

Vaginisenegalia massiliensis gen. nov., sp. nov., a new bacterium isolated from the vagina flora and its taxono-genomic description

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Abstract

Strain Marseille-P5643^T was isolated from a vaginal sample of a healthy Senegalese woman. It is an anaerobic Gram-negative, rod-shaped bacterium. Strain Marseille-P5643^T exhibits 93.7% similarity levels with the *Facklamia hominis* strain ATCC 700628^T, the phylogenetically closest related species with standing in nomenclature. The draft genome size of strain Marseille-P5643^T is 1.79 Mb with 39.0 mol% of G+C content. We propose here the creation of *Vaginisenegalia massiliensis* gen. nov., sp. nov., as a new bacterial genus from the phylum Firmicutes.

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Introduction

The vagina has a diverse and complex microbiota comprising a wide variety of microorganisms [1,2]. A healthy woman usually has a vaginal flora dominated by *Lactobacillus*, usually named Döderlein's bacteria [3,4]. Hence an imbalance of the complex ecosystem dominated by species belonging to the genus *Lactobacillus* can lead to vaginosis [5].

Since 1921, bacterial vaginosis is considering as a dysbiosis characterized by an increase in the pH of the vaginal mucosa, a decrease in lactobacilli, and a proliferation of Gram negative anaerobic bacteria [6,7]. Currently, it is important to understand the role played by the vaginal microbiota, especially in the prevention of bacterial vaginosis, sexually transmitted infections and urinary tract infections, to better manage women of childbearing age [8].

The strain described here is a bacterium isolated for the first time from the vagina of a healthy woman, suggesting that exploration of the vaginal ecosystem could help to prevent or manage associated diseases. It is important to study the involvement of microbial diversity in normal physiological functions and exposure to certain diseases [9]. Culturomics, a new method to determine all human-associated microbes using different bacterial growth conditions mimicking natural conditions, was used here [10–12]. The description of this new species is based on a method combining genotypic and phenotypic characteristics of the bacterium, supplemented by the taxono-genomic strategy previously described [13,14]. In this report, we provide a brief classification and set of features for *Vaginisenegalia massiliensis* gen. nov., sp. nov., strain Marseille-P5643^T, together with a description of the complete genomic sequencing and annotation.

Isolation and growth conditions

In 2017, as part of a study of the vaginal flora microbiome, the strain Marseille-P5643^T was isolated from a swab sample from a woman living in Dielmo, a Senegalese village (West Africa). An attempt to identify this bacterium using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

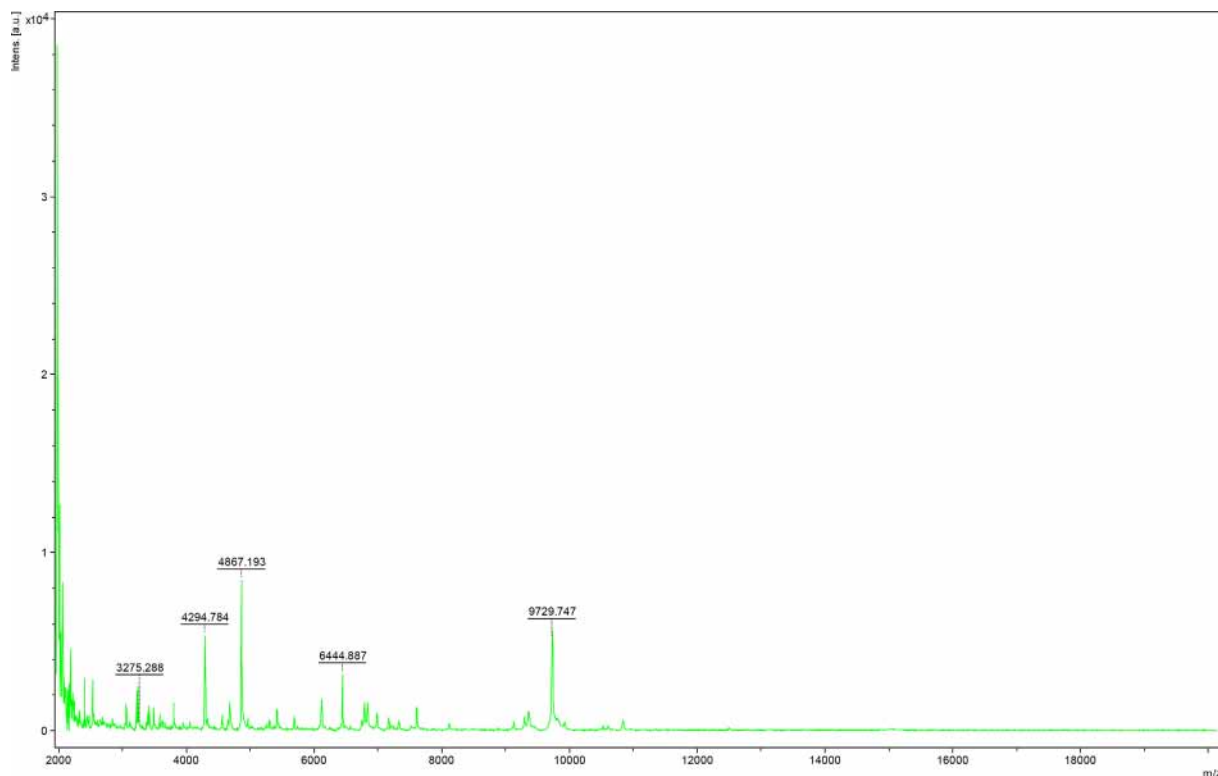


FIG. 1. MALDI-TOF MS reference spectrum of *Vaginisenegalia massiliensis* gen. nov., sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

(MALDI-TOF MS) did not provide a result. The identification process was performed on a Microflex LT spectrometer (Bruker, Daltonics, Bremen, Germany) as previously described [15]. Spectra obtained were imported and analysed using the BIOTYPER 3.0 software against the Bruker database, permanently improved with the local MEPHI database (Fig. 1). The vaginal swab was placed directly in liquid medium enriched with sheep's blood and rumen. Initial growth of bacterial cells was obtained after 15 days of pre-incubation in an anaerobic environment. Then, 10 μ L of this liquid was seeded on 5% sheep's blood agar (bioMérieux, Marcy l'Etoile, France) under anaerobic conditions at 37°C.

Phenotypic characteristics

Strain Marseille-P5643^T grew anaerobically; its first growth was observed after 15 days of incubation at 37°C on 5% sheep's blood–Columbia agar medium (bioMérieux) in an anaerobic atmosphere generated using the GENbag anaer system (bioMérieux). Strain Marseille-P5643^T (= CSUR P5643) is a Gram-negative bacterium. Its colonies appear transparent on agar with a mean diameter of 0.5 mm. Cells are not motile and

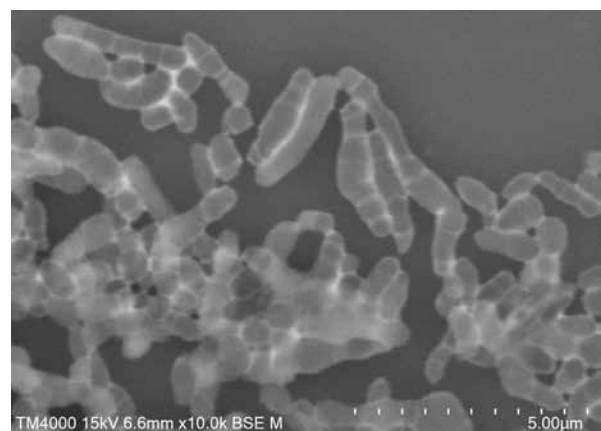


FIG. 2. Scanning electron microscopy (SEM) of stained *Vaginisenegalia massiliensis* gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 min to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000). Scales and acquisition settings are shown of figures.

TABLE 1. Phenotypic characterization of *Vaginisenegalia massiliensis* gen. nov., sp. nov., based on analytical profile index (API) tests

| Tests | Characteristics | Results | |
|----------------|----------------------------------|-------------------|---|
| API ZYM | Alkaline phosphatase | - | |
| | Esterase (C4) | + | |
| | Esterase lipase (C8) | + | |
| | Lipase (C14) | - | |
| | 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 | - | |
| | API 20 A | Indole production | - |
| | | Urease | - |
| Glucose | | + | |
| Mannitol | | - | |
| Lactose | | - | |
| Sucrose | | - | |
| Maltose | | - | |
| Salicin | | - | |
| Xylose | | - | |
| Arabinose | | - | |
| Gelatin | | - | |
| Esculin | | + | |
| Glycerol | | - | |
| Cellobiose | | - | |
| Mannose | | + | |
| Melezitose | | - | |
| Raffinose | | - | |
| Sorbitol | - | | |
| Rhamnose | - | | |
| Trehalose | + | | |

present no catalase and oxidase activities. The shape of this bacterium was highlighted with the Hitachi TM4000 instrument (Hitachi Group, Krefeld, Germany) (Fig. 2).

Biochemical characteristics of strain Marseille-P5643^T were tested using the API ZYM and 20A strips (bioMérieux) and are

presented in Table 1. A comparative study of the differential characteristics of this strain with other closely related species is displayed in Table 2. The major fatty acid found for this strain was hexadecanoic acid (57.7%), followed by 9-octadecenoic acid (25.5%). Minor amounts of unsaturated, branched and other saturated fatty acids were also detected (Table 3). A microscopic image of the bacterial cells was taken with the Hitachi TM4000 instrument (Hitachi Group, Krefeld, Germany) (Fig. 2).

Strain identification

To identify strain Marseille-P5643^T, the 16S rRNA gene was amplified using the primer pair fDI 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 reported [16]. The 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (<http://www.codoncode.com>). The 16S rRNA (accession number LT971014) gene sequence analyses showed 93.7% similarity with *Facklamia hominis* strain ATCC 700628, confirming the status of strain Marseille-P5643^T as a new bacterium [17,18]. We accordingly proposed to classify *Vaginisenegalia massiliensis* as a new genus within the family *Aerococcaceae* belonging to the phylum Firmicutes (Fig. 3).

Genome sequencing

Using EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany), the genomic DNA of strain Marseille-P5643^T was extracted and then sequenced on a MiSeq sequencer (Illumina Inc, San Diego, CA, USA) with the Nextera

TABLE 2. Differential characteristics of *Vaginisenegalia massiliensis* gen. nov., sp. nov., *Facklamia languida* [25], *Facklamia miroungae* [26], *Enterococcus asini* [27]

| Property | <i>Vaginisenegalia massiliensis</i> | <i>Facklamia languida</i> | <i>Facklamia miroungae</i> | <i>Enterococcus asini</i> |
|----------------------|-------------------------------------|---------------------------|----------------------------|---------------------------|
| Cell diameter (µm) | 0.2 | NA | 0.8–0.9 | NA |
| Oxygen requirement | Anaerobic | Anaerobic | Facultatively anaerobic | Facultatively anaerobic |
| Gram stain | - | + | + | + |
| Spore formation | - | - | - | - |
| Motility | - | NA | - | - |
| Production of: | | | | |
| Alkaline phosphatase | - | + | w | - |
| Catalase | - | - | - | - |
| Nitrate reductase | - | - | - | - |
| Urease | - | - | + | NA |
| β-galactosidase | - | - | - | - |
| N-acetyl-glucosamine | - | - | NA | + |
| Acid from: | | | | |
| Mannitol | - | - | - | - |
| Glucose | + | NA | + | + |
| Lactose | - | - | - | + |
| Raffinose | - | - | - | - |
| G+C (mol%) | 39.0 | 43.9 | 35.6 | 44.7 |
| Source | Vagina | Clinical sample | Juvenile elephant seal | Donkey |

+, positive result; -, negative result; NA, data not available; w, weakly positive.

TABLE 3. Fatty acid profiles (%) of *Vaginisenegalia massiliensis* strain Marseille-P5643^T

| Fatty acids | Name | Mean relative % ^a |
|-------------|---------------------------|------------------------------|
| 16:00 | Hexadecanoic acid | 57.7 ± 2.0 |
| 18:1n9 | 9-Octadecenoic acid | 25.5 ± 1.9 |
| 18:2n6 | 9,12-Octadecadienoic acid | 7.5 ± 1.2 |
| 18:00 | Octadecanoic acid | 6.3 ± 0.6 |
| 14:00 | Tetradecanoic acid | 3.1 ± 1.0 |

^aMean peak area percentage.

Mate Pair sample prep kit and Nextera XT Paired End (Illumina), following a previously described protocol [19]. Genome assembly was carried out using a pipeline containing various softwares (VELVET [20], SPADES [21], and SOAP DENOVO [22]), and trimmed (MiSEQ and TRIMMOMATIC [23] softwares) or untrimmed (only MiSEQ software) data. GAPCLOSER was used to decrease assembly gaps. Scaffolds with a base pair number <800 bp and those with a depth value <25% at mean depth have been

removed. Therefore, the best assembly was chosen by using different criteria (number of scaffolds, N50, number of N).

Comparison of genome properties

The genome of strain Marseille-P5643^T has a length of 1 754 973 bp with a 39.0 mol% G+C content (Fig. 4). It is composed of 1613 proteins and 1698 genes with 55 RNA genes (6 rRNA, 48 tRNA and 1 tmRNA). By comparing its genome with other closer genomes, we find that the strain Marseille-P5643^T (1.75 Mb) was smaller than *Trichococcus collinsii*, *Enterococcus asini*, *Facklamia miroungae*, *Facklamia hominis* and *Dolosicoccus paucivorans* (3.29, 2.57, 2.03, 1.89 and 1.76 Mb, respectively), but larger than those of *Facklamia languida* (1.71 Mb).

Furthermore, its G+C content (39 mol%) is higher than that of *D. paucivorans* and *F. miroungae* (37.9 and 35.7 mol%,

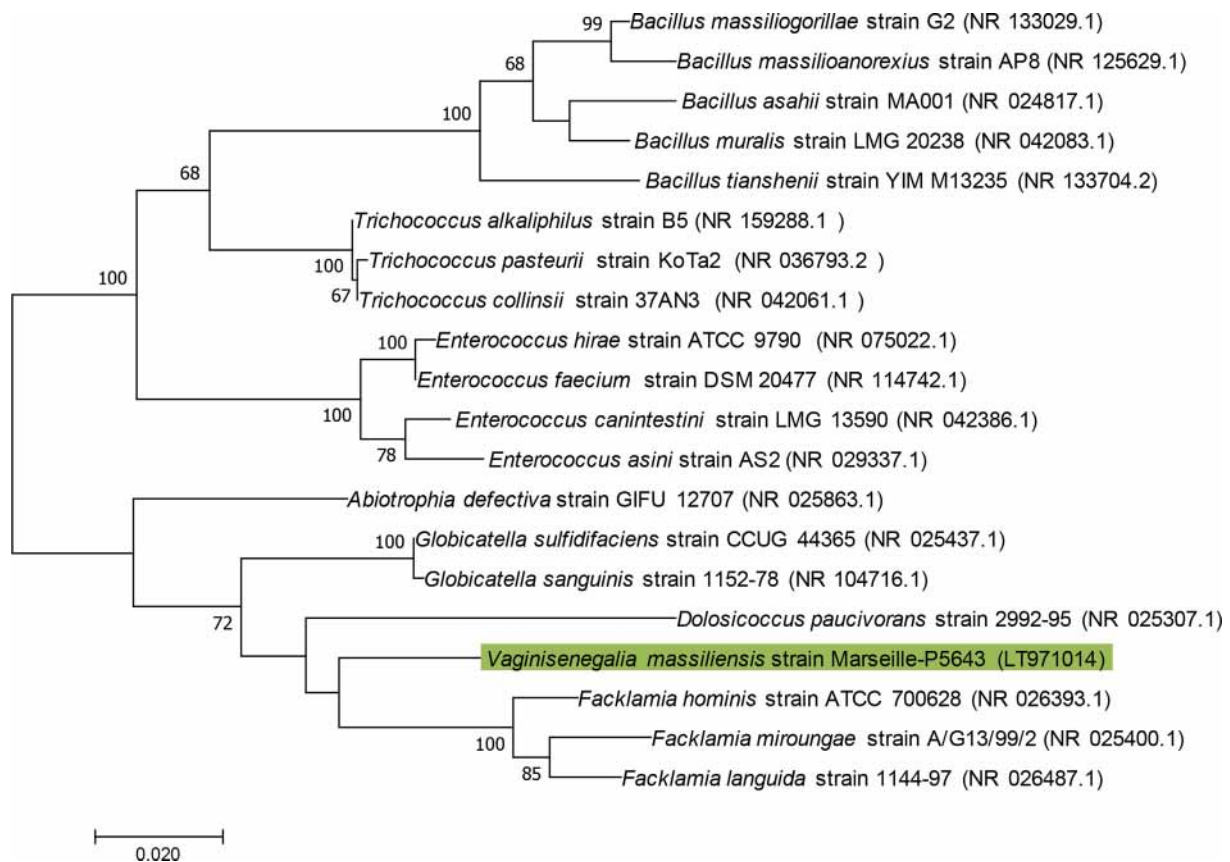


FIG. 3. Phylogenetic tree highlighting the position of *Vaginisenegalia massiliensis* gen. nov., sp. nov., relative to the most closely related type strains within the genus *Vaginisenegalia*. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inferences were obtained using the maximum likelihood method and MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 2% nucleotide sequence divergence.

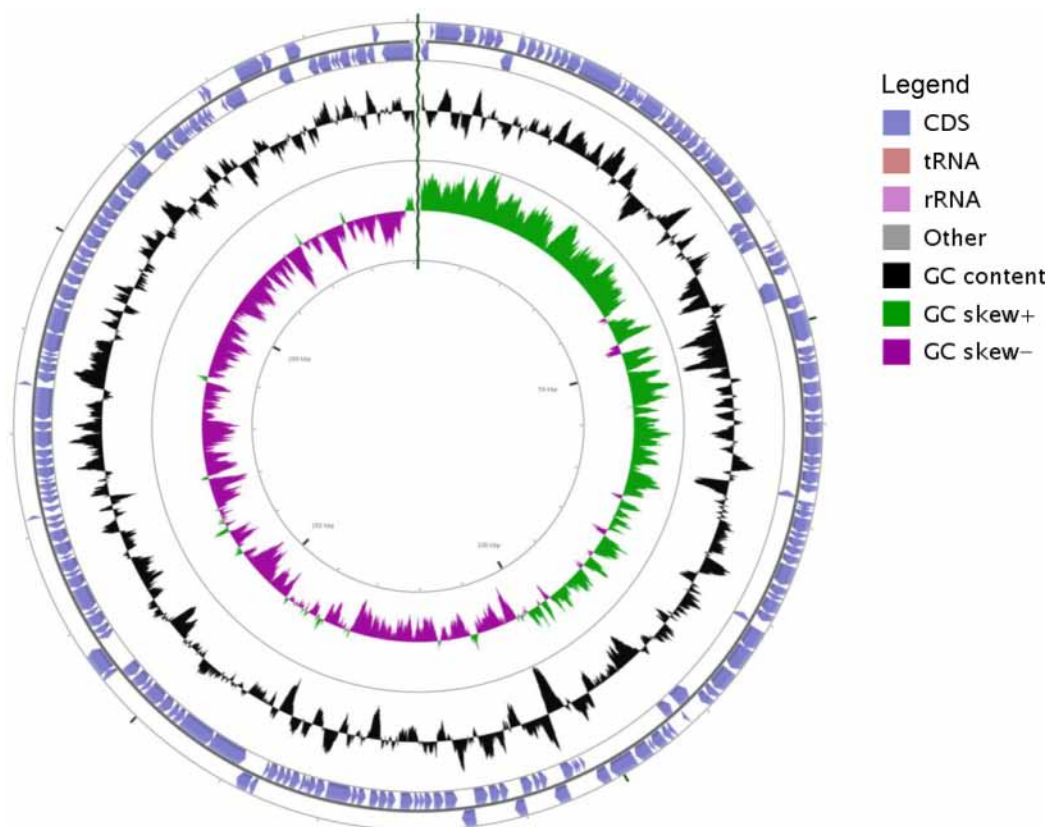


FIG. 4. A circular map generated using the CGVIEW SERVER [28], showing a full view of the genome of *Vaginisenegalia massiliensis* (1 754 973 bp). From outside to the centre: region-coding genes and RNA genes (tRNA/rRNA) from the forward and reverse strands, respectively, GC content (black) and GC skew (green/mauve).

TABLE 4. Number of genes associated with general COGs functional categories of *Vaginisenegalia massiliensis* gen. nov., sp. nov., strain Marseille-P5643^T

| Code | Description | Value | % ^a |
|---|---|-------|----------------|
| Information storage and processing | | | |
| [J] | Translation, ribosomal structure and biogenesis | 189 | 11.6 |
| [A] | RNA processing and modification | 0 | 0 |
| [K] | Transcription | 116 | 7.1 |
| [L] | Replication, recombination and repair | 91 | 5.6 |
| [B] | Chromatin structure and dynamics | 0 | 0 |
| Cellular processes and signalling | | | |
| [D] | Cell cycle control, cell division, chromosome partitioning | 34 | 2.1 |
| [Y] | Nuclear structure | 0 | 0 |
| [M] | Defence mechanisms | 67 | 4.1 |
| [T] | Signal transduction mechanisms | 84 | 5.2 |
| [M] | Cell wall/membrane/envelope biogenesis | 110 | 6.8 |
| [N] | Cell motility | 15 | 0.9 |
| [Z] | Cytoskeleton | 1 | 0.1 |
| [W] | Extracellular structures | 3 | 0.2 |
| [U] | Intracellular trafficking, secretion and vesicular transport | 17 | 1.0 |
| [O] | Post-translational modification, protein turnover, chaperones | 69 | 4.2 |
| [X] | Mobilome: prophages, transposons | 16 | 1.0 |
| Metabolism | | | |
| [C] | Energy production and conversion | 53 | 3.3 |
| [G] | Carbohydrate transport and metabolism | 108 | 6.6 |
| [E] | Amino acid transport and metabolism | 100 | 6.1 |
| [F] | Nucleotide transport and metabolism | 80 | 4.9 |
| [H] | Coenzyme transport and metabolism | 56 | 3.4 |
| [I] | Lipid transport and metabolism | 41 | 2.5 |
| [P] | Inorganic ion transport and metabolism | 77 | 4.7 |
| [Q] | Secondary metabolites biosynthesis, transport and catabolism | 16 | 1.0 |
| Poorly characterized | | | |
| [R] | General function prediction only | 154 | 9.5 |
| [S] | Function unknown | 117 | 7.2 |
| | Hypothetical protein | 196 | 12.0 |

COGs, Clusters of Orthologous Groups.

respectively) but similar to *F. hominis* (38.9 mol%) and smaller than *T. collinsii*, *E. asini* and *F. languida* (45.8, 44.7 and 43.9 mol%, respectively). The comparison of gene numbers shows that the number of genes of *Vaginisenegalia massiliensis* (1613) was lower than those of *T. collinsii*, *E. asini*, *F. miroungae*, *D. paucivorans* and *F. hominis* (3163, 2512, 1949, 1794 and 1767, respectively). The genome analysis of strain Marseille-P5643^T allowed us to study the distribution of genes into Clusters of Orthologous Groups categories, which showed the importance of the function of translation, ribosomal structure and biogenesis (Table 4).

Finally, the degree of genomic similarity of strain Marseille-P5643^T with closely related species was estimated using the ORTHOANI software [24]. OrthoANI values among closely related species (Fig. 5) ranged from 63.52% between *F. languida* and *T. collinsii* to 86.79% between *F. hominis* and *F. languida*. When *V. massiliensis* was compared with these closely related species, values ranged from 64.82% with *E. asini* to 74.79% with *F. languida*.

Conclusion

Taxono-genomics, based on phenotypic and genotypic data, has been used to describe this new bacterium. This allowed detection of clear differences between our strain and those described previously. Therefore, we formally propose the

creation of *Vaginisenegalia massiliensis* gen. nov., a new genus in the *Aerococcaceae* family within the phylum Firmicutes. *Vaginisenegalia massiliensis* sp. nov., contains the type strain Marseille-P5643^T (CSURP5643), which was isolated from the vagina of healthy Senegalese woman.

Description of *Vaginisenegalia* gen. nov.

(*Va.gi.ni.se.ne.ga.lia* N.L. fem. n. *Vaginisenegalia*, is a compound name between vagina and Senegal specifying the type and location of sampling). Cells are Gram-negative bacilli anaerobic and non-motile. Colony growth is obtained in anaerobic conditions at 37° C. The DNA G+C content is about 38 mol%. The type species of the genus is *Vaginisenegalia massiliensis*.

Description of *Vaginisenegalia massiliensis* sp. nov.

'*Vaginisenegalia massiliensis*' gen. nov., sp. nov. (*mas.si.li.en'sis* N.L. fem. adj. *massiliensis*, to Massilia, the Latin name of Marseille where the type strain was first isolated and characterized) is classified as a member of the family *Aerococcaceae* in the phylum Firmicutes. Strain Marseille-P5643^T is the type strain of the new species '*Vaginisenegalia massiliensis*' gen. nov., sp. nov. It is a strictly anaerobic, Gram-negative bacterium, non-spore-forming and non-motile. Colonies of strain Marseille-P5643^T observed on blood agar medium are transparent with a mean diameter of 0.5 mm. This bacterial strain does not present any catalase and oxidase activities. The genome size of

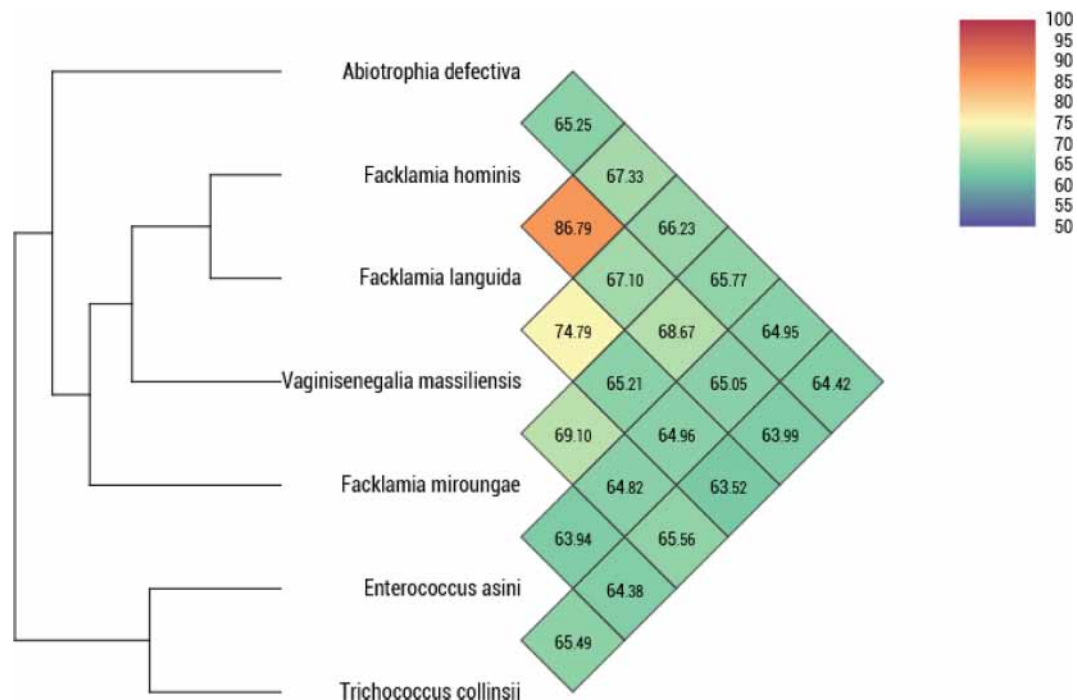


FIG. 5. Heatmap generated with ORTHOANI values calculated using the OAT software between *Vaginisenegalia massiliensis* gen. nov., sp. nov., and other closely related species with standing in nomenclature.

Vaginisenegalia massiliensis strain Marseille-P5643^T is 1 754 973 bp with 39.0 mol% G+C content. The GenBank accession number for the 16S rRNA gene sequence of strain Marseille-P5643^T is LT971014 and for the whole genome shotgun project is UWPC00000000. It was isolated from the vagina of a Senegalese woman living in a rural area.

Nucleotide sequence accession numbers

The 16S rRNA and genome sequences were deposited in GenBank under accession numbers LT971014 and UWPC00000000, respectively.

Deposit in culture collections

Strain Marseille-P5643^T was deposited in two different strain collections under the following number (CSURP5643).

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Conflicts of interest

None to declare.

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