Urinicoccus massiliensis gen. nov., sp. nov., a new bacterium isolated from a human urine sample from a 7-year-old boy hospitalized for dental care

E. K. Yimagou[1], H. Anani[2], A. Yacouba[1], I. Hasni[1], J.-P. Baudoin[1], D. Raoult[3] and J. Y. Bou Khalil[1]

1) *Aix Marseille University, IRD, AP-HM, MEPHI,* 2) *Aix Marseille Univ, IRD, AP-HM, VITROME, IHU-Méditerranée Infection, Marseille, France and* 3) *Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

Abstract

Urinicoccus massiliensis strain Marseille-P1992^T (= CSURP1992 = DSM100581) is a species of a new genus isolated from human urine.

Keywords: Culturomics, new species, taxonogenomics, urine, *Urinicoccus massiliensis*
Original Submission: 16 July 2019; **Revised Submission:** 10 October 2019; **Accepted:** 17 October 2019
Article published online: 30 October 2019

Corresponding author. J. Y. Bou Khalil, MEPHI, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin 13385 Marseille Cedex 05. France
E-mail: boukhaliljacques@gmail.com

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 *Urinicoccus 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 *Urinicoccus,* family *Peptoniphilaceae*, phylum *Firmicutes.*

https://doi.org/10.1016/j.nmni.2019.100615

TABLE 1. Description of Urinicoccus massiliensis according to the digitalized protologue TA00972 on the www.imedea.uib.es/dprotologue website

TAXONUMBER	TA00972
DATE OF THE ENTRY	2019-05-30
DRAFT NUMBER/DATE	001
VERSION	Submitted
SPECIES NAME	*Urinicoccus massiliensis*
GENUS NAME	Urinicoccus
SPECIFIC EPITHET	*Urinicoccus massiliensis*
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	edmondkuete@yahoo.fr
SUBMITTER	KUETE YIMAGOU EDMOND
E-MAIL OF THE SUBMITTER	edmondkuete@yahoo.fr
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

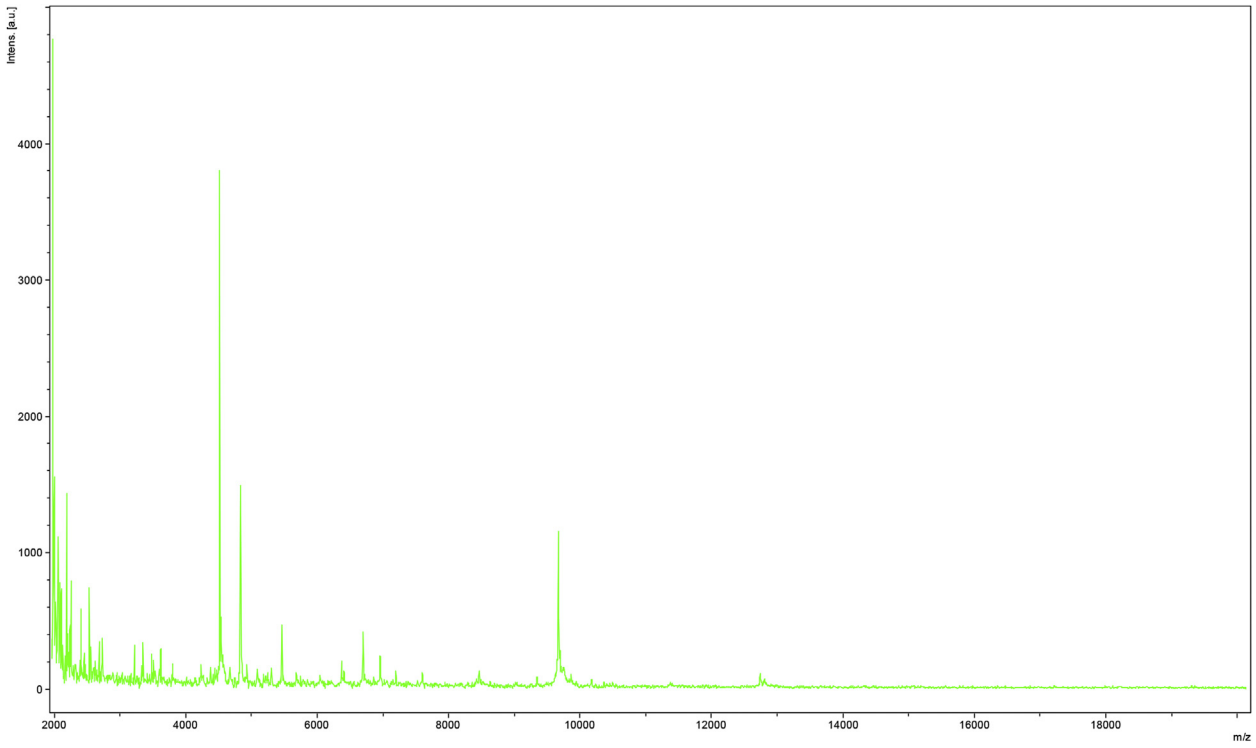

FIG. 1. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

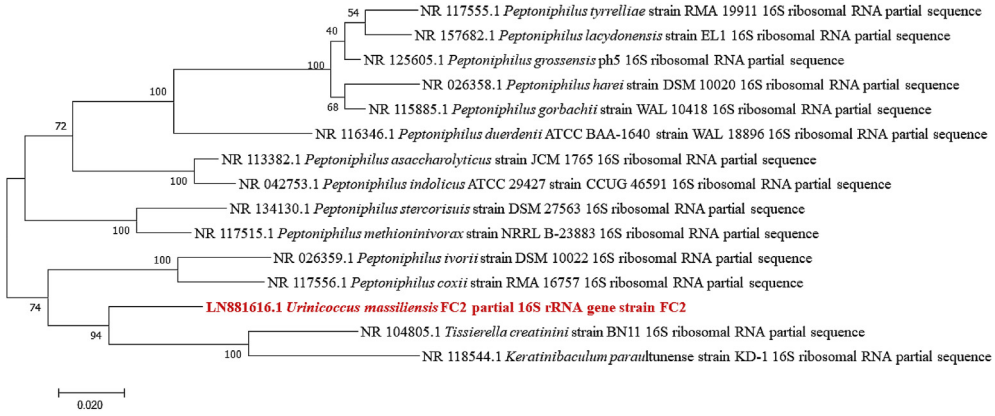

FIG. 2. Phylogenetic tree showing the position of *Urinicoccus massiliensis* strain Marseille-P1992^{T} relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using Muscle v3.8.31 with default 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.

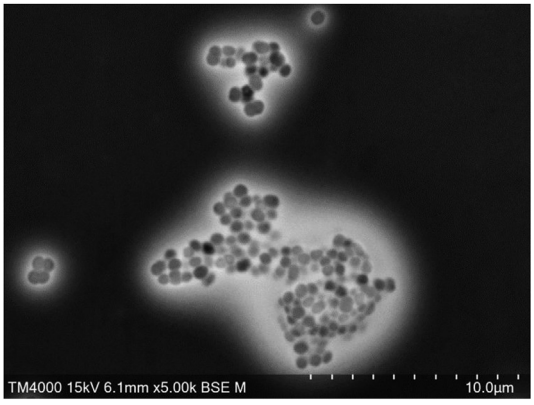

FIG. 3. Electron micrograph of *Urinicoccus massiliensis* strain Marseille-P1992^T obtained with a Hitachi TM4000Plus tabletop scanning electron microscope.

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	−

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^T 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.

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 (Trimmomatic [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: ***Urinicoccus massiliensis***			
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	+

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

Characteristics	*Urinicoccus massiliensis*	*Peptoniphilus asaccharolyticus*	*Peptoniphilus coxii*	*Peptoniphilus duerdenii*	*Peptoniphilus harei*	*Peptoniphilus indolicus*	*Peptoniphilus ivorii*	*Peptoniphilus lacydonensis*	*Peptoniphilus senegalensis*
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
Urinicoccus massiliensis	FPLH00000000	2.08	41.7	2047
Peptoniphilus harei	AENP00000000	1.84	34.4	1766
Peptoniphilus duerdenii	AEEH00000000	2.08	34.2	2018
Peptoniphilus senegalensis	CAEL00000000	1.84	32.3	1726
Peptoniphilus coxii	LSDG00000000	1.84	44.6	1783
Peptoniphilus lacydonensis	FNWF00000000	1.85	29.9	1788
Peptoniphilus asaccharolyticus	FWWR00000000	2.23	32.3	2268
Peptoniphilus ivorii	LR134523.1	1.59	53.2	1569
Peptoniphilus indolicus	AGBB00000000	2.24	31.7	2145

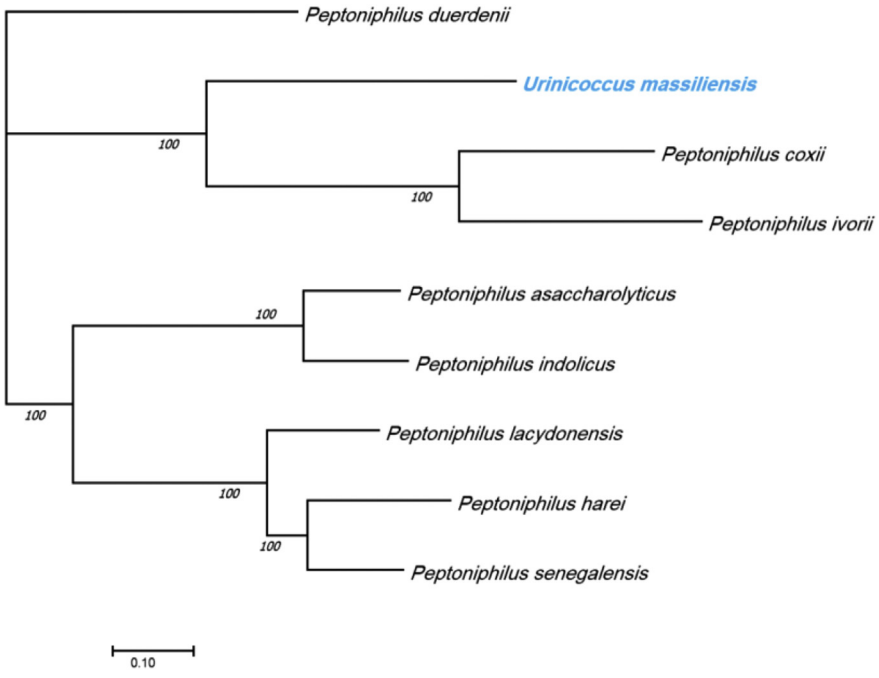

FIG. 4. Phylogenetic tree based on core genes highlighting the position of *Urinicoccus massiliensis* (blue) relative to other closely related bacterial taxa. The annotated GFF3 file of reference genomes was used as matrix in Roary version 3.10.2 on galaxy online site (http://www.usegalaxy.org.au) choosing 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^T 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 duerdeni.*

The degree of genomic similarity of strain Marseille-P1992^T 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*.

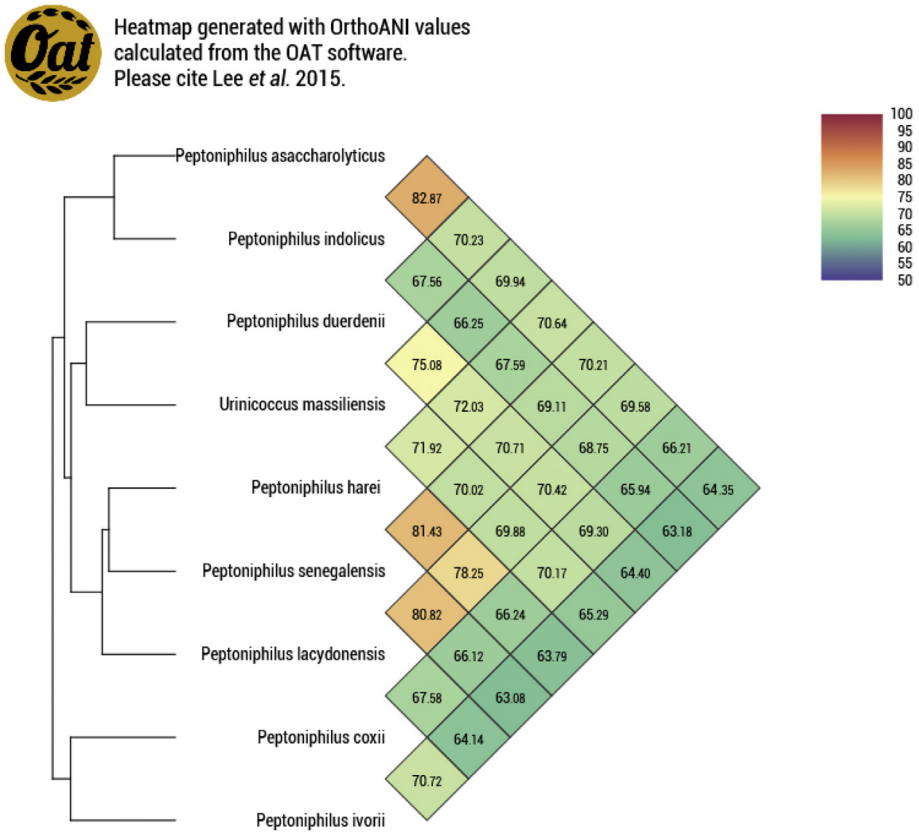

FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software between genus species and other closely related species with standing in nomenclature.

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

The words in blod 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-P1992^{T} was deposited in two different strain collections (= CSURP1992 = 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).

Acknowledgements

The authors thank Hitachi Corporation for providing the TM4000Plus Tabletop microscope. They also thank Aurelia Caputo from IHU-Méditerranée Infection, Marseille, France for submitting the genomic sequences to GenBank.

References

[1] Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93. https://doi.org/10.1111/1469-0691.12023.

[2] Lagier J-C, Hugon P, Khelaifia S, Fournier P-E, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64. https://doi.org/10.1128/CMR.00014-14.

[3] Lagier J-C, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203. https://doi.org/10.1038/nmicrobiol.2016.203.

[4] Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015;28:208–36.

[5] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.

[6] Ramasamy D, Mishra AK, Lagier J-C, Padhmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91. https://doi.org/10.1099/ijs.0.057091-0.

[7] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51. https://doi.org/10.1086/600885.

[8] Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70. https://doi.org/10.1007/s10096-014-2263-z.

[9] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier P-E, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of *Gracilibacillus massiliensis* sp. nov. Microb Ecol Health Dis 2016;27. https://doi.org/10.3402/mehd.v27.32049.

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

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

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

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

[14] Anani H, Khodor M, Raoult D, Fournier P-E. Whole-genome sequence of French clinical *Olivibacter jilunii* strain P8502. Microbiol Resour Announc 2019;8. https://doi.org/10.1128/MRA.00701-19.

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

[16] Auch AF, von Jan M, Klenk H-P, Göker M, et al. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genom Sci 2010;2:117–34. https://doi.org/10.4056/sigs.531120.

[17] Anani H, Abou Abdallah R, Chelkha N, Fontanini A, Ricaboni D, Mailhe M, et al. Draft genome and description of *Merdibacter massiliensis* gen.nov., sp. nov., a new bacterium genus isolated from the human ileum. Scientific Rep 2019;9:7931. https://doi.org/10.1038/s41598-019-44343-8.