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

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► To cite this version:

M. L. Tall, C. I. Lo, E. Kuete Yimagou, A. Fontanini, J. Delerce, et al.. Genome sequence and description of *Urinicoccus timonensis* gen. nov., sp. nov., a new bacterium isolated from a human stool sample. *New Microbes and New Infections*, 2020, 37, pp.100720. 10.1016/j.nmni.2020.100720 . hal-03149718

HAL Id: hal-03149718

<https://amu.hal.science/hal-03149718>

Submitted on 7 Sep 2022

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1 **Abstract**

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.

8 **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,
11 based on the increase of culture conditions, has considerably broadened the repertoire of the
12 human gut microbiota [2-4]. The isolation, culture and characterization of microorganisms are
13 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.

16 **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)
21 were imported and analyzed using the Biotyper 3.0 software against the Bruker database that
22 was continually incremented with MEPHI database. The strain was isolated on 5% sheep
23 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-
26 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
31 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
35 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),
46 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

50 lower than 25% of the mean depth were removed. The best assembly was selected using
51 different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-
52 P3926^T was 1,978,908 bp-long with a 41.1 mol% G+C content (Figure 4; Table 2). The
53 values dDDH obtained from *U. timonensis* strain Marseille-P3926 by comparison with other
54 close strains were detailed in Table 3. These dDDH values were below 70% of the
55 recommended threshold for species demarcation [13], confirming that the strain studied is
56 representative of a new species. Of the 1,931 predicted genes, 1,871 were protein-coding
57 genes and 60 were RNAs. A total of 1,619 genes were assigned as putative function and 427
58 genes were annotated as hypothetical proteins (Figure 5; Table 4). The degree of genomic
59 similarity of strain Marseille-P3926^T with closely related species was estimated using the
60 OAT software [14]. OrthoANI values among closely related species (Figure 5) ranged from
61 62.22% between *Tissierella creatinini* and *Peptoniphilus ivorii* to 82.9 % between
62 *Peptoniphilus asaccharolyticus* and *Peptoniphilus indolicus*. When *Urinicoccus timonensis*
63 was compared to that of these closely species, values ranged from 63.78 % with *T. creatinini*
64 to 70.75 % with *Peptoniphilus grossensis*.

65 **Conclusion**

66 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.

71 **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
73 and *coccus* which is the name of bacteria with around shape. Colonies were white and smooth
74 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
76 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
80 of the family *Peptoniphilaceae* in the phylum *Firmicutes*. Strain Marseille-P3926^T is the type
81 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
86 sequence of strain Marseille-P3926^T is LT908436 and for the whole genome shotgun project
87 is NZ_OCTU00000000.1. It was isolated from human feces sample.

88 **Nucleotide sequence accession number.** The 16S rRNA gene and genome sequences were
89 deposited in Genbank under accession number LT908436 and NZ_OCTU00000000.1,
90 respectively.

91 **Acknowledgements**

92 The authors thank A. Caputo for submitting the genomic sequence to GenBank. We also
93 thank T. Irie, K. Imai, S. Matsubara, T.Sakazume, Y. Ominami, H. Akiko and the Hitachi
94 team of Japan (Hitachi High-Technologies Corporation, Science & Medical Systems Business
95 Group 24-14, Tokyo, Japan) for the installation of a TM4000 microscope at the IHU
96 Méditerranée Infection.

97 **Conflicts of interest:**

98 None to declare

99 **Funding sources:**

100 The research was funded by the Mediterranee-Infection foundation and the French National
101 Research Agency under the program “Investissements d’Avenir”, reference ANR-10-IAHU-
102 03. This research was supported by a grant from the Institut Universitaire de France (IUF,
103 Paris, France) to Professor Anthony Levasseur.

104 **Ethics and consent**

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

107 **References**

- 108 [1] The Human Microbiome Project | Nature n.d.
109 <https://www.nature.com/articles/nature06244> (accessed May 4, 2018).
- 110 [2] Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial
111 culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect*
112 2012;18:1185–93. doi:10.1111/1469-0691.12023.
- 113 [3] Lagier J-C, Hugon P, Khelaifia S, Fournier P-E, La Scola B, Raoult D. The rebirth of
114 culture in microbiology through the example of culturomics to study human gut microbiota.
115 *Clin Microbiol Rev* 2015;28:237–64. doi:10.1128/CMR.00014-14.
- 116 [4] Lagier J-C, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of
117 previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol*
118 2016;1:16203. doi:10.1038/nmicrobiol.2016.203.
- 119 [5] Ramasamy D, Mishra AK, Lagier J-C, Padhmanabhan R, Rossi M, Sentausa E, et al.
120 A polyphasic strategy incorporating genomic data for the taxonomic description of novel
121 bacterial species. *Int J Syst Evol Microbiol* 2014;64:384–91. doi:10.1099/ijs.0.057091-0.
- 122 [6] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing
123 revolution in bacteriology: routine identification of bacteria by matrix-assisted laser
124 desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009;49:543–51.
125 doi:10.1086/600885.
- 126 [7] Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, et al.
127 Complementarity between targeted real-time specific PCR and conventional broad-range 16S
128 rDNA PCR in the syndrome-driven diagnosis of infectious diseases. *Eur J Clin Microbiol*
129 *Infect Dis* 2015;34:561–70. doi:10.1007/s10096-014-2263-z.
- 130 [8] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier P-E, Raoult D, et al. Microbial
131 culturomics unravels the halophilic microbiota repertoire of table salt: description of

132 *Gracilibacillus massiliensis* sp. nov. *Microb Ecol Health Dis* 2016;27.
133 doi:10.3402/mehd.v27.32049.

134 [9] Zerbino DR, Birney E. Velvet: Algorithms for de novo short read assembly using de
135 Bruijn graphs. *Genome Res* 2008;18:821–9. doi:10.1101/gr.074492.107.

136 [10] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al.
137 SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J*
138 *Comput Biol* 2012;19:455–77. doi:10.1089/cmb.2012.0021.

139 [11] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically
140 improved memory-efficient short-read de novo assembler. *Gigascience* 2012;1:18.
141 doi:10.1186/2047-217X-1-18.

142 [12] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina
143 sequence data. *Bioinformatics* 2014;30:2114–20. doi:10.1093/bioinformatics/btu170.

144 [13] Meier-Kolthoff, J. P., Auch, A. F., Klenk, H.-P., & Göker, M. (2013). Genome
145 sequence-based species delimitation with confidence intervals and improved distance
146 functions. *BMC Bioinformatics*, **14**, 60. doi:10.1186/1471-2105-14-60

147 [14] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: An improved algorithm and software
148 for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–3.
149 doi:10.1099/ijsem.0.000760.

150 [15] Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular
151 genomes. *Nucleic Acids Res.* 2008;36(Web Server issue):W181–W184.
152 doi:10.1093/nar/gkn179

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158 **Table 1:** Phenotypic characterization of *Urinicoccus timonensis* based on the biochemical
 159 tests.

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

161 (A)

Bacteria : <i>Urinicoccus timonensis</i>			
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-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	+

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163 (B)

Bacteria: <i>Urinicoccus timonensis</i>	
API ZYM	

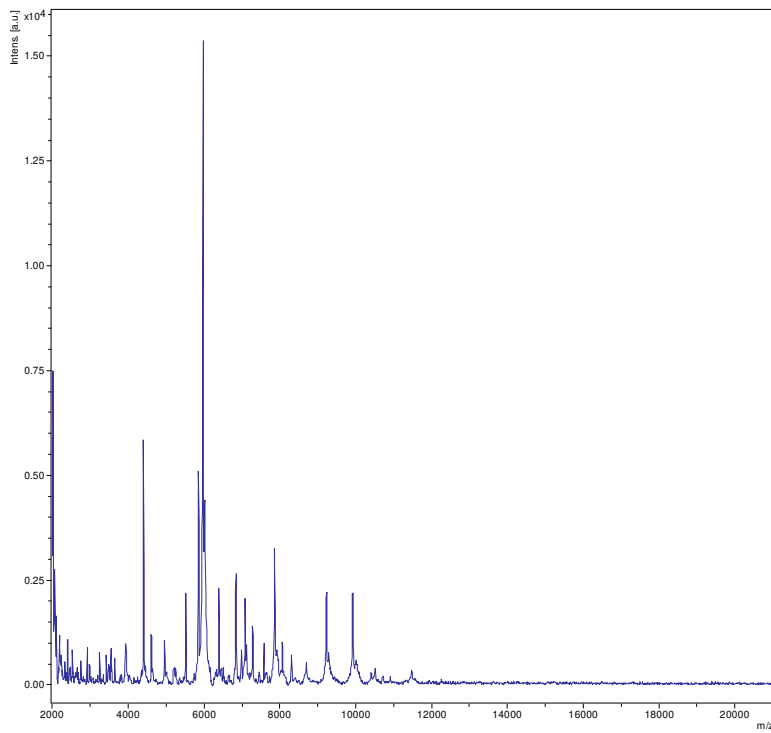
Test	Results (+/-)
Control	-
Alkaline phosphatase	-
Esterase (C 4)	-
Esterase Lipase (C 8)	-
Lipase (C 14)	-
Leucine arylamidase	-
Valine arylamidase	-
Cystine arylamidase	-
Trypsine	-
α -chymotrypsine	-
Acid phosphatase	-
Naphtalo-AS-BI-phosphohydrolase	-
α -galactosidase	-
β -galactosidase	-
β -glucuronidase	-
α -glucosidase	-
β -glucosidase	-
N-acetyl- β -glucosaminidase	-
α -mannosidase	-
α -fucosidase	-

165 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 (%)
<i>Urinicoccus timonensis</i>	OCTU01000001.1	1,978,908	60	1871	1,931	41.1
<i>Tissierella creatinini</i>	SUSSS01000001.1	2,611,442	58	2471	2,533	37.7
<i>Peptoniphilus indolicus</i>	NZ_JH165061.1	2,237,864	33	2133	2166	29.7
<i>Peptoniphilus grossensis</i>	NZ_HE978566.1	2,101,866	29	1983	2012	32.7
<i>Peptoniphilus coxii</i>	NZ_KQ960154.1	1,837,050	39	1713	1752	44.5
<i>Peptoniphilus asaccharolyticus</i>	NZ_FWWR0100017.1	2,232,586	32	2283	2315	32.2
<i>Keratinibaculum paraultunense</i>	SWAE01000001.1	2,248,613	55	2156	2211	29.4
<i>Peptoniphilus ivorii</i>	NZ_LR134523.1	1,587,771	64	1488	1574	53.2

168 Table 4 : Number of genes associated with the 25 general clusters of orthologous group (COG) functional categories.

	<i>Urinicoccus_timonensis</i>	<i>Peptoniphilus_ivorii</i>	<i>Tissierella_creatinini</i>	<i>Peptoniphilus_indolicus</i>	<i>Peptoniphilus_grossensis</i>	<i>Peptoniphilus_coxii</i>	<i>Peptoniphilus_asaccharolyticus</i>	<i>Keratinibaculum_paraaltunense</i>	<i>Description</i>
[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
[O]	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
[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 cog



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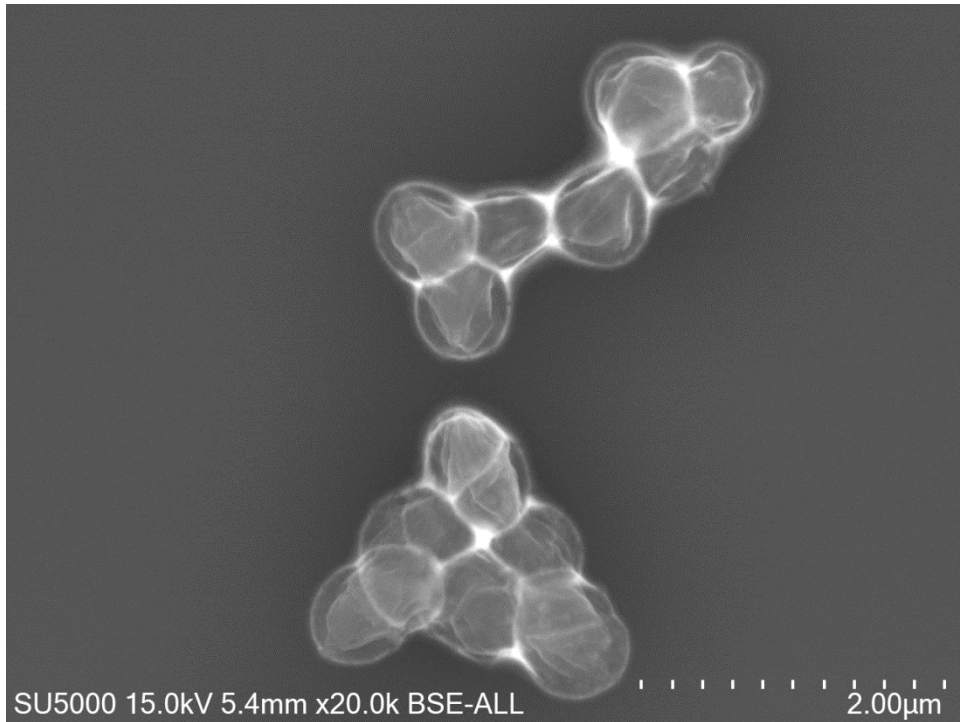
171 **Figure 1:** MALDI-TOF MS reference spectrum of *Urinicoccus timonensis* gen. nov., sp. nov.

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

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177 **Figure 2:** Scanning electron microscopy of stained *Urinicoccus timonensis* gen. nov., sp. nov.

178 A colony was collected from agar and immersed into a 2.5 % glutaraldehyde fixative solution.

179 Then, a drop of the suspension was directly deposited on a poly-L-lysine coated microscope

180 slide for 5 minutes and treated with 1 % phosphotungstic acid (PTA) aqueous solution (pH

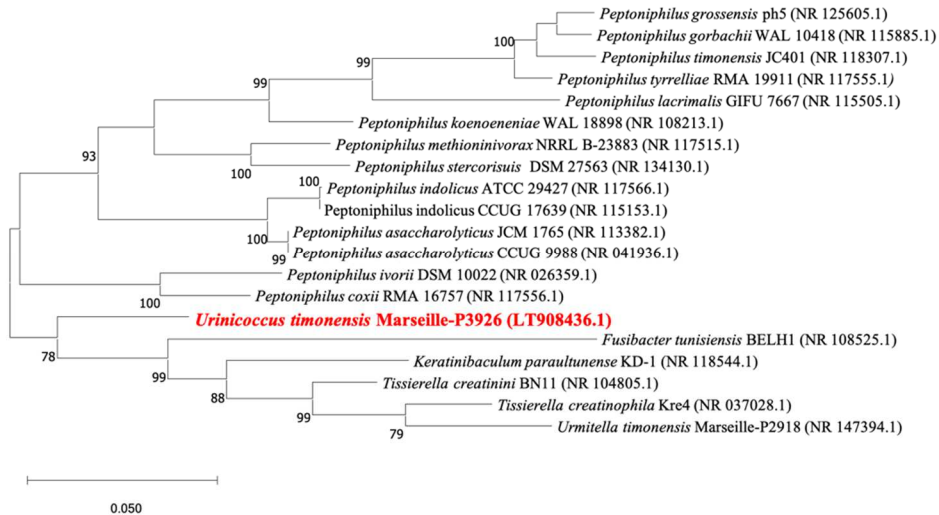
181 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water; air

182 dried and examined in a tabletop SEM (Hitachi SU5000) with approximately 60 centimeters

183 in height and 33 cm in width to evaluate bacteria structure. Scales and acquisition settings are

184 shown in figure.

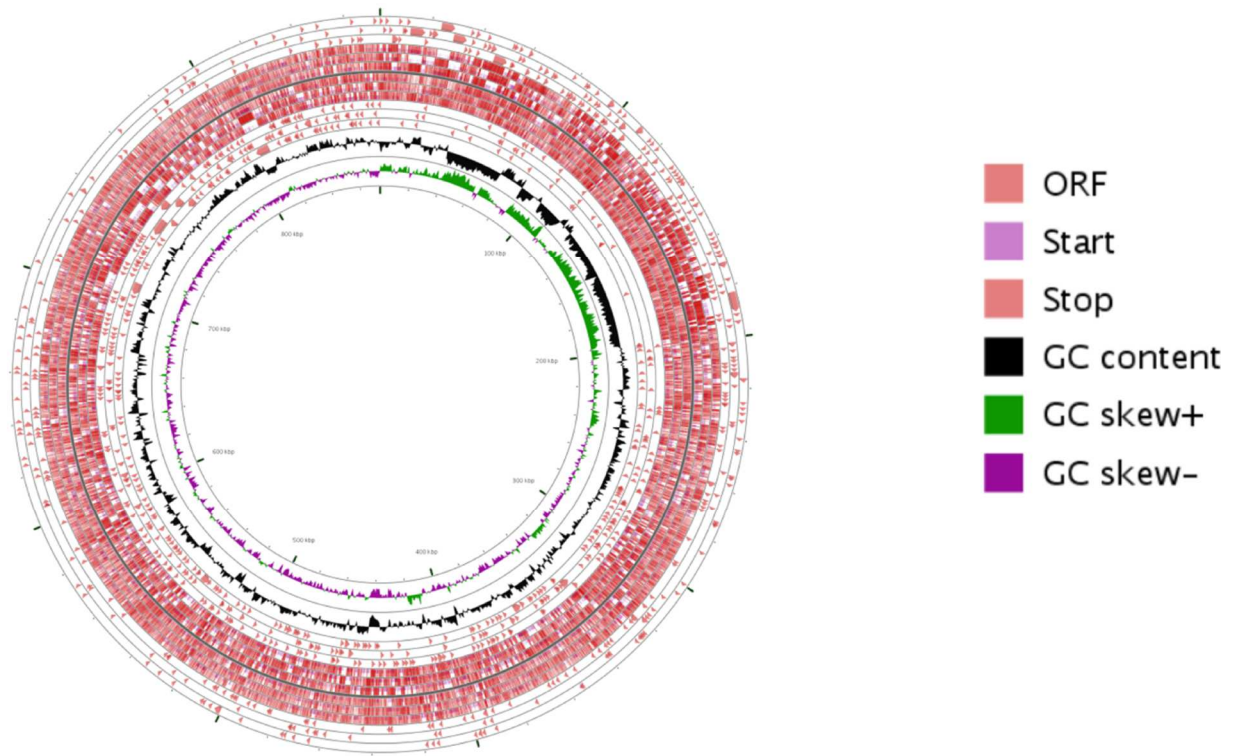
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 187 **Figure 3:** Phylogenetic tree highlighting the position of *Urinicoccus timonensis* gen. nov., sp.
 188 nov. with regard to other closely related species. Genbank accession numbers of 16S rRNA
 189 are indicated in parentheses. Sequences were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

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198 **Figure 4:** A circular map generated using the CGView [15] Server showing a complete view

199 of the genome of *Urinicoccus timonensis* gen. nov., sp. nov.

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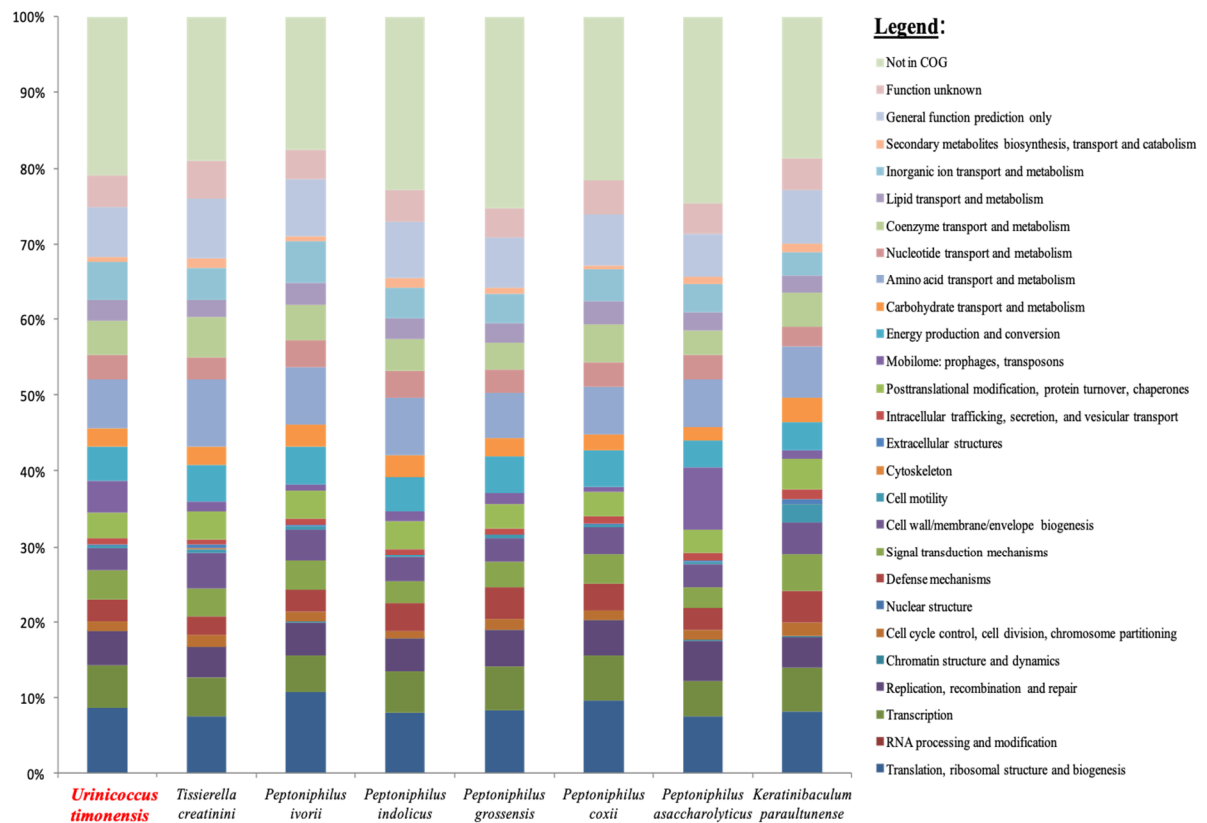
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Figure 5: Distribution of functional classes of predicted genes according to the clusters of

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orthologous groups of proteins of *Urinicoccus timonensis* gen. nov., sp. nov., among other

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closely related species.

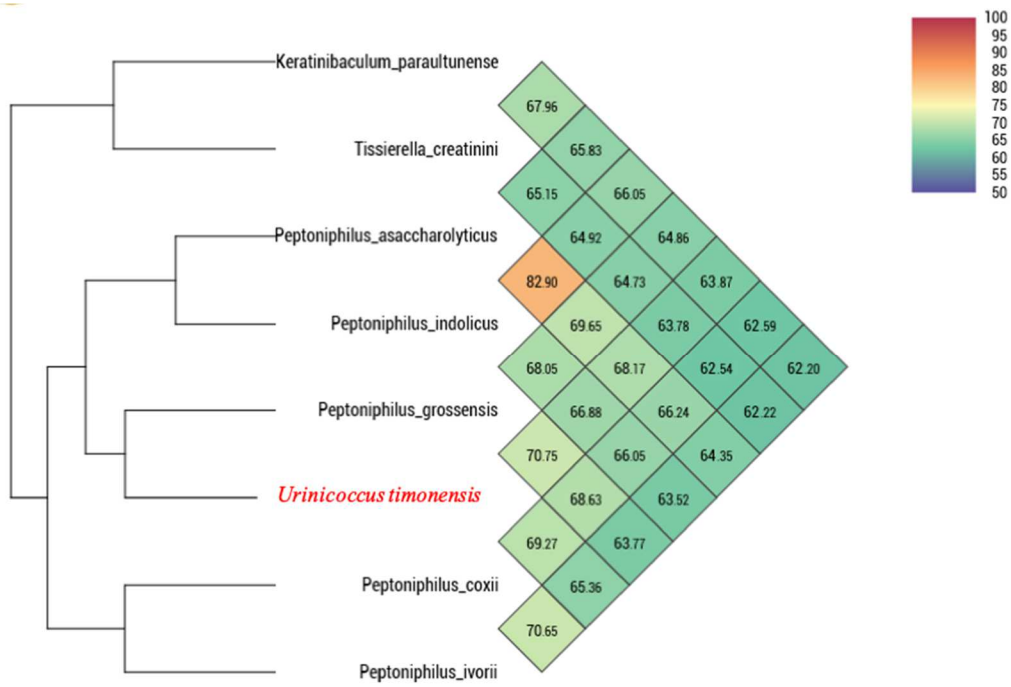
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216 **Figure 6:** Heatmap generated with OrthoANI values calculated using the OAT software

217 between *Urinicoccus timonensis* gen. nov., sp. nov. and other closely related species with

218 standing in nomenclature.