Taxonogenomics description of *Arcanobacterium urinimassiliense* sp. nov., a new bacterial species isolated from urine sample

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Abstract

Strain Marseille-P3248^T is a new species from the order *Actinomycetales* that was isolated from the urine sample of a girl aged 20 months with rotavirus gastroenteritis. It is a facultative anaerobic Gram-positive rod-shaped bacterium. Strain Marseille-P3248^T exhibits 94.73% sequence similarity with *Arcanobacterium pluranimalium* strain M430/94/2, a phylogenetically related species with standing in nomenclature. Its genome size is 1 667 964 bp with 49.1% G + C content. Strain Marseille-P3248^T (= CSURP3248) is the type strain of the new species *Arcanobacterium urinimassiliense* sp. nov.

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Introduction

Members of the genus *Arcanobacterium* are Gram-positive rods and coccoid bacteria, non-motile and spore forming. They are facultatively anaerobic. Many of the described *Arcanobacterium* species have been isolated from animal or human samples, such *Arcanobacterium bialowiezense* [1], *Arcanobacterium canis* [2], *Arcanobacterium hippocoleae* [3], *Arcanobacterium haemolyticum* [4] and *Arcanobacterium pyogenes* [5]. However, *A. haemolyticum* is an opportunistic pathogen responsible for septicaemia in immunocompromised persons [6,7]. Several strains of this species have been isolated from many clinical samples [8]. It is important to understand the involvement of bacterial diversity in normal physiological functions and in susceptibility to diseases [9]. As a result, the culturomics approach, based on diversified culture conditions, has made it possible to isolate species that have never previously been cultured. This new strategy was designed to complete the metagenomics of 16S rRNAs and to study the diversity of human intestinal bacteria [10–12]. In parallel, the taxonogenomic method has been developed to describe bacterial species by associating the analysis of complete sequences of the genome with the phenotypic characteristics of the species [13]. By applying these new approaches, we perform here a brief description of *Arcanobacterium urinimassiliense* sp. nov., a new species isolated from a urine sample.

Materials and methods

Strains isolation and identification

Strain Marseille-P3248 was isolated from a urine sample of a 20-month-old French girl with rotavirus gastroenteritis using culturomics [10,11]. The guardian of the girl supplied signed and informed consent. The study was authorized by the ethics committee of the Institut Federatif de Recherche IFR48 under agreement number 09-022. The sample was pre-incubated and treated as reported previously [14].

Distinct and pure colonies of strain Marseille-P3248 were tested by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany). No identification was obtained but

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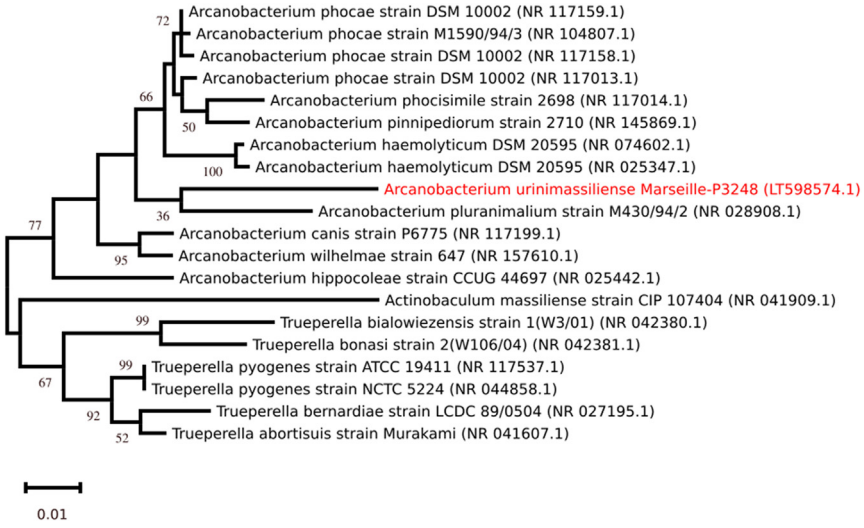


FIG. 1. Phylogenetic tree highlighting the position of *Arcanobacterium urinimassiliense* strain Marseille-P3248 with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inferences were obtained using the maximum likelihood method and the MEGA X software [29]. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes.

the generated spectra were compared with those of a regularly updated local database (https://www.mediterranee-infection.com/urms-data-base). The failed identification by MALDI-TOF MS led us to systematically sequence the 16S rRNA gene, as described previously [15]. Then, CodonCode Aligner software (http://www.codoncode.com) was used to assemble and correct the different short sequences to obtain a consensual and suitable sequence for comparison with the NCBI database using the BLASTn algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Growth conditions and phenotypic characterization

In order to assess the best growth conditions of this new strain, different temperatures and atmospheres (aerobic, anaerobic and microaerophilic) were tested as described by Diop et al. [16]. To determine biochemical criteria, API ZYM and API 50 CH strips (bioMérieux, Marcy l'Étoile, France) were tested, according to the manufacturer's instructions. Sporulation and Gram-staining, as well as catalase and oxidase tests, were performed following standard procedures [17]. The morphological structure of strain Marseille-P3248 was observed with a scanning electron microscope using a Tecnai G20 device (FEI Technologies Inc., Hillsboro, OR, USA).

Extraction and sequencing genome

On the basis of its phylogenetic position, the similarity of 16S rRNA and phenotypic differences with other close members of the *Actinomycetaceae* family, we decided to sequence strain Marseille-P3248. Currently, there are six available genomes

TABLE 1. Differential characteristics of *Arcanobacterium urinimassiliense* sp. nov. strain Marseille-P3248 compared with other *Arcanobacterium* species

Properties	AUr	APh	AHa	ACa	AHi	APl
Cell diameter (μm)	0.3–0.4	0.2–0.9	na	0.5	na	na
Oxygen requirement	FA	FA	FA	FA	FA	FA
Gram stain	+	+	+	+	+	+
Motility	—	—	—	—	—	—
Endospore formation	—	—	—	—	—	—
Catalase	—	+	—	—	—	+
Urease	—	—	—	—	—	—
β-Galactosidase	+	+	+	+	+	—
Ribose	+	+	+	+	—	+
Mannose	+	+	na	na	—	na
Mannitol	—	+	—	—	—	—
Sucrose	—	+	—	+	—	—
Glucose	+	+	+	+	+	+
Fructose	+	+	na	na	na	na
Maltose	+	+	+	+	+	variable
Starch	—	+	na	na	na	na
Lactose	+	+	+	+	+	—
G + C content (mol%)	49.1	50.0	53.1	na	na	57.0
Source	Human	Seals	Human	Dog	Horse	Deer

Abbreviations: AUr, Arcanobacterium urinimassiliense sp. nov.; APh, Arcanobacterium phocae; AHe, Arcanobacterium haemolyticum; ACa, Arcanobacterium canis; AHi, Arcanobacterium hippocoleae; APl, Arcanobacterium pluranimalium; FA, facultative anaerobic; +, positive reaction; –, negative reaction; na, data not available.

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TABLE 2. Cellular fatty acid composition (%) of *Arcanobacterium urinimassiliense* sp. nov., strain Marseille-P3248 and others members of the genus *Arcanobacterium*

Fatty acids	Name	AUr	AHe	APh	API	AHi	ACa
$C_{16:0}$	Hexadecanoic acid	33.3	31.5	28.0	9.5	42.8	30.7
$C_{18:0}$	Octadecanoic acid	20.7	23.0	28.1	12.1	15.0	18.2
$C_{18:1n9}$	9-Octadecenoic acid	32.4	21.0	25.4	29.1	23.1	19.1
$C_{18:2n6}$	9,12-Octadecadienoic acid	12.2	2.0	1.8	4.9	1.1	9.9
$C_{17:0\ anteiso}$	14-methyl-hexadecanoic acid	1.5	0.9	1.0	0.7	0.9	0

Abbreviations: AUr, Arcanobacterium urinimassiliense sp. nov.; APh, Arcanobacterium phocae; AHe, Arcanobacterium haemolyticum; ACa, Arcanobacterium canis; AHi, Arcanobacterium hippocoleae; API, Arcanobacterium pluranimalium.

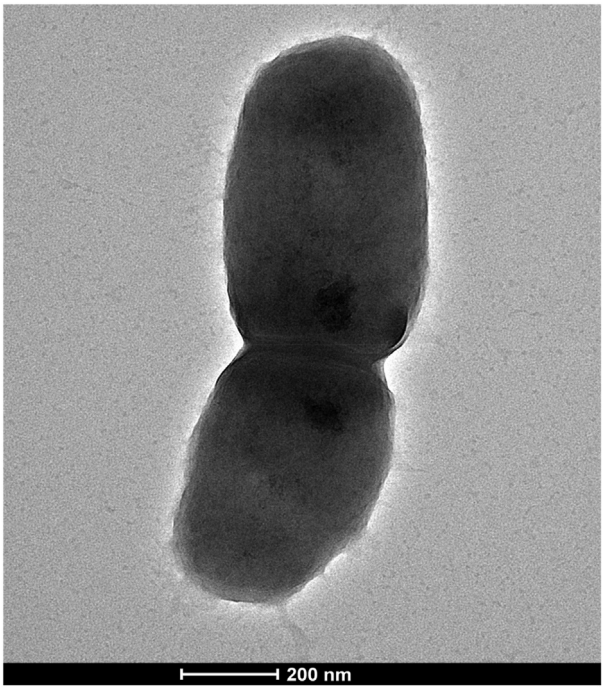


FIG. 2. Transmission electron microscopy of *Arcanobacterium urinimassiliense* sp. nov. Cells are observed on Tecnai G20 transmission electron microscope operated at 200 keV. Scales are displayed on the figure.

within the genus *Arcanobacterium*, among them the first genome of *A. urinimassiliense* sp. nov. Its GenBank accession number is FPJH00000000 and consists of 11 contigs.

The genomic DNA of strain Marseille-P3248 was extracted with an EZ1 biorobot using an EZ1 DNA Tissue kit (Qiagen, Hilden, Germany), after being pretreated with lysozyme and incubated at 37°C for 2 hours. Genomic DNA of *Arcanobacterium urinimassiliense* strain Marseille-P3248 was sequenced on a MiSeq sequencer (Illumina, San Diego, CA, USA) using the mate-pair strategy. Genomic DNA (gDNA) was bar coded in order to be mixed with 11 other projects with the Nextera Mate-Pair sample prep kit (Illumina). The mate-pair library was prepared with 1 µg of genomic DNA using the Nextera Mate-Pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with Mate-Pair junction adapters. Then, sequencing was launched taking into account all the parameters described previously [18]. The 1,613,495 paired reads were filtered according to the read qualities. These reads were trimmed then assembled with CLC Genomics Workbench 21.0.3. Finally, the draft genome of *Arcanobacterium urinimassiliense* sp. nov., which consists of two scaffolds made of 11 contigs, was generated with a genome size of 1.66 Mbp with G + C content of 49.1 mol%.

Genome assembly, annotation and comparison

The genome assembly of strain Marseille-P3248 was performed using pipeline softwares such as Spades [19], Velvet [20] and Soap Denovo [21]. Sequences recovered from Illumina MiSeq were trimmed with Trimmomatic software [22] or untrimmed using MiSeq software. To minimize the gaps, we used GapCloser software [23] to remove scaffolds <800 bp and scaffolds with a depth value less than 25% of the mean depth. Parameters such as number of scaffolds, N50, and number of N were chosen to obtain the best assembly. Finally, genome annotation was obtained through the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.0 [24].

The genomic comparison was carried out by evaluating the degrees of similarity of the genomic sequences between the studied genomes using the suitable tools. Indeed, the GGDC (Genome-to-Genome Distance Calculator) web server (available online at http://ggdc.dsmz.de) allows calculation of DNA–DNA hybridization [25]. The analysis of the mean nucleotide identity was estimated using OAT software [26].

Results

Phylogenetic analysis

An analysis of the sequences of the 16S rRNA gene of strain Marseille-P3248 showed that it presented nucleotide similarities of 94.73% with *Arcanobacterium pluranimalium* strain M430/94/2 (GenBank accession number NR_028908.1). This was also

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TABLE 3. Antimicrobial susceptibility tests applied to strain Marseille-P3248

Antimicrobial susceptibility discs	Zone diameter (mm)[a]	Marseille-P3248	Reading
Amoxicillin/clavulanic acid (30 µg)	≥23 to <16	25	Susceptible
Trimethoprim/ sulfamethoxazole (25 µg)	≥16 to <10	18	Susceptible
Gentamycin (15 µg)	≥15 to <15	18	Susceptible
Imipenem (10 µg)	≥22 to <17	26	Susceptible
Ceftriaxone (30 µg)	≥21 to <15	23	Susceptible
Oxacillin (5 µg)	≥8 to <14	10	Susceptible
Gentamicin (500 µg)	≥17 to <22	19	Susceptible
Fosfomycin (50 µg)	≥24 to <24	24	Susceptible
Rifampicin (30 µg)	≥26 to <23	24	Susceptible
Doxycyclin (30 µg)	≥19 to <17	20	Susceptible
Colistin (50 µg)	≥15 to <15	17	Susceptible
Vancomycin (30 µg)	≥17 to <17	15	Resistant
Penicillin (10 µg)	≥29 to <18	16	Resistant
Amoxicillin (25 µg)	≥21 to <14	12	Resistant
Erythromycin (15 µg)	≥22 to <17	15	Resistant

[a]Values recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST).

TABLE 4. Genomic comparison of *Arcanobacterium urinimassiliense* Marseille-P3248 with phylogenetically related species

	Reference sequence	Size (bp)	Protein-coding genes	Total genes	RNAs	G + C content (%)
AUr	FPJH00000000.1	1,667,964	1405	1480	55	49.1
APh	LT629804.1	1,998,342	1717	1809	68	50.0
AHa	NC_014218.1	1,986,154	1761	1859	65	53.1
TBe	LNIZ00000000.1	2,028,874	1747	1836	55	65.4
TBi	LR134476.1	2,043,249	1794	1860	55	59.6
TPy	CP007519.1	2,338,390	2044	2128	55	59.5

Abbreviations: AUr, Arcanobacterium urinimassiliense sp. nov.; APh, Arcanobacterium phocae strain DSM 10002; AHa, Arcanobacterium haemolyticum DSM 20595; TBe, Trueperella bernardiae LCDC 89-0504; TBi, Trueperella bialowiezensis NCTC13354; TPy, Trueperella pyogenes TP6375.

the closest phylogenetic species with a validly published name (Fig. 1). Given that this percentage of similarity is much lower than the recommended threshold value [13,27] for predicting a new bacterium, we consider that the strain Marseille-P3248 is potentially a new species belonging to the genus *Arcanobacterium*.

Strain phenotypic and biochemical characterization

Gram staining showed a Gram-positive rod-shaped bacterium with length ranging from 0.4 to 0.6 µm and width ranging from 0.3 to 0.4 µm. Motility test was negative. We report that the Marseille-P3248^T strain has catalase and oxidase activity. Colonies of strain Marseille-P3248^T were circular, opaque and white on blood agar after 48 hours of incubation with a mean diameter of 0.5 mm. The biochemical characteristics of strain Marseille-P3248^T obtained using the API ZYM and 50 CH strips (bioMérieux) allowed us to perform a comparative study of phenotypic criteria of this strain with other closely related *Arcanobacterium* species (Table 1). The major fatty acids found from this strain were hexadecanoic acid (33.3%), 9-octadecenoic acid (32.4%) and octadecanoic acid (20.7%). Minor amounts of unsaturated, branched and other saturated fatty acids were also detected (Table 2). Scanning electron micrograph showed a rod-shaped bacterium (Fig. 2). In addition, antimicrobial tests revealed that strain Marseille-P3248 was susceptible to amoxicillin/clavulanic acid, trimethoprim/

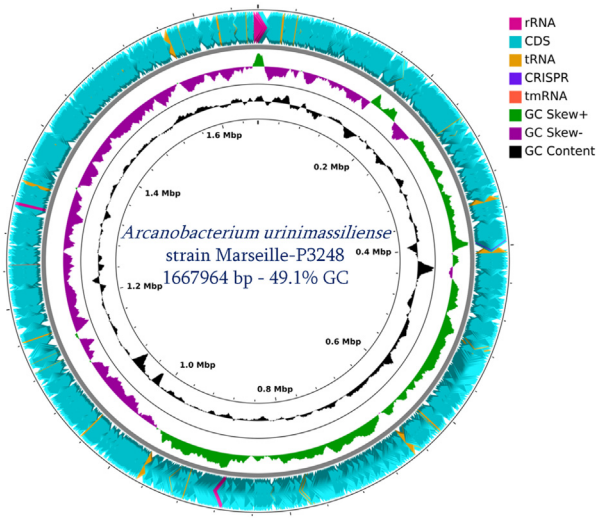


FIG. 3. A circular map of the genome of *Arcanobacterium urinimassiliense* sp. nov. strain Marseille-P3248 showing its different components with coloured bands. It is performed using the CGView server by submitting the genomic sequence [30].

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TABLE 5. Number of genes associated with the 26 general clusters of orthologous groups functional categories of *Arcanobacterium urinimassiliense* compared with those of related species

Code	Description	AUr	AHa	APh	TBe	TBi	TPy
[J]	Translation, ribosomal structure and biogenesis	143	155	154	151	152	157
[A]	RNA processing and modification	0	0	0	0	0	0
[K]	Transcription	59	64	65	66	62	94
[L]	Replication, recombination and repair	59	68	64	69	66	67
[B]	Chromatin structure and dynamics	0	0	0	0	0	0
[D]	Cell cycle control, cell division, chromosome partitioning	13	13	14	17	16	16
[Y]	Nuclear structure	0	0	0	0	0	0
[V]	Defence mechanisms	22	34	23	34	30	35
[T]	Signal transduction mechanisms	26	40	43	33	37	43
[M]	Cell wall/membrane/envelope biogenesis	73	65	64	70	71	81
[N]	Cell motility	3	4	3	3	4	4
[Z]	Cytoskeleton	0	0	0	0	0	0
[W]	Extracellular structures	0	0	1	1	1	1
[U]	Intracellular trafficking, secretion, and vesicular transport	9	10	8	11	13	9
[O]	Posttranslational modification, protein turnover, chaperones	34	44	43	47	45	46
[X]	Mobilome: prophages, transposons	2	26	7	8	5	8
[C]	Energy production and conversion	54	72	66	82	73	75
[G]	Carbohydrate transport and metabolism	80	98	109	103	93	186
[E]	Amino acid transport and metabolism	53	59	61	103	102	93
[F]	Nucleotide transport and metabolism	44	48	55	62	43	58
[H]	Coenzyme transport and metabolism	44	52	48	49	55	59
[I]	Lipid transport and metabolism	28	28	28	34	42	39
[P]	Inorganic ion transport and metabolism	49	69	57	66	59	74
[Q]	Secondary metabolites biosynthesis, transport and catabolism	2	7	6	3	5	4
[R]	General function prediction only	29	39	42	49	54	58
[S]	Function unknown	22	21	25	23	14	23

sulfamethoxazole, gentamycin, imipenem, ceftriaxone, oxacillin, gentamicin, fosfomycin, rifampicin, doxycyclin and colistin, but resistant to vancomycin, penicillin, amoxicillin and erythromycin (Table 3).

Analysis and interpretation of genomic data

The genome of strain Marseille-P3248^T was 1 667 964 bp long with a 49.1 mol% G + C content (Table 4). Its assembly was achieved on 11 contigs (Fig. 3). Of the 1480 predicted genes, 1425 were protein-coding genes and 55 were RNAs (three 5S rRNA, three 16S rRNA, one 23S rRNA, 45 tRNA and three ncRNA genes). There were 848 putative function genes for the strain Marseille-P3248. Finally, 245 genes were annotated as hypothetical proteins. Analysis of the Clusters of Orthologous Groups categories showed that the translation elements appeared to be more numerous within the genome of strain Marseille-P3248 (Table 5). DNA–DNA hybridization analysis showed values ranged from 18.6% between *Trueperella bernardiae* and *Trueperella bialowiezensis* to 33% between *T. bernardiae* and *A. urinimassiliense* sp. nov., strain Marseille-P3248 (Table 6). These values are lower than the 70% threshold used for delineating prokaryotic species, which confirmed that this strain represented a new species within the genus *Arcanobacterium* [25].

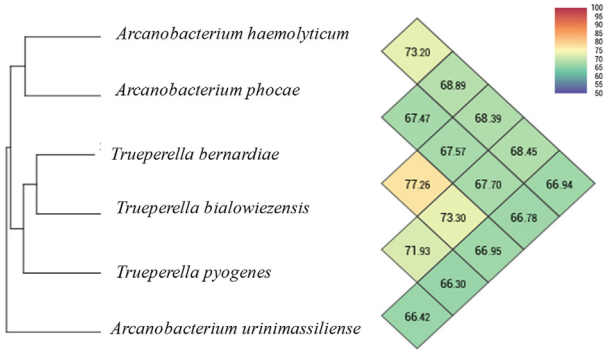


FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Arcanobacterium urinimassiliense* Marseille-P3248 and other related species.

Finally, in order to measure the overall similarity between genome sequences, an OrthoANI analysis (Fig. 4) was performed among closely related *Actinomycetaceae* species. The highest value of identity was 77.26%, shared between *Trueperella pyogenes* and *T. bernardiae* and the lowest value of similarity obtained was 66.30% between *T. pyogenes* and *A. urinimassiliense* strain Marseille-P3248. When strain Marseille-P3248 was compared with these closely related species, values ranged from 66.30% with *T. pyogenes* to 66.94% with *A. haemolyticum*. These values are below the recommended threshold value for being of the same species [28].

TABLE 6. Numerical DNA–DNA hybridization values (%) obtained by comparison genome of strain Marseille-P3248 with closely related species using GGDC formula 2 software (DDH estimates based on HSP identities/length)

	AUr	APh	AHa	TBe	TBi	TPy
AUr	100%	24.1 ± 4.8%	25.0 ± 4.8%	33.0 ± 4.9%	24.0 ± 4.7%	22.5 ± 4.7%
APh		100%	19.4 ± 4.6%	22.6 ± 4.7%	23.8 ± 4.8%	19.7 ± 4.6%
AHa			100%	22.7 ± 4.8%	22.1 ± 4.7%	24.0 ± 4.8%
TBe				100%	18.6 ± 4.6%	20.3 ± 4.7%
TBi					100%	18.8 ± 4.6%
TPy						100%

Abbreviations: AUr, Arcanobacterium urinimassiliense sp. nov.; APh, Arcanobacterium phocae strain DSM 10002; AHa, Arcanobacterium haemolyticum DSM 20595; TBe, Trueperella bernardiae LCDC 89-0504; TBi, Trueperella bialowiezensis NCTC13354; TPy, Trueperella pyogenes TP6375.

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Conclusion

Phenotypic criteria, morphological characteristics and genomic features of strain Marseille-P63248 led us to conclude that this strain is a new member of the *Arcanobacterium* genus. In addition, comparative analysis with the phylogenetically closest species with standing in nomenclature support formally the creation of *Arcanobacterium urinimassiliense* sp. nov., of which its type strain is strain Marseille-P3248.

Description of *Arcanobacterium urinimassiliense* sp. nov. *Arcanobacterium urinimassiliense* sp. nov. (u.ri.ni.mas.sil.ien'se, N.L. u.ri.no, N.L. gen. fem. urini, 'urine', from which this bacterium was first cultivated; and mas.si.li.en'se. L. masc. adj., massiliense, of Massilia, the Latin name of Marseille, where the type strain was first isolated). It is a Gram-positive rod shaped bacterium. Cells are not motile and present a mean diameter of 0.5 µm. It is catalase and oxidase positive. On blood agar, bacterial colonies appear circular and white after 48 hours of incubation. Hexadecanoic acid, octadecanoic acid and 9-octadecenoic acid were the major fatty acids present in the bacterial cells. The genome size of *A. urinimassiliense* strain Marseille-P3248^{T} is 1.66 Mbp with 49.1 mol% G + C content. The genome and the 16S rRNA sequences have been deposited in the GenBank database under accession numbers FPJH00000000 and LT598574, respectively. The type strain is strain Marseille-P3248^{T}, which was isolated from the urine sample of a child with rotavirus gastroenteritis.

Conflicts of interest

None to declare.

Acknowledgements

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