analysis 1,000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 5 % nucleotide sequence divergence.

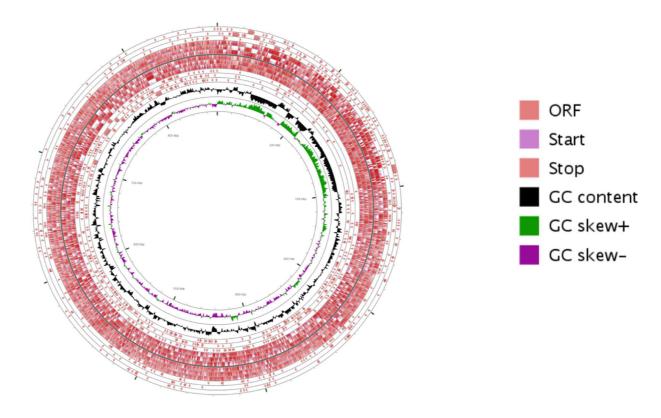


Figure 4: A circular map generated using the CGView [15] Server showing a complete view of the genome of *Urinicoccus timonensis* gen. nov., sp. nov.

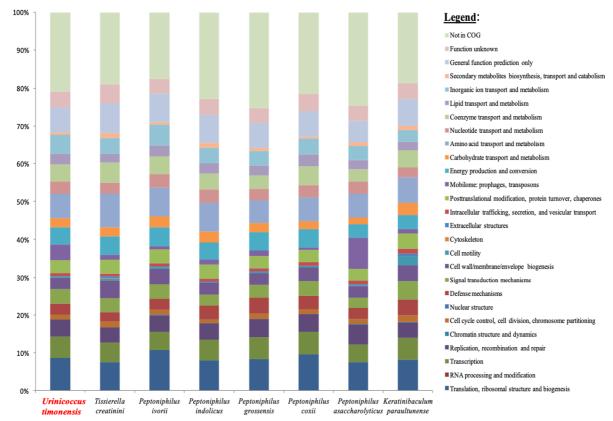


Figure 5: Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Urinicoccus timonensis* gen. nov., sp. nov., among other closely related species.

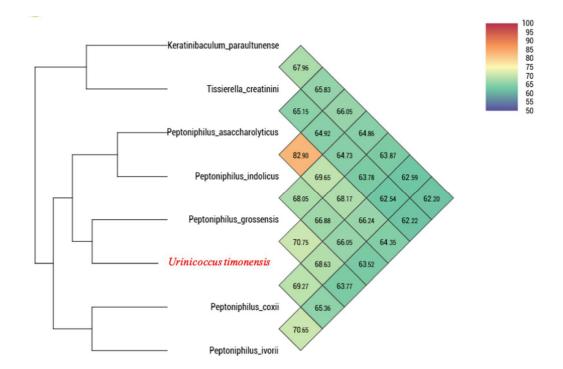


Figure 6: Heatmap generated with OrthoANI values calculated using the OAT software between *Urinicoccus timonensis* gen. nov., sp. nov. and other closely related species with standing in nomenclature.